SOME ASPECTS OF THE CHEMISTRY OF THE DRIED FRUIT AROMA

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This thesis is dedicated to Edith Staubach, my grandmother, whose interest throughout my education has been of great value to me. Thank you Nain.

ABSTRACT

Many compounds of alleged dried fruit-like aroma have been prepared with a view to a structure-activity relationship (S.A.R.) analysis of dried fruit odorants. Of the oxaspiro[2,5]octane type compounds, all the diastereoisomers of ethyl 4,4,7-trimethyl-2-(1-oxaspiro[2,5]octyl)carboxylate were prepared. The glycidate esters, derived from the Darzens condensation of α-ionone with methyl and ethyl chloroacetate, were also prepared. Megastigma-5,7-(E),9-trien-4-one was synthesised from ß-ionone. All of the compounds were claimed to have dried fruit-like or related aromas, but these claims were not confirmed by us. Some novel chemistry and compounds were prepared during the syntheses of these compounds. In a serendipitous discovery by workers at Gallahers Ltd., cyclohexylacetic acid was found to possess a dried fruit note. In order to investigate the stereoelectronic requirements of the presumed dried fruit aroma receptor, several commercial compounds were assessed and several analogues were prepared and assessed. The 2-, 3- and 4- methyl substituted acids were synthesised by two novel routes from a suitable methyl substituted cyclohexanone. The ring methyl substituted α,ß-unsaturated acids were also prepared and assessed, as were the α,ß-, ß,γ-and γ,δ-unsaturated analogues of cyclohexylacetic acid itself. In addition, several bicyclic acetic acids and their unsaturated analogues were prepared and assessed. Thus, 9-bicyclo-[3,3,1]nonylacetic acid and 9-bicyclo[3,3,1]nonylideneacetic acid were made. Likewise, the isomers of 2-bicyclo[2,2,1]heptyl acetic acid and 2-bicyclo[2,2,1]heptylideneacetic acid were assessed. Furthermore, a variety of the intermediates were prepared and assessed. The results of the S.A.R. study of these substituted acetic acids were inconclusive. The most realistic dried fruit-like odorant was cyclohexylacetic acid and

the odorant of lowest threshold was exo-2-bicyclo[2,2,1]heptyl acetic acid. Of interest, some methyl esters of these types of compound had dried fruit-like notes in their aroma profile on smoking in a cigarette.

C O N T E N T S

INTRODUCTION

The flavour of tobacco is a complex mixture of various modalities of which the dried fruit aspect in both unsmoked tobacco and tobacco smoke is discernible. This thesis is an investigation of compounds which relate to the dried fruit aroma and hence potentially mimics this aspect in tobacco itself. In view of the broad and interdisciplinary nature of this area of research, it is intended, in the introduction, to take a general overview of the biological and histological aspects of olfaction and to review briefly some of the aroma theories. The known constituents of tobacco and dried fruit which are of relevance to this work will then be considered.

The human nose comes in a variety of shapes and size, its raison d'être spanning the realms of cosmetic appeal to its use as a reference point in vision, but its primary function is as an organ for chemoreception. The physiology of the mammalian nasal sensory apparatus has been studied and reviewed by many authors[1-9] whose work shall be summarised below.

With regard to chemoreception, the human nose has two symmetric areas of so-called olfactory sensory epithelium (OSE), each about $2.5cm^2$ in area and in line with the nostrils. The morphology of the nose itself directs a stream of inhaled (or exhaled) air over the OSE. Just as two eyes allow stereovision, two nostrils enable a degree of directional specification of an odorant source.

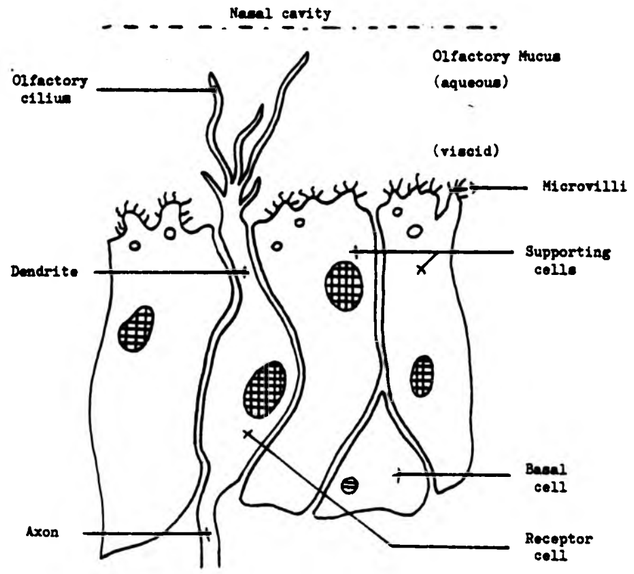
To suggest that chemical sensitivity occurs uniquely on the OSE is misleading. Information is also obtained from other tissues in the respiratory system; for example, the bronchial constriction caused by strong acids like hydrogen chloride. Also, the taste buds in the mouth can provide information about inhaled chemicals. However, whereas the taste buds of man can detect sour, sweet, salt and bitter chemicals, the

OSE can detect and differentiate an enormous variety of odours, and is designed by nature purely for this function.

The degree of development of olfactory sense in man is comparatively low. It has been suggested that as human intelligence developed, the sense of smell became less important, with regard to survival.[10] Smell is still an important sense, however. We detect a rotten egg by its odour and we know the difference in aroma of petrol and water. Olfactory sensing such as this may still be of some survival value. However, when we think of odorants nowadays, it is usually fragrances and perfumes that spring to mind. The role of aroma in social decorum is quite formidable. A person is more likely to find attractive a mate who smells of roses rather than rotting garlic. Aroma is not as important in humans as lower forms of life. Our knowledge of insect pheromones is rapidly expanding, with insects displaying a high reliance on such chemical communication. Perhaps the evolution of other communicative skills has made man less dependent on smell.

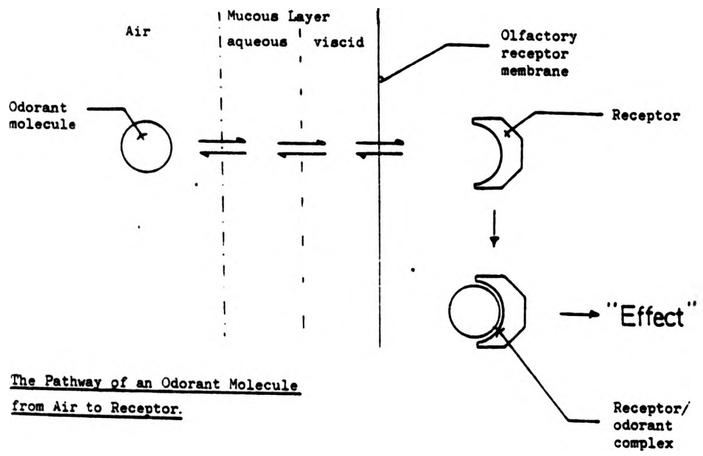
The question of how odorants are detected is quite complex. One must consider the large variation between people in their ability to detect odorants. Indeed, an individual may be anosmic to an odour readily detected by someone else. The example of androsterone is frequently cited, the incidence of anosmia to this urine-smelling compound being close to 50%. It has been shown that variation in anosmic occurrence exists between races and the sexes.[11]

Another problem with olfactory assessment is the subjectivity of smell. Two people experienced in smelling, say, onions may differ in their detective mechanisms. It may be that compound X is the primary chemical stimulant for one of them, whereas it is compound Y for the other. Likewise, compound Z may be stimulating identical detective receptors, but the smell described differently by different people.



The Olfactory Epithelium.

Diagram 1.



The Pathway of an Odorant Molecule from Air to Receptor.

Diagram 2.

Moreover, the same person may describe the same odorant in a different way when challenged a second time.

Other potential hazards of in vivo odour assessments include the subject person's state. Obviously, if the person has a cold, access to the OSE may be restricted. Certain medicines are known to affect olfaction and, perhaps less obviously, certain foodstuffs.

The OSE is made up of essentially three cell types, as shown in diagram 1.[12] The basal and sustentacular cells appear to be of mainly structural importance. The mucus layer continuously bathes the surface with secretions from Bowman's glands. Secretion can be increased considerably when irritants contact the epithelium.

The average person has ~10^8 olfactory receptor neurons (ORNs) and it is these cells that convert chemical contacts into electrical signals along the neuronal axons. It was originally thought that there would be a variety of different ORNs with odour type specificity, but electrophysiological experiments disproved this. An ORN can be responsive to various odorants and presumably interpretation by the brain must be based on an overview of all ORN responses; that is a smell profile.

With respect to odorant receptor sites, it is thought that membranous particles, probably proteins, on the cilia may be involved. It is possible that odour differentiation could be, at least partially, via receptor position, either by position on the surface or depth under the surface. A schematic representation of the relationship of the ORN to the air flowing over the OSE is shown in diagram 2.[13]

Some of the problems of biochemical analysis of the olfactory system have been highlighted earlier. Beets[2] summarises the situation thus: "....having looked around in wonder, we are forced to admit that we are still far removed from the molecular level where the peripheral processes

of chemoreception take place, starting with an interaction between a molecular species of which we know nearly everything and a molecular species of which we know almost nothing. It is obvious that our present techniques may permit us to narrow but never to bridge the gap."

This synopsis excludes several finer points however. It is evident that an odorant molecule can be well defined and that the effect generally considered is the subjective evaluation by a panel of assessors. It would be more helpful if information could be derived from a shorter path than the odorant challenge to the panel's assessment. This can be achieved by isolation of the OSN and information can be derived directly from the shorter pathway of odorant challenge to neuron stimulation. Electrophysiology experiments have shown that at the cellular level, OSN's are more complex than originally thought, and of limited value in providing information about odorant binding. It would be more useful if the odorant molecule receptors themselves could be analysed and hence shortening the pathway further. Attempts to isolate the presumed ciliary proteins and evaluation of their binding properties is, however, in its infancy. Work by Getchell and Getchell[14] on the olfactory tissue of frogs seems to imply receptor proteins as binding agents and a specific site for ethyl n-butyrate was demonstrated. Other workers have also implicated protein binding sites[15] but analogous results with human chemoreception have yet to be proven.

Other important factors in chemoreception are the partition coefficients between the various phases of the epithelium.[16] As previously shown in diagram 2, an odorant must show volatility, mucus solubility and probably lipophilicity in order to physically reach the receptor species, and only at this point will odorant molecule/receptor binding become relevant. Correlations between partition

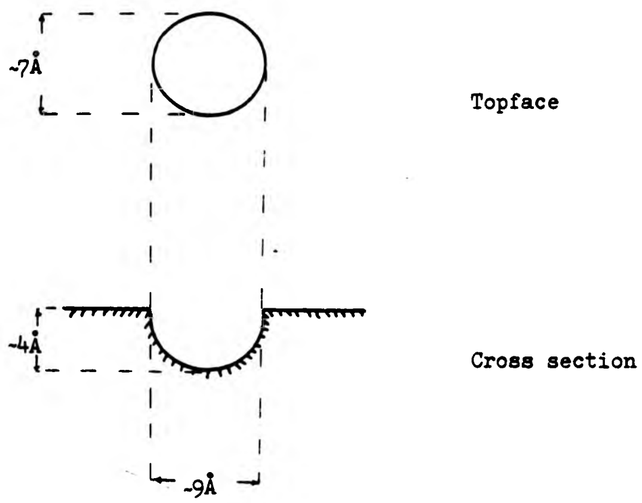
coefficients,[17] generally with the water:n-octanol system, and odorant activity have been attempted with limited success. The model relevance of the water:n-octanol system must be questioned, however, especially with regard to the achiral nature of these two phases, when the biosystem is chiral in both the aqueous and the lipophilic phases.[18]

Another potential pitfall is the metabolism of an odorant along this pathway, the products of which may be more or less able to effect a response.

Despite all the difficulties, great advances have been made in recent years with regard to structure-activity relationships (SAR) analysis in human chemoreception.[2,9,19] The use of computers,[20,21] especially pattern recognition and graphic programmes, has contributed enormously to the understanding of many biological systems where the receptor is not defined, but its active site is inferred by SAR analysis. Chemoreception is not an exception.[22] The structural requirements of sweet tasting compounds are now quite well understood, due to SAR analysis.[23-25] The precise nature of the human 'sweet' receptor and its mechanism for neuron response induction is not known.

With respect to the application of SAR techniques to odorants, an example is the analysis of many steroids and synthetic compounds that display a urine type smell. Ohloff and coworkers have demonstrated the structural and configurational factors required for this effect.[26,27] This work and related work on ambergris-type odorants invokes the so-called "triaxial rule of odour sensation" which will be discussed later.

One approach into the field of receptor specificity is by analysis of specific anosmia, a method championed by Amoore,[5,16] and does not necessitate isolation of the receptors. The method is akin to other biochemical techniques such as the detection of protein binding sites in



The Camphoraceous Receptor Site.

Diagram 3.

the chemotactic response of E.coli to various sugars.[28] By considering subjects without certain receptors (i.e. specific anosmics) there appears to be ~30 primary odours which may correspond to ~30 discrete receptor proteins or combinations of a smaller number of receptors. This approach will also be considered further below.

In summary, the biochemistry of the receptor cells is poorly understood with probably the most promising area for future discoveries being the isolation and detailed examination of receptor proteins from ciliary membranes. Until such time as the odorant/receptor system is directly described, SAR and aroma theories assist in our understanding of human chemoreception, and various of these theories will be briefly discussed in the following section. Once the receptor system is understood, perhaps it will then be possible to illucidate the wonder of brain perception of odorants.

Supposing an SAR study has been carried out for some odorant effect, the result must then be interpreted. This is generally achieved by pattern recognition and invoking an olfaction theory to explain the results. A large number of such theories have been presented and some of the more important will be mentioned here.

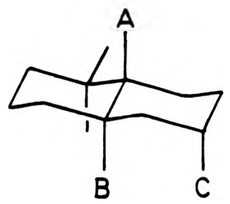
Amoore's stereochemical theory[5,16] suggests that the receptor sites, represented as an indentation on a membrane, are sensitive to the structural properties of an odorant molecule. The theory assumes perhaps ~30 primary odours of which Amoore has defined eight; ethereal, camphoraceous, musky, floral, minty, pungent, putrid and sweaty. These were deduced from specific anosmia studies. Predictions about the aroma of an untested molecule using this theory can be quite impressive. The camphor receptor site, for example, is claimed to be an oval cup shaped structure as shown in diagram 3.[29,30] Substances which can interact with this cup with some degree of fit, will effect a response by some

undefined mechanism.

The membrane puncturing theory presented by Davies[31] implies a change in membrane permeability caused by vacation of an adsorbed odorous molecule. The hole left in the membrane allows the flow of ions and hence a neuron response is initiated. Experimental evidence provides little support for this theory as originally suggested, but perhaps the concept per se is not so far fetched. It may be that the membrane permeability is changed by an odorant molecule/receptor protein complex via the formation of ion channels. This revised concept would seem more plausible.[32]

The vibrational theory has been supported by Dyson[33] and Wright.[34,35] In essence, an odorant molecule transfers vibrational energy from itself to the receptor when complexed. Correlation between vibrational spectra and odorous materials has been presented and predictions made regarding the aroma properties of untested compounds. Dyson considered the range 1500-3000 cm^{-1} to be important whereas Wright was concerned with the somewhat lower wave numbers of 100-700 cm^{-1}. The latter would seem more credible in that vibrations of the whole molecule are considered, rather than individual functionalities, and the concept does infer a method of energy transfer from the odorant to the receptor molecule.

The profile-functional group concept of Beets[2] considers the steric and functionality properties of the odorant molecule to be critical for binding to some receptor site which may effect a neuron response. The affinity of the functional group determines the orientation of the odorant molecule at the receptor site and hence provides the profile of the odorant molecule when receptor interaction takes place. Unlike Amoore's theory, specific receptors were not invoked but accessible functionality was considered to be the key to



Odour qualities:-

Wet earth, musty
Sea water, seaweed
Tobacco
Sandelwood-type
Animal, note of musk
Faecal

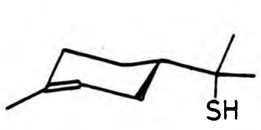
The Triaxial Rule of Odour Sensation - Ambergris.

Diagram 4.

strong odorant perception.[16] For bifunctional compounds, the stronger binding of two interactions becomes possible. This approach can be illustrated by the AH, B concept. Ohloff and Giersch[36,37] describe this system with compounds possessing a menthane type structure, AH being a hydrogen donor function (such as a hydroxyl) and B a hydrogen acceptor function (such as a ketone). The concept provides a possible energy transfer mechanism, if proton exchange were to occur, and the concept has been applied to the sweet taste receptor in a modified form.[24,38]

Further work by Ohloff and coworkers led to the so called "triaxial rule of odour sensation" found applicable to ambergris smelling compounds.[34-41] By synthesis and assessment of a formidable number of compounds, it was demonstrated that for molecules of the type shown in diagram 4, the requirements for ambergris-type odorant activity were, in general, a trans-fused ring junction, an oxygen functionality and substituents with axial orientation in positions A, B and C. This rule was later extended to urine-type odorants[26,27] and the woody character of some compounds in the eudesmane series.[42] It is clear that a computer graphics simulation of the active site(s) for all these compound types would facilitate visualisation of active site/odorant interaction and odour quality predictions.

The theories of Amoore and Beets may be closely related in that both involve a receptor with some ability to bind compounds more or less readily and hence possess a selectivity presumably reflected by a corresponding dissociation constant. Given this, an alternative approach to the Amoores specific anosmia studies could be quantitative SAR,[21] on the assumption that the changing of an odorous molecules structure, such that it tends to optimisation of the response, should decrease the detection threshold. This approach, in collaboration with specific anosmia studies, has been used in the identification of the



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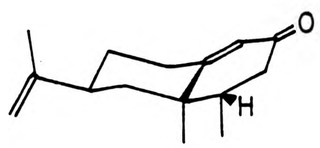

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sebaceous primary odour.[43]

An interesting development has recently been reported by Mason and Morton.[44] Their work has involved the artificial production of specific anosmia in salamandas, in a fashion analogous to the suicide substrate principle used in enzymology. Their approach was the nucleophilic attack of a Schiff base formed *in vivo* by an odorant and receptor species and follows earlier significant work by Menevse *et al.*[45] in which specific protection was used to defend the receptors of pentyl acetate from a thiol specific binding reagent. Deprotection enables these receptors to be studied, without interference from other receptors, using electrophysiological methods. Clearly, further work in this area would prove rewarding.

TOBACCO

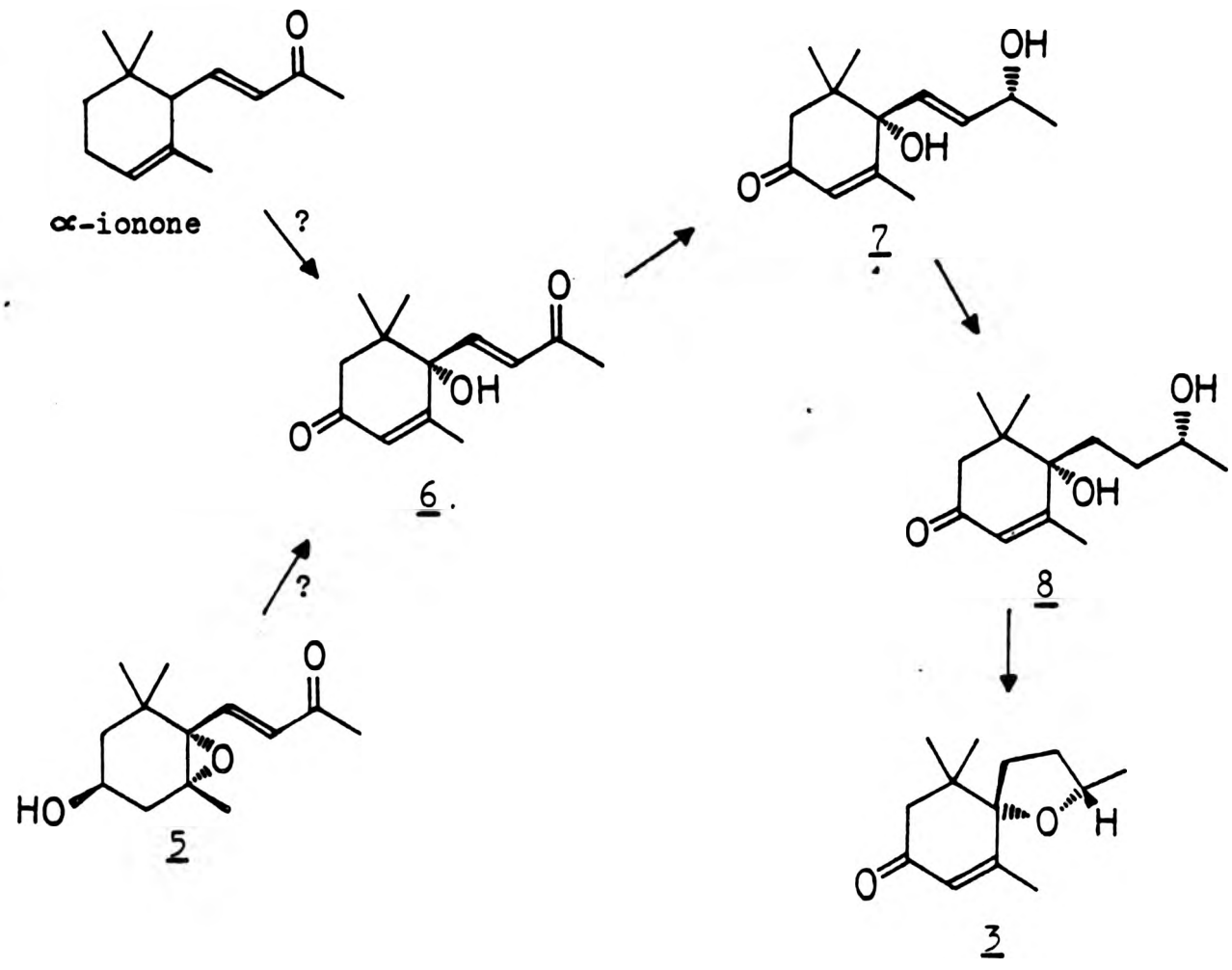
Apart from food producing plants and cotton, tobacco was reported in 1978 to be the most cultivated plant in the world, being produced in the quantity of about 5 million tons *per annum*.[46] Research into its constituents has been naturally quite vigorous especially in the last two decades, inspired by the links between smoking and detrimental health. The number of compounds detected in the oils and smoke of various tobacco types is more than 3000,[47] many of which contribute more or less to the flavour of the tobacco concerned. Key flavour compounds have been suggested and certain compounds implicated as differentiating particular aromas. The primary flavour impact compound, if there is such a compound, has yet to be discovered for tobacco unlike, for example, grapefruit.[48] The isolation of the thiol 1, a compound with the remarkably low threshold of 2×10^{-5} parts *per* billion, from grapefruit has replaced nootkatone 2 as the primary impact compound in grapefruit. In tobacco, it seems more likely that various compounds provide the basic profile of tobacco aroma, especially in view of the critical dependence

of flavour on factors such as curing, harvesting and infection as well as tobacco variety. One aspect that has been investigated is that which gives Turkish tobacco its distinctive quality, and this is discussed below.

The use of additives to tobacco in order to modify flavour characteristics is well known, including vanillin, coumarin, lavender and bergamot oil in this category,[49] The desire for potentially less harmful tobacco substitutes requires low toxicity flavourants with genuine aspects of tobacco taste and smell. The search for novel additives and substitutes has stimulated research in this field,[50] despite the commercial failure of the so-called new smoking material (NSM). This was partially due to the inadequate mimicry of tobacco, which could perhaps be overcome by further research leading to better synthetic materials.

A review listing the known constituents of tobacco was published in 1968[51] and was updated in 1977 with respect to isoprenoids and alkaloids.[46] It is not intended to discuss or list the constituents of tobacco here, except in the areas of specific interest to this work.

The carotenoids as a class of compounds are well known as precursors to odorous materials.[52] Drying processes appear to facilitate the breakdown of carotenes to smaller, volatile products.[46] Simple cleavages and functionality changes can be invoked to explain the occurrence of most of these, and the processes are not confined to tobacco. Other dried plant products, such as tea, are known to contain flavoursome carotene degradation products. Interestingly, carotenoid pigments are known to be present in the human olfactory epithelium but their function, if any, is unknown.[53]



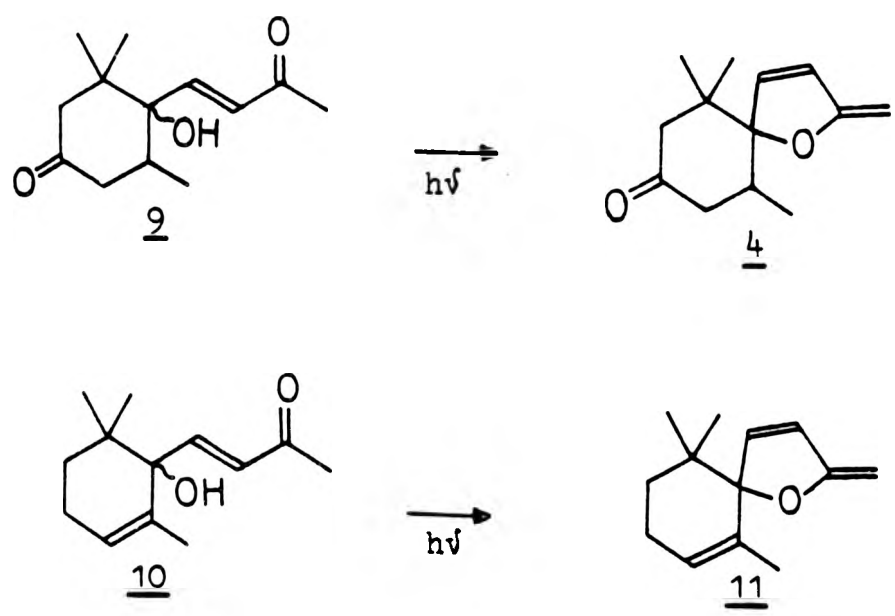
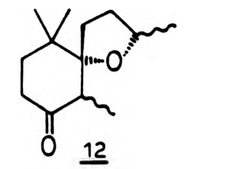


Diagram 5.



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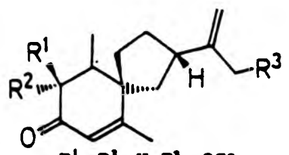
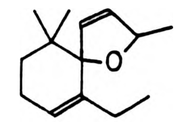


$R^1 = R^3 = H, R^2 = OGluc$ 15
$R^2 = R^3 = H, R^1 = OGluc$ 16
$R^1 = R^2 = H, R^3 = OGluc$ 17



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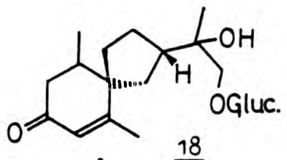

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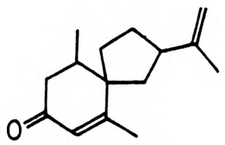

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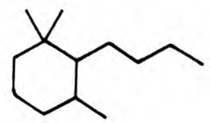

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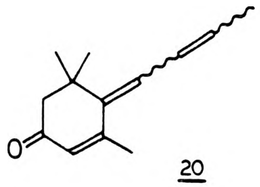

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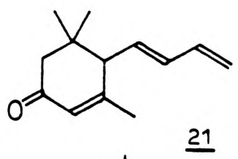

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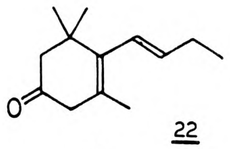

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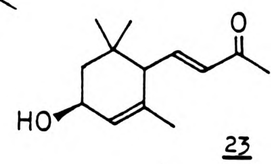

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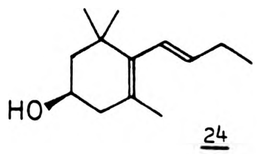

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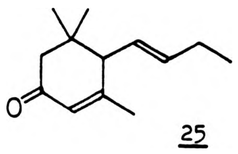

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Diagram 6.

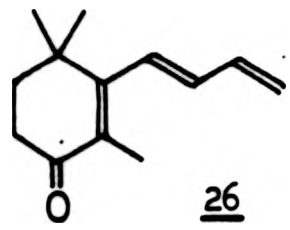
Simple spirane compounds of presumed carotenoid origin

Enzell et al.[46] described two simple oxaspirane type structures known to be tobacco constituents, 3 and 4. Diagram 5 shows the proposed biosynthetic pathway to 3 from 6, itself derived from possibly α-ionone or 5. Note that α-ionone, 5 and 7 are also compounds identified in tobacco. Compound 4 could originate similarly, although the alternative mechanism shown in diagram 5 was suggested, by analogy to the known transformation 10 to 11. Interestingly, spirane compounds such as these are claimed to be characteristic aroma constituents of black tea.[54] Also, the spirane compounds 12 have been identified in Osmanthus-absolute,[55] a fragrance material used in the flavouring of chinese tea. Spiranes 12 are claimed to possess cedarwood aromas with aspects of dried fruit and patchouli. Furthermore, compounds such as 13 have been patented as fruity, blackcurrant smelling compounds.[56]

The spirane compound 14 was isolated from tobacco mosaic virus infected tobacco leaves, and is described as a useful tobacco flavourant.[57] The related compounds 15, 16, 17 and 18 are reported to give a realistic tobacco aroma on pyrolysis[58] and were isolated from flue-cured Virginia tobacco, the absolute stereochemistry resulting from X-ray analysis of a derivative.

Megastigmane-type compounds

Presumed of carotenoid origin, many compounds with the megastigmane skeleton, 19, are known. Indeed, the well known perfumery materials, the ionones, fall into this category. Compounds 20 and 21 have been identified in tobacco[59,60] in quantitites up to 10% of the condensate,[61,62] and are shown in diagram 6. Compounds 20 and 21 have been claimed as key tobacco flavourants[47] with a tobacco-like, balsamic, woody smell, and all the compounds in diagram 6, except 23,



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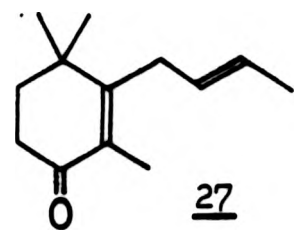

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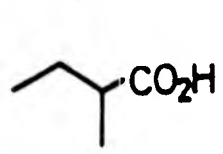
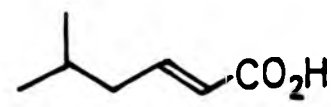
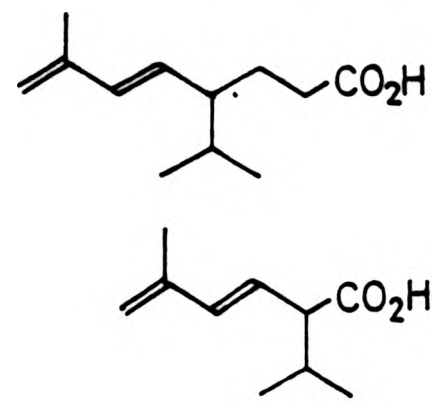
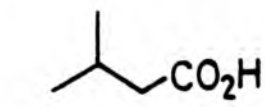
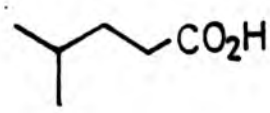
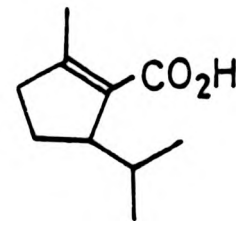


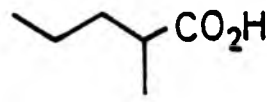


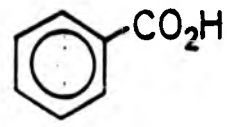












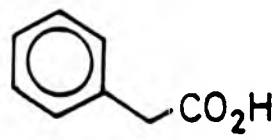
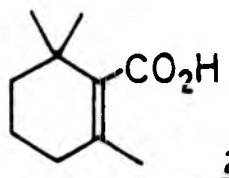


Diagram 7.



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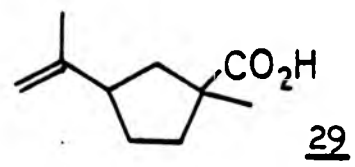

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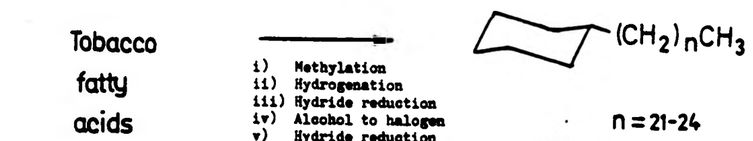
Diagram 8.



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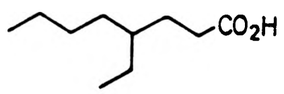

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have been identified in tobacco. Recently, some compounds of this type have been reported to be present in tobacco as glucoside derivatives.[63]

Also of interest are compounds 26 and 27 found in Osmanthus - absolute. Compound 27 has been isolated from tobacco and passionfruit, and possesses a "flowery-fruity odour";[64] Compound 26, on the other hand, possesses a "tea, spicy and dried fruit reminiscent" aroma.[65]

Acids

In 1968 Stedman[51] listed all the known acid constituents in tobacco. He noted the importance of branched aliphatic acids, such as ß-methyl valeric acid and isovaleric acid, in the aroma profile of Turkish tobacco. Indeed, the addition of these acids to other types of tobacco imparts a distinct Turkish tobacco flavour. It was also noted that formic and acetic acids make up ~75% of the volatile acids in tobacco.

Enzell et al.[46] made additions to Stedman's list and some of the compounds on the revised list are shown in diagram 7.

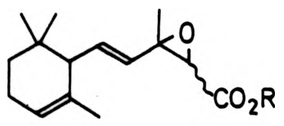
Some other interesting acids have been described by Fujimori and Kaneko[66] in their studies on tobacco aroma. Noteworthy are 28 and 29.

Work by Mold et al.[67] seemed to imply the presence of monosubstituted cyclohexane fatty acids in some unknown oxidation state. The acidic compounds of tobacco were subjected to the reduction processes shown in diagram 8 such that the carbon skeleton was retained, but as the fully saturated hydrocarbon. The cyclohexyl alkanes obtained by this procedure were characterised as 30. It should be noted, however, that volatile compounds produced at any stage would be lost in this procedure and so compounds such as phenyl acetic acid would not have been detected.

Anderson and Kelly[43] have recently detected the powerful odorant

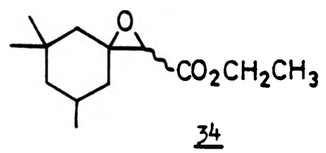
Table 1.

→ Hexanal	1-Octen-3-ol
Heptanal	1-Octanol
Octanal	1-Nonanol
Nonanal	Hexanoic acid
→ (E)-2-Heptenal	Heptanoic acid
(E)-2-Octenal	Octanoic acid
(E)-2-Nonenal	Nonanoic acid
(E)-2-Decenal	Decanoic acid
(E)-2-Undecenal	Geranyl acetone
(2E,4E)-2,4-Heptadienal	2-Pentylfuran
(2E,4E)-2,4-Decadienal	N-Ethyl-2-formylpyrrole
Benzaldehyde	



R=CH_3 32

R=CH_2CH_3 33



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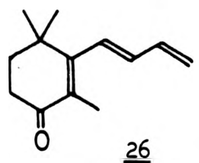

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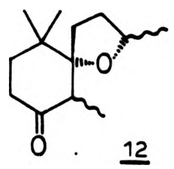

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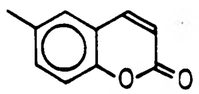

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31 in flue-cured Virginia tobacco: This compound interacts with the proposed sebaceous primary odour or modality receptor,[43] activation of which contributes significantly to the flavour of tobacco smoke. A novel method of threshold detection was developed, involving the smoking of treated cigarettes.[43]

DRIED FRUIT AROMA

One of the discernible features of tobacco aroma is the dried fruit note, most evident in the odour of unsmoked tobacco, but also important in the taste when smoked. This note can be described as warm, fruity and saliva-inducing and is best represented by the smell of dried sultanas, dried figs, dates or raisins. Other fruits, such as apricots and bananas, when dried, possess the same aspect in their aroma profile but the topnote is more characteristic of the fruit itself: Notably, the use of a fermented fig extract has been patented as a tobacco flavourant.[57]

The volatiles of dried fruits have not been extensively studied. Buttery et al.[68] have examined by glc/ms the steam volatile oils of raisins and dried figs, ostensibly in search of insect attractants. Table 1 shows the compounds listed as present in both extracts. A similar study on dried sultanas has been presented by Ramshaw and Hardy[69] and those compounds identified and appearing in Table 1 are highlighted. Although these compounds will contribute to the aroma profile of dried fruit, the primary flavour impact compound(s) of dried fruit has not yet been identified, if there is any.

There are reports in the literature of compounds that possess dried fruit-type odours. Arctander[70] refers to compounds 32 and 33 as fig, date or banana-like in aroma: Similar compounds such as 34 are reported to have a tobacco odour[71] and other glycidate esters are alleged to

have fruity, often strawberry-like, smells:

As mentioned earlier, compound 26 is reported to be "tea, spicy and dried fruit reminiscent" in odour[65] and 12, "cedarwood-like with aspects of dried fruit and patchouli":[55]

Another compound reported, by Arctander,[69] to be "fig or date-like in its fruity deep sweetness" behind a tonka topnote, is the coumarin 35:

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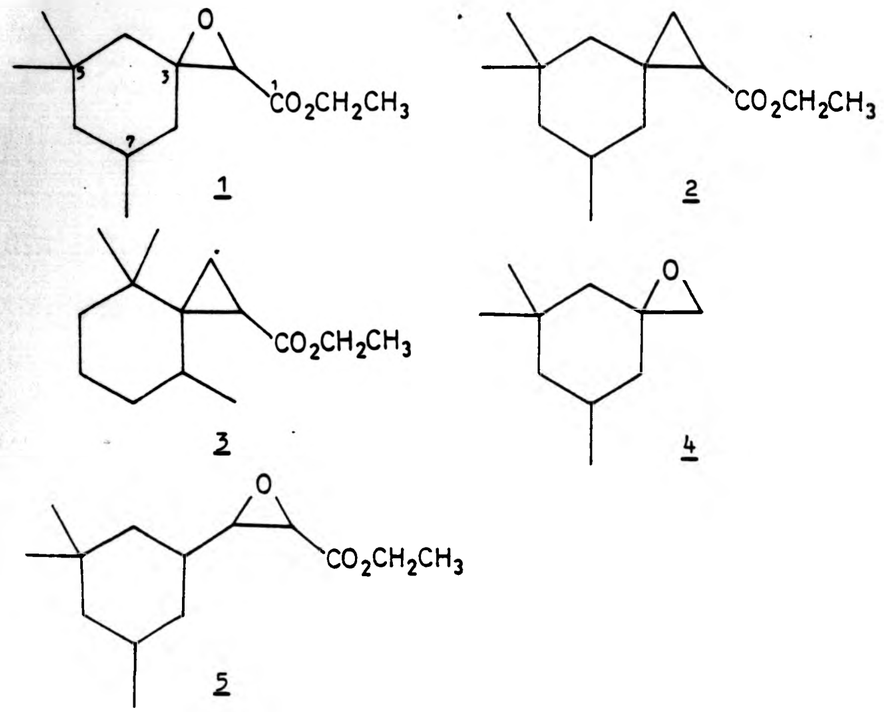

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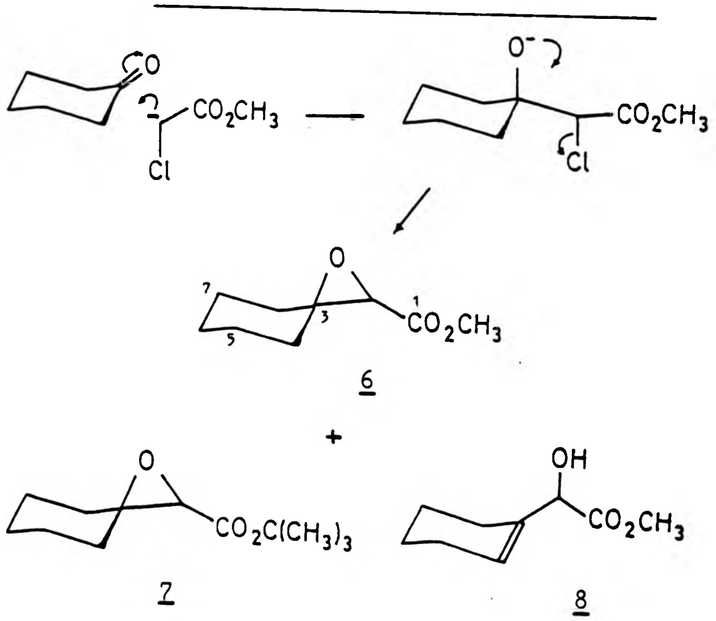

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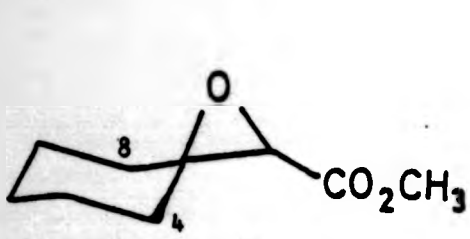
Diagram 1.

RESULTS AND DISCUSSION

Consideration of the spirane compounds detailed in the Introduction and the reported aroma characteristics of the oxaspirane esters 1, led to the belief that compounds with spirane structures could be dried fruit or tobacco-like odorants. The structure-activity study envisaged would systematically modify the functionality and steric factors of 1. Replacing the epoxide ring by cyclopropane gives the novel compounds 2, which could be prepared by analogy to the preparation of 3 by Mousseron-Canet et al.[2] The importance of the ester moiety could be examined by the preparation of 4[3,4] and the spatial relationship of the two rings could be investigated by preparing the novel compounds 5.

Clearly, at the outset, it was necessary to prepare 1 and to confirm its alleged odorous properties, which have been described by Sfiras[5] and Arctander[6] as tobacco-like, although neither author had defined the stereochemistry nor the method of preparation of the compound referred to. In the first instance, the preparation of 6 was undertaken. This model system enabled reaction conditions to be optimised and there are no complications with diastereoisomers. The compound also assisted in the spectral assignments of some of the more complex structures. The ethyl ester analogue of 6 has been prepared by Johnson et al.[7] in good yield using the Darzens reaction.[8,9] Using similar conditions, potassium t-butoxide, methyl chloroacetate and cyclohexanone provided 6, as shown in diagram 1. Better yields were obtained using tetrahydrofuran[10] instead of t-butanol as solvent. The reaction also provided the t-butyl ester 7,[11] as 5% of the volatile products, and the allylic alcohol 8 as a relatively involatile product, which could be separated from 6 by column chromatography and distillation. Compound 7 was probably formed by the attack of the t-butoxide anion on the ester carbonyl of either the



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Catalysed by KO^tBu or heat or $LiClO_4$

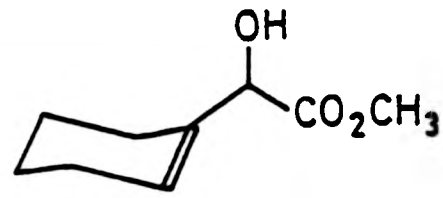

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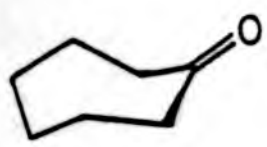
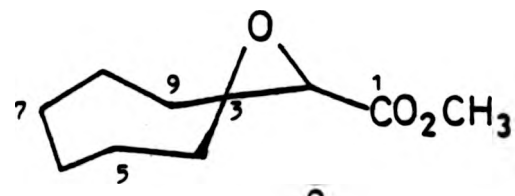
Diagram 2.



$ClCH_2CO_2CH_3$
KO^tBu



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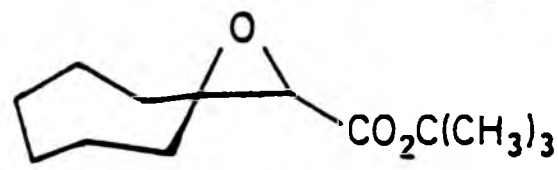

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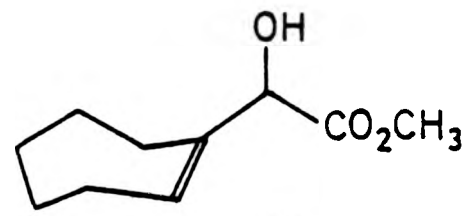

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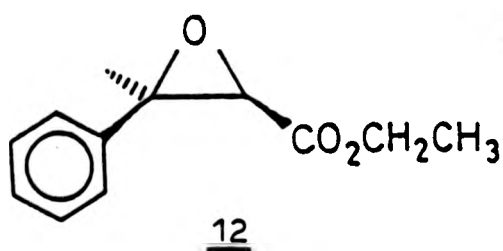
Diagram 3.



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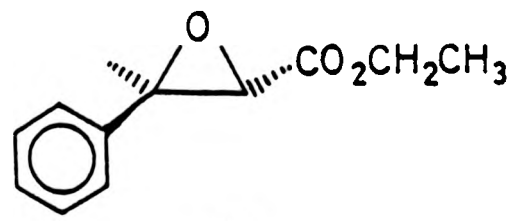

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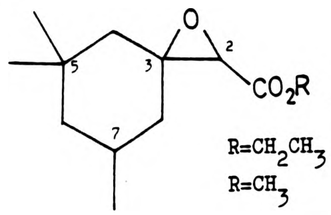
Diagram 4.



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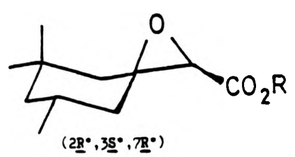
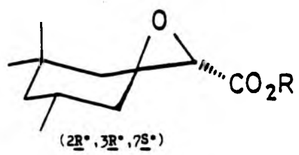
R=CH_3 23



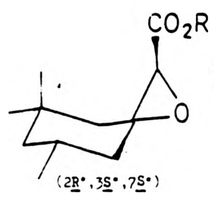
(2R*,3S*,7R*)

R=CH_2CH_3 14
R=CH_3 24



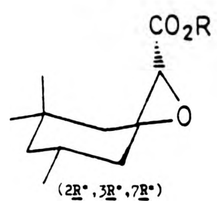
(2R*,3R*,7S*)

R=CH_2CH_3 15
R=CH_3 25



(2R*,3S*,7S*)

R=CH_2CH_3 16
R=CH_3 26



(2R*,3R*,7R*)

R=CH_2CH_3 17
R=CH_3 27

methyl chloroacetate starting material or 6, followed by loss of methoxide. This transesterification reaction with potassium t-butoxide has literature precedent.[12] The allylic alcohol 8 was presumably formed via the abstration of one of the mildly acidic protons attached to C-4 and C-8, followed by ring opening of the epoxide function, as shown in diagram 2. This rearrangement was also observed on storage, proceeding slowly at ambient temperature, but was quite fast in refluxing tetrahydrofuran. The structure of 8 was confirmed by lithium perchlorate-catalysed rearrangement[13] of 6 to 8, also shown in diagram 2.

A second model system using cycloheptanone was investigated, as shown in diagram 3. The methyl glycidate 9 was obtained in 60% yield together with 10 in 4% and 11 in 1%. Compounds 10 and 11 were presumed to be formed by mechanisms analogous to the cyclohexane compounds 7 and 8. Similarly, 9 rearranged on storage at ambient temperature to 11 and this reaction could be catalysed by lithium perchlorate.[13]

The aromas of both 6 and 9 were sweet, fruity and reminiscent of strawberries. Indeed, they were adjudged to be more strawberry-like in odour than the better known, if curiously named, strawberry smelling glycidates "Aldehyde C16", 12/13. Interestingly, it has been reported that only 12 smells strawberry-like, 13 being of a nonspecific, sweet odour.[15]

Structures with substituents are more complicated to prepare. Compound 1 represents four enantiomeric pairs of diastereoisomers, as shown in diagram 4. As stated above, the stereochemistry of the tobacco-like smelling compound was not defined and so it could be any one or a combination of 14, 15, 16 or 17.

The attack of α-halo ester carbanions in the Darzen reaction is

Diagram 5.

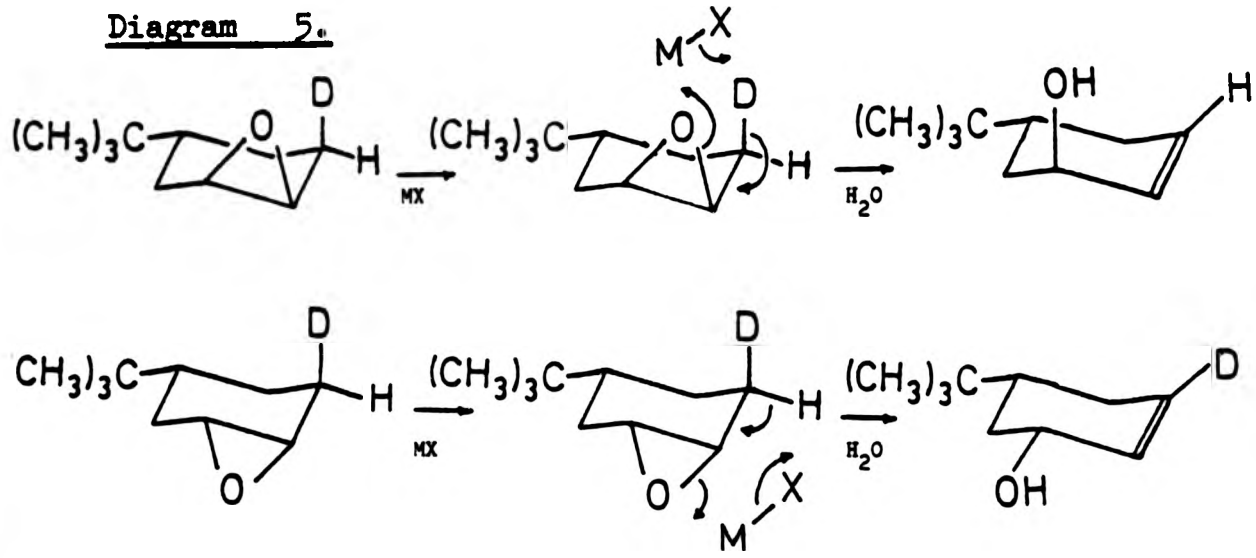
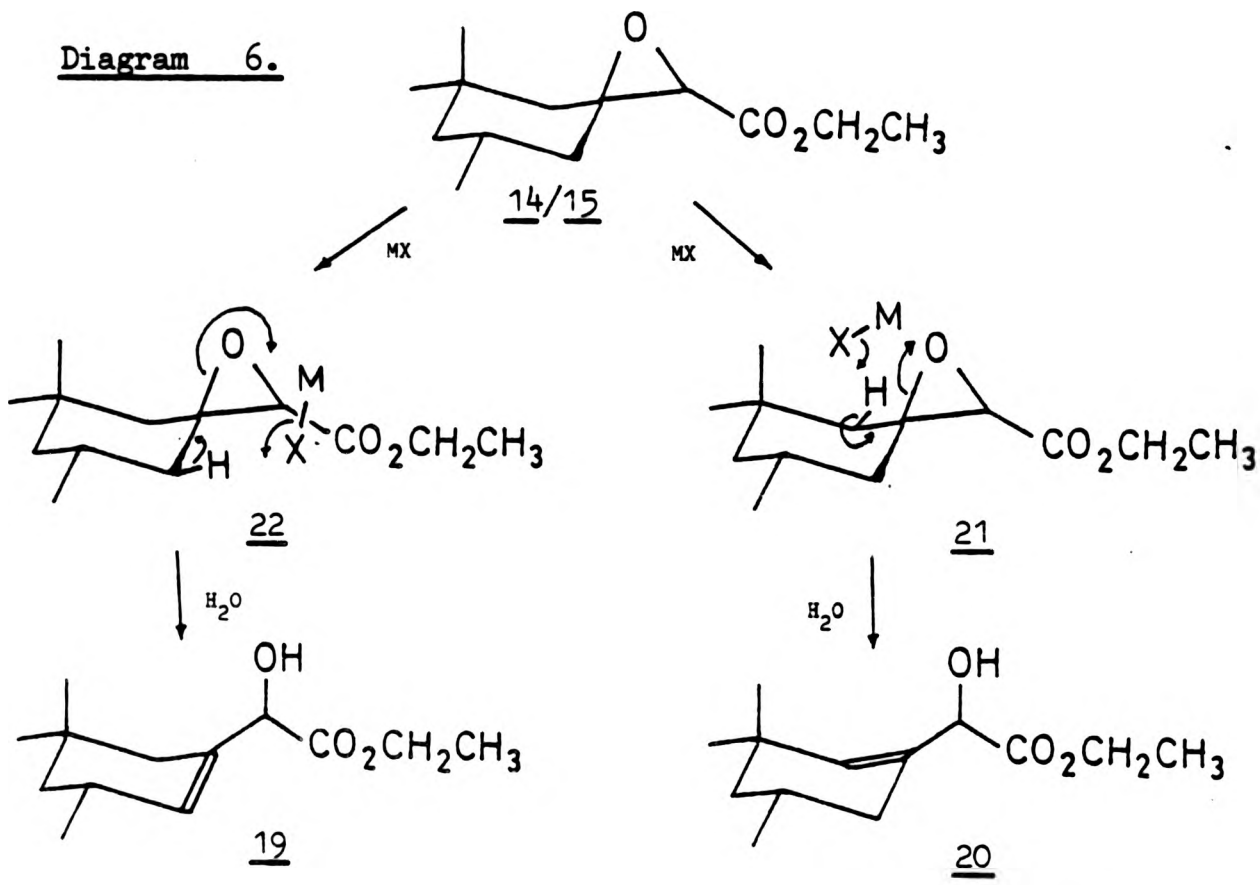
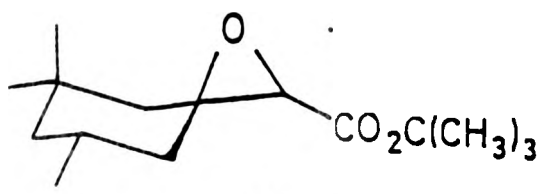


Diagram 6.





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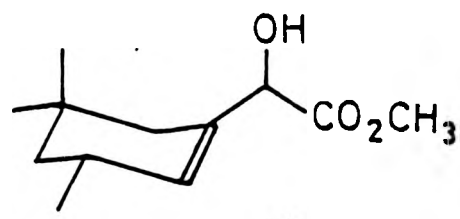

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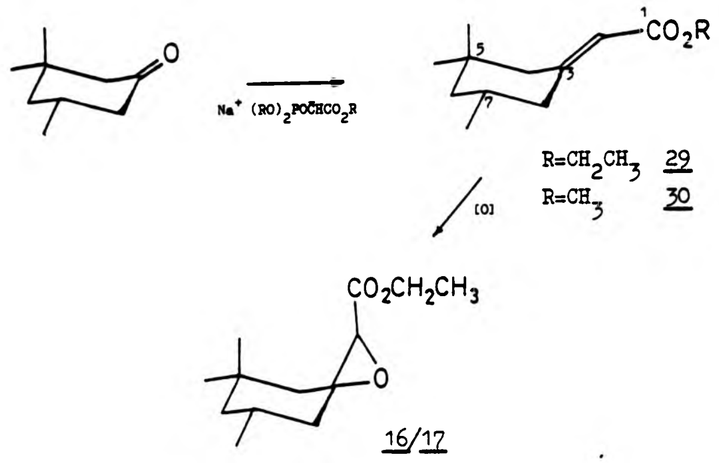


Diagram 7.

known to occur predominantly from the equatorial orientation in substituted cyclohexanones.[16] This result was confirmed when 14 and 15 were isolated as the sole ethyl glycidate products when 3,3,5-trimethyl cyclohexanone was subjected to the same reaction conditions as the model compounds, but using ethyl chloroacetate. The chromatographic and spectral data showed no detectable amounts of 16 or 17. The tertiary butyl ester analogues 18 were detected by g.c. and the allyl alcohols 19 were also isolated. Compound 19 was also obtained by the lithium perchlorate-promoted rearrangement of 14 and 15.[13] The isomeric alcohols 20 were not detected in either the Darzens or the lithium perchlorate reactions, which lends weight to the proposed six electron six centre cyclic transition state for the base induced rearrangements of epoxides to allylic alcohols,[17] as shown in diagram 5.[18] By analogy, the proposed transition states for the metal salt rearrangement of the glycidate esters 14 and 15 are shown in diagram 6. Molecular models show that the proposed transition state 21 has a disfavoured steric interaction between the axial methyl and the metal salt. The alternative transition state 22 does not suffer this interaction. The mixture of 14 and 15 were separated by careful spinning band distillation providing a sample of pure 15 and an enriched sample of 14 containing 13% 15.

Similar work with methyl chloroacetate provided the 23 type compounds, 24 and 25, as pure compounds. The isomeric compounds 26 and 27 were not detected. The transesterification products 18 were isolated along with the isomeric allylic alcohols 28.

It was envisaged that the other two isomers 16 and 17 could be prepared as shown in diagram 7. Using Wittig-Horner conditions,[19,20] the (Z)- and (E)- alkenes 29 were prepared as a mixture in the ratio 2.5:1, the (E)-isomer predominating.[21] The methyl analogues 30 were

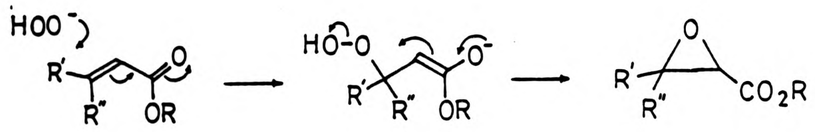


Diagram 8.

6

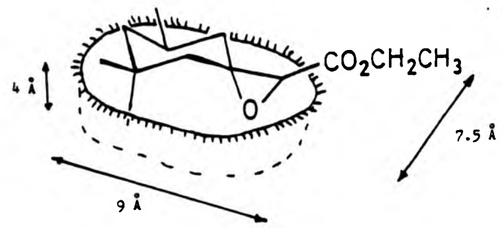
prepared similarly. Interestingly, when the Wittig-Horner reagent employed was methyl diethylsodiophosphonoacetate, the products obtained, on coupling in similar conditions, are both 29 and 30. There is presumably an exchange of alkoxy groups between the ester and the phosphonate.[22]

The epoxidation was expected to be achieved using hydrogen peroxide in the presence of base via the mechanism shown in diagram 8, with equatorial attack being preferred.[23] Basic hydrogen peroxide[24] or sodium tungstate-catalysed[25] conditions were surprisingly ineffective in a variety of experimental conditions, despite the electron poor nature of the alkene double bond. After this work was completed, a paper describing improved conditions for sodium tungstate-catalysed epoxidation was published by Kirshenbaum and Sharpless.[26]

The use of the electrophile m-chloroperoxybenzoic acid has been reported to react with less hindered acrylates,[27] but proved inefficient at epoxidising the systems described here.[28] The more powerful electrophile p-nitroperoxybenzoic acid[24] was more successful, giving a mixture of 14, 15, 16 and 17. Silica chromatography separated the major products 16 and 17 as a 2:1 mixture.

The aromas of 14, enriched 15 and 16/17 were assessed. Compound 14 and enriched 15 were both sweet, fruity and camphoraceous, 14 being slightly less sweet. The 16/17 mixture was sweet, oily and vaguely fruity, but not camphoraceous. The strawberry note of 6 was not evident. The methyl analogues 24 and 25 were also sweet and camphoraceous, 24 being less sweet and sharper. None of these compounds had tobacco-like or dried fruit-like aromas.

It is interesting that the 14, 15, 24 and 25 glycidate isomers give camphoraceous notes. Using the camphor receptor site defined by Amoore[29] and discussed in the Introduction, models of these compounds



Fit of 14/15 in camphor receptor site.

Diagram 9.

were found to fit the proposed site with reasonable surface contact and volume filling. The isomers 16, 17, 26 and 27 do not fit so tightly. The situation is represented in diagram 9. The value of the discriminant function described by Eminet and Chastrette[30] was not calculated for any of these compounds.

The threshold detection concentrations on cigarettes of the compounds 14, enriched 15, 16/17, 24 and 25 were assessed to be greater than 1000 p.p.m.

With regard to structural assignments, the model oxaspirane 6 is well known. Published data include the ^{13}C n.m.r.[31] and mass[32] spectra. The spectral data obtained for the Darzens condensation product were in complete agreement with the structure given.

The other model compound 9 has also been reported[32] and again the spectral data were consistent with the proposed structure.

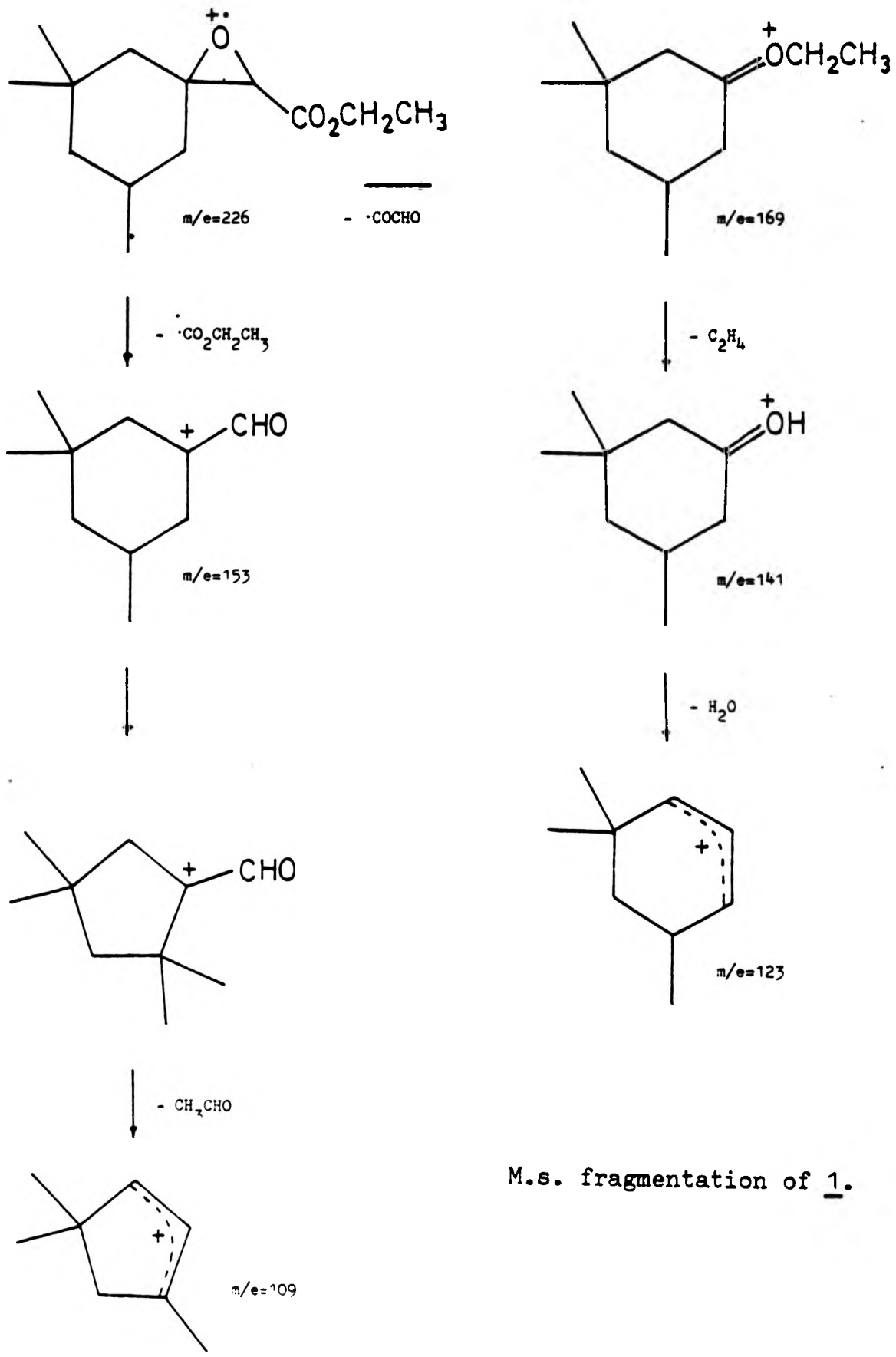
The t-butyl ester analogue 7 was observed as an impurity in crude 6 and was assigned from its ^{1}H n.m.r. spectrum by comparison with 6 and 9. Likewise, the t-butyl ester 9 was assigned from its spectral characteristics.

The ethyl ester analogues of the hydroxy esters 8 and 11 have been described by Hartman and Rickborn.[13] The methyl esters themselves were identified from their spectra, which were very similar to the ethyl analogues.

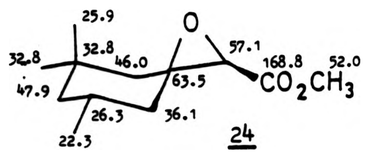
The structures of the various glycidates derived from 3,3,5-trimethylcyclohexanone were characterised by analysis of their spectral properties. Although the Darzens products have been previously reported,[5,16,33] no spectral data have been published.

With regard to 14 and 15, the m.s. of each were virtually identical. The fragmentations described for cyclic glycidates by Baldas et al.[32] were evident and so the loss of $^{\bullet}COCHO$ from the parent ion,

Diagram 10.



M.s. fragmentation of 1.



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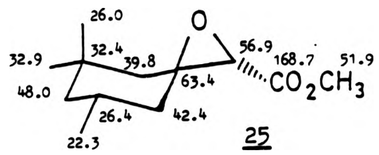

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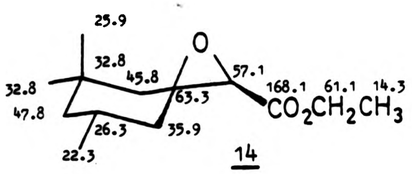

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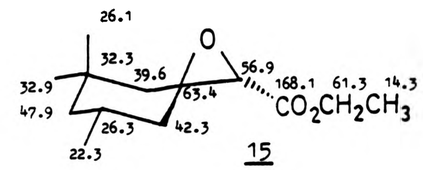

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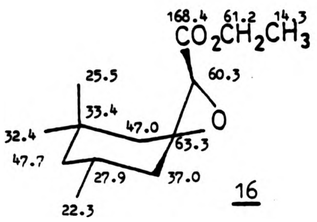

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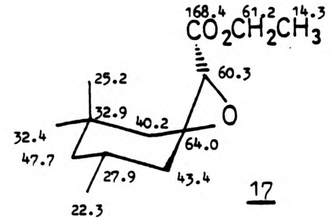

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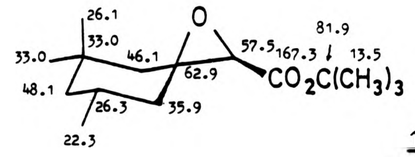

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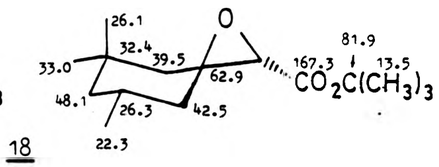

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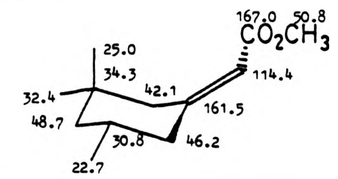

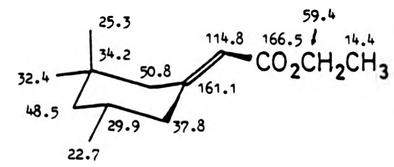

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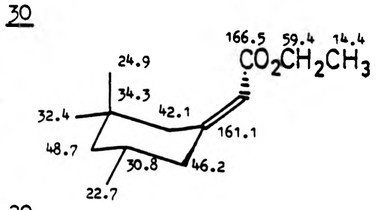


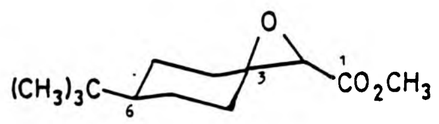
^{13}C n.m.r. data. For experimental details, see p. 89.

corresponding to $C_{13}H_{22}O_3$, gave the base peak at m/e = 169, as shown in diagram 10. The peaks of mass 153, 123 and 109 can all be related to simple fragmentations, but the second most abundant ion at m/e = 83 cannot be explained similarly. This peak was not resolvable and could not, therefore, be accurately measured, but presumably corresponds to either $C_5H_7O^+$ or $C_6H_{11}^+$. The i.r. spectra were very similar with the typical glycidate splitting[10] of the carbonyl stretching band at ~1755 and ~1730 cm^{-1}. This splitting has been explained by invoking two low energy conformers,[34] Fermi resonance being rejected.[35] The ^{1}H n.m.r. spectra were also very similar, noteworthy differences being the chemical shift of the axial methyl substituent which occurs as a singlet at 1.04δ in the (2R*, 3S*, 7R*)- and at 1.08δ in the (2R*, 3R*, 7S*)-isomer. Changes in the fine structure between 2.2 - 1.5δ were also observed. The singlet at 3.19δ in both compounds is characteristic of the glycidate C-2 proton. The ^{13}C n.m.r. spectra were virtually identical except for the shifts of the methylene carbons C-4 and C-8. The chemical shift and long range coupling information allowed the assignment of each isomer to be deduced. Comparison with the deoxygenated analogues 29 also aided the assignments. It is interesting to note the downfield shift of the trans[36] methylene group in both the α,β-unsaturated and α,β-epoxy carboxylic esters.

The methyl esters, 24 and 25, were likewise assigned from their spectra. The mass, i.r. and n.m.r. spectra were similar to the ethyl esters and few differences were discernible between 24 and 25. Their mass spectra, almost identical, showed the peaks from simple cleavages at m/e = 153, 123 and 109, and a base peak at m/e = 155, presumably arising from the loss of ·COCHO from the parent ion, which corresponded to $C_{12}H_{20}O_3$. Just as with the ethyl esters, a fragment at m/e = 83

was observed, but, again, accurate mass evaluation proved impossible. The infrared spectra showed the typical glycidate carbonyl stretch absorptions at ~1760 and ~1740 cm^{-1}. The ^{1}H n.m.r. spectra were more different than the ethyl esters. The unresolved region at 2.2-1.2δ was different, as were the chemical shifts of the epoxide proton and the axial methyl. The (2R*, 3R*, 7S*)-isomer had singlets at 3.20 and 1.07δ respectively, whereas the (2R*, 3S*, 7R*)-isomer showed the singlets at 3.22 and 1.03δ. The ^{13}C n.m.r. spectra showed deshielding of the C-4 and C-8 methylene carbon resonances comparable to the ethyl ester. Indeed, the ^{13}C n.m.r. spectra of 14 and 24 were nearly identical, as were those of 15 and 25, except for the ester alkyl peaks.

The novel glycidates 16 and 17, prepared as a mixture, were identified from their spectral characteristics. The mass spectrum was similar to those of 14 and 15 with fragments at m/e = 153, 123, 109 and 83 and a base ion at 169. The ion at m/e = 83 was not resolvable. The molecular ion at m/e = 226 corresponded to the formula $C_{13}H_{22}O_3$. The i.r. spectrum was also very similar to those of 14 and 15, with the glycidate carbonyl stretching bands at ~1760 and ~1730 cm^{-1}, but a new strong band was observed at 1155 cm^{-1}. The ^{1}H n.m.r. spectrum was very complex. The ester methylene was apparent as two quartets centred at 4.26 and 4.22δ, and the epoxide proton appeared as two singlets at 3.33 and 3.31δ. The region 1.1 - 0.7δ was very complex with seven discrete peaks, presumably reflecting the effect of a more proximal isomeric centre on the ring methyl chemical shifts. This proximity effect was reflected in the ^{13}C n.m.r. spectrum, where the axial methyl group was perturbed by the orientation of the ester function to the order of 0.3 p.p.m. The chemical shifts of the C-4 and C-8 resonances implied that the major isomer had the (2R*, 3S*, 7S*)-configuration, by correlation with the 14 and 15 spectra and by consideration of long range



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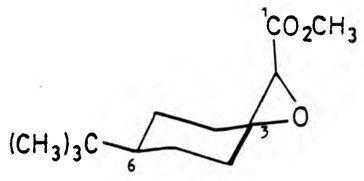


32

coupling information. The rest of the ^{13}C spectrum implied the preferred conformation to be as drawn. The only comparable work published is that of Bottin-Strzalko and Roux-Schmitt,[37] in which the carbon shifts of 31 and 32 were reported. The remarkable chemical shift change of C-3 from 64.3 p.p.m. in 31 to 65.6 p.p.m. in 32 is not paralielled by the 1 compounds. However, the ring carbon shift changes are comparable. It is clear that further work is required in this area to clarify the preferred conformations of these compounds.

The t-butyl esters 18 were reported by Maroni-Bernaud et al.[16] and Idriss et al.[38] with virtually no spectroscopic justification. These esters were identified from their spectra which were similar to the methyl and ethyl analogues. Although not separated, the ^{13}C n.m.r. assignments of the isomers were deduced.

The novel allylic alcohols 28 obtained impure were assigned from i.r. and ^{1}H n.m.r. spectral evidence. The broad i.r. absorption at 3600-3100 cm^{-1} and the band at ~1740 cm^{-1} support the hydroxyl ester assignment. The alkene protons resonate as a broad doublet at 5.53δ (J ~10Hz). Deuterium oxide exchange caused considerable sharpening of the signal at 4.47δ, which corresponds to the proton on C-2. The ring methyl signals were poorly resolved in the region 1.1-0.8δ. The methyl ester protons were observed at 3.77δ.

The novel ethyl allylic alcohols 19 gave a similar ^{1}H n.m.r. spectrum except for the ethyl protons which were observed as a quartet at 4.25δ and a triplet at 1.27δ (J ~7Hz). The infrared spectrum confirmed the assignment with a broad hydroxyl absorption at 3600-3200 cm^{-1} and a carbonyl band at ~1735 cm^{-1}.

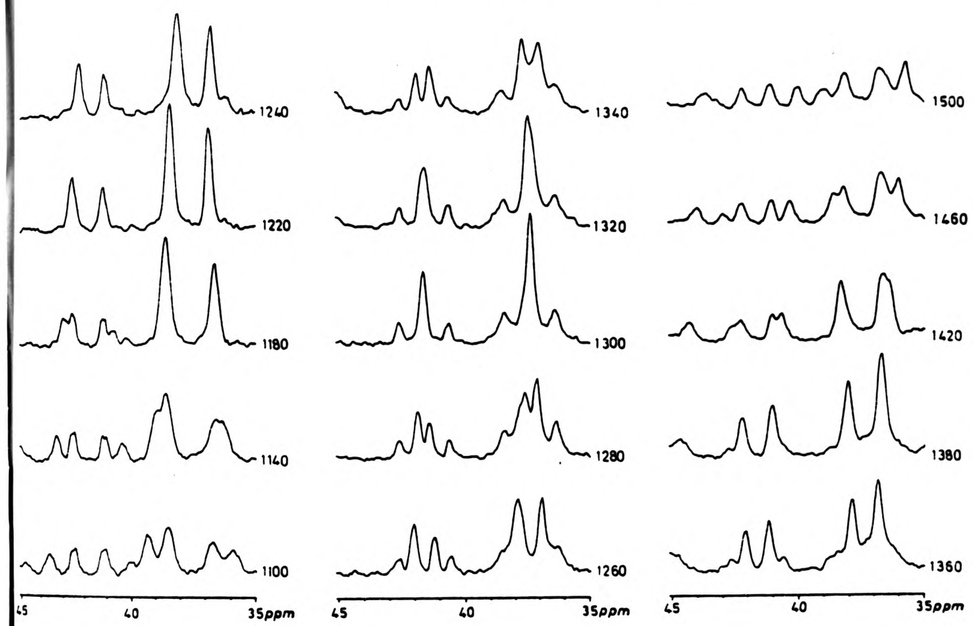
The precursor to 16 and 17, 29 has been described by Tullar and Lorenz.[20] Analysis of the spectroscopic properties of 29 implied the structure shown. The infrared spectrum of the mixture showed carbonyl

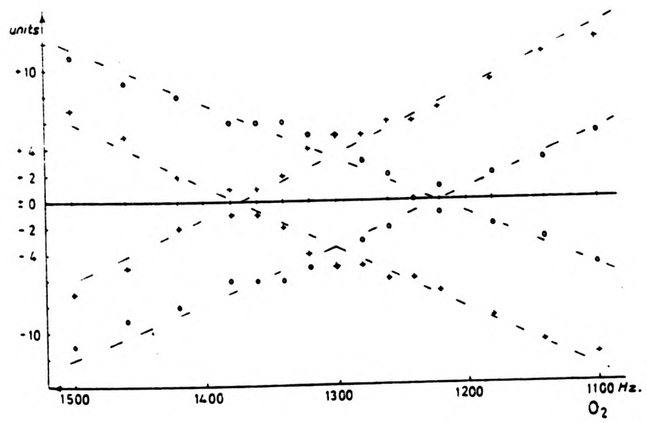
and alkene stretching bands at ~1720 and ~1650 cm^{-1} respectively. The ^{1}H n.m.r. spectrum showed two singlets for the isomeric alkene protons at 5.66δ for the (Z) isomer and 5.55δ for the (E) isomer. Signals between 1.1 and 0.7δ were attributed to the ring methyls. The ethyl ester methylene was seen as a quartet at 4.12δ (J-7Hz). A broad multiplet was also seen unresolved partially under this quartet, which corresponds to the equatorial proton on the allylic methylene cis to the ester moiety. The ^{13}C n.m.r. spectrum showed the major isomer to be (E), assigned by comparison with literature values,[27,39] and expected from literature precedent.[21] Notably, the C-4 methylene carbon resonance showed an off-resonance decoupled signal considerably simpler than the other methylenes, which was considered to be due to an absence of ^{2}J type long range coupling. Also, the cis allylic methylenes were not split into triplets in the off-resonance decoupled spectrum, but appeared as AB quartets, reflecting the non-equivalence of the attached protons.

The methyl esters 30 were virtually identical to 29 spectroscopically, except for the n.m.r. spectral differences intrinsic to the ester alkyl. The infrared spectrum showed bands at ~1725 and ~1650 cm^{-1}. The n.m.r. data were nearly identical to 29 except that the ester methyl signal was observed at 3.62δ as a sharp singlet. The absence of the ethyl ester quartet enabled the equatorial protons, on the allylic methylene cis to the ester functionality, to be observed as a multiplet at 4.0-3.6 . The ^{13}C n.m.r. spectrum displayed a quartet, for the methylene cis to the ester moiety, in the off-resonance decoupled mode. We examined this quartet using the single frequency off-resonance decoupled (S.F.O.R.D.) technique. Varying the decoupling frequency input at somewhat lower power, enabled the collapsing and re-emerging of coupling to be observed in the ^{13}C n.m.r. spectra of 30,

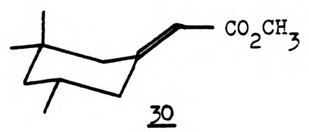
Diagram 11.

S.F.O.R.D. Analysis of the ^{13}C n.m.r. spectrum of 30 between 45 and 35 p.p.m.





(Proton decoupling frequency)



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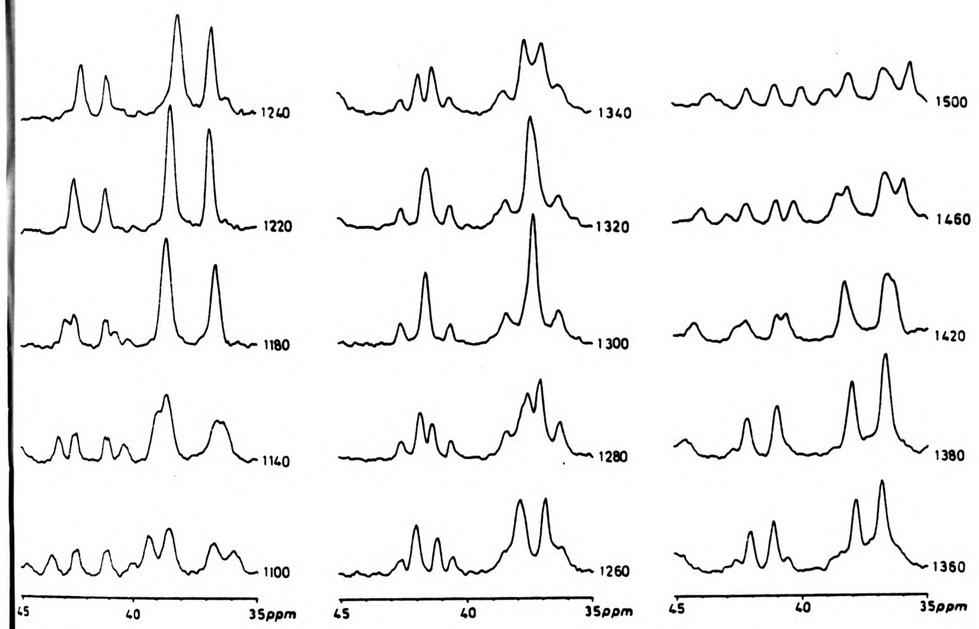
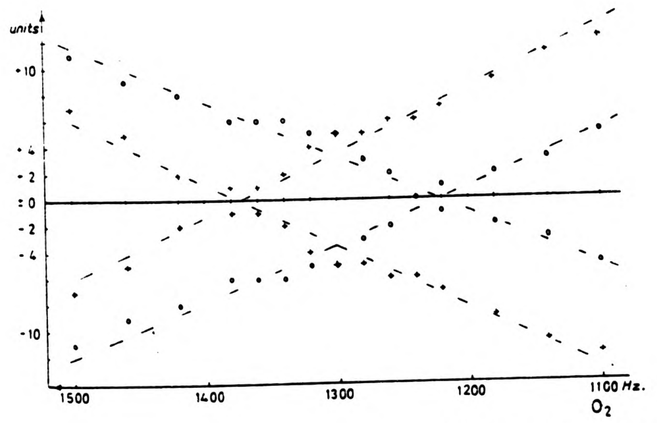
The assistance of T. Southon in running and interpreting these experiments is acknowledged.

Diagram 11.

S.F.O.R.D. Analysis of the ^{13}C n.m.r. spectrum of 30 between 45 and 35 p.p.m.





(Proton decoupling frequency)

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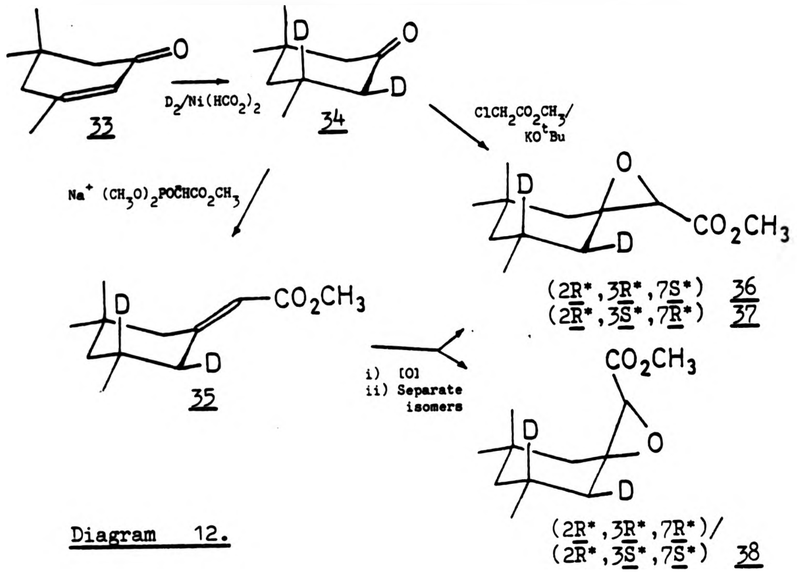
The assistance of T. Southon in running and interpreting these experiments is acknowledged.

as shown in diagram 11. Plotting the coupling constant against decoupler frequency gives the graph shown. From the baseline intersection values, the protons attached to the cis methylene carbon would resonate at ~1380 and ~1220 Hz. An ^{1}H n.m.r. spectrum of the same material on the same machine provided absolute frequency values for the chemical shift of the proton resonances. The strongly deshielded equatorial proton of this cis methylene was shown to resonate at ~1395 Hz. in absolute terms. This multiplet corresponds to the ~1380 Hz. resonance derived from the S.F.O.R.D. experiment. The corresponding axial proton, therefore, appears at ~1220 Hz, which translates as ~1.75δ. This resonance appears in the envelope 2.3 - 1.1δ and was not resolved in the ^{1}H n.m.r. spectrum.

The work on oxaspirane structures of this type proved disappointing with regard to the dried fruit-like aromas, but could be of use with regard to strawberry flavours. Our results with oxaspirane compounds, in conjunction with the findings regarding the strawberry aroma aspects of several phenyl glycidate compounds published by Mosandl,[15] provides a foundation for the structure-activity relationships to be deduced for these compounds. This study might be undertaken along the lines of the originally proposed modifications of the trimethyl substituted oxaspirane 1, as described at the beginning of this section, or perhaps involve the preparation of ring methyl substituted systems, akin to the sort of work reported herein (vide infra) for the cyclohexylacetic acid analogues. Epoxidation of the ring methyl substituted cyclohexylideneacetic acid esters prepared by us might provide interesting strawberry smelling compounds.

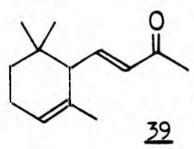
The oxaspirane to allylic alcohol rearrangement could be an area for valuable future research from a mechanistic standpoint. Preparation of specifically labelled compounds could establish whether such



33 34 35 36 37 38

(2R*,3R*,7S*) 36
(2R*,3S*,7R*) 37
(2R*,3R*,7R*)/
(2R*,3S*,7S*) 38

Diagram 12.



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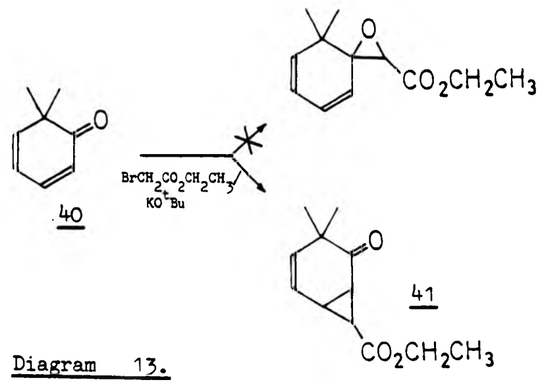


40 41

Diagram 13.

rearrangements indeed involve a cyclic transition state or whether an anti-coplanar elimination mechanism is important. The preparation of suitably labelled material might be obtained by reduction of isophorone 33, as shown in diagram 12. Catalytic hydrogenation of isophorone using a nickel catalyst has been described by Podlejski and Wilozynska.[33] Such hydrogenations occur by predominantly cis addition to the alkene and, thus, substituting deuterium for hydrogen should yield the dideuterated compound 34. The Wittig-Horner condensation of 34 with trimethyl sodiophosphonoacetate should provide (Z)- and (E)- 35. The ^{13}C n.m.r. spectra of the (E)-isomer would provide conformation of the equatorial orientation of the deuterium on C-8 by single frequency off-resonance decoupled spectral analysis, with respect to the C-4 resonance. The Darzens condensation with methyl chloroacetate would give 36, but the basic conditions of this reaction may give rise to the loss or scrambling of deuterium label from C-8. An alternative procedure would be the epoxidation of 35 using a peracid, followed by separation of the products 36, 37 and 38. Having obtained a suitably labelled substrate, the rearrangement of 37 into the corresponding allylic alcohol could be studied. Loss of the C-8 deuterium would imply syn-elimination, whereas retention of deuterium would imply anti-elimination.

The preparation of the α-ionone derived glycidates was predictably difficult. The functionality of the α-ionone 39 stating material provides the inherent potential for side reactions. For example, Spreitzer and Buchbauer[40] described the attempted Darzens condensation of the dienone 40 with ethyl bromoacetate. As diagram 13 shows, the product was the bicycloheptane 41 presumably via either a carbene addition or a conjugate addition and elimination reaction. A similar reaction was documented by Maroni-Bernaud et al.[16]

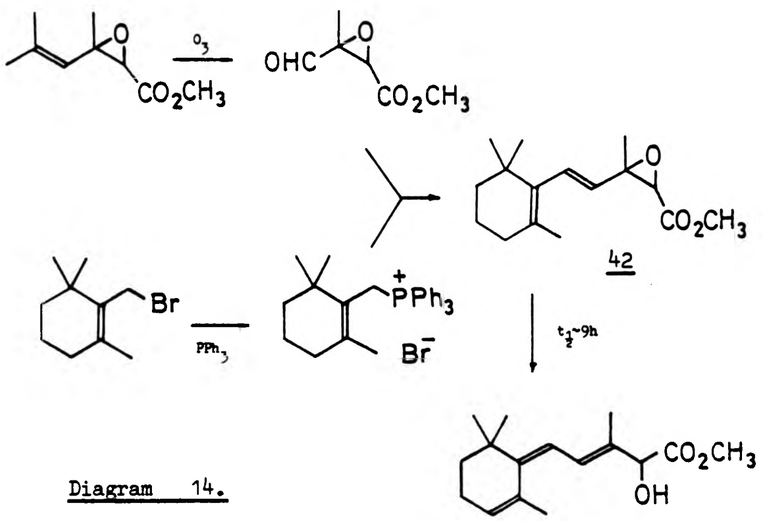
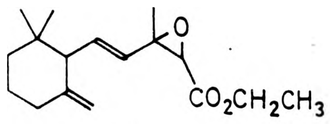


Diagram 14.



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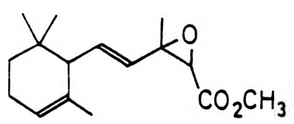

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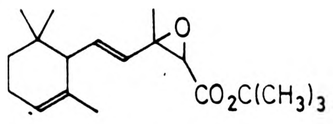

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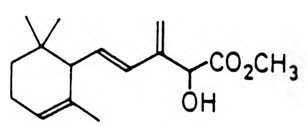
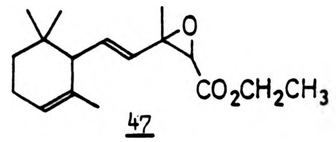


46

Despite the potential for side reactions, ionone glycidates have been reported. In 1942, Heilbron et al.[41] described the preparation of such materials and Milas et al.[42] reported the synthesis of 42,[1] using the Darzens condensation, in 1948. However, the purity and structural assignments of these early references must be treated with scepticism, in view of some of the work discussed below. More recently, a paper was presented by Schulte-Elte et al.[43] describing the preparation of 43 from γ-ionone using the Darzens conditions. Another quite recent paper by Oediger and Eiter[44] described 42 and highlighted some of the decomposition products. It was Davalian and Heathcock,[45] however, who pointed out the intrinsic instability of ionone derived glycidates to the Darzens reaction conditions. Indeed, they claimed that even at neutral pH in methanol, 42 has a half life of about nine hours at ambient temperature. These authors proposed an alternative method of preparation as shown in diagram 14. This ingenious synthesis enables isomers to be prepared, in principle, by using the appropriate starting material.

In our laboratory, it was found that the Darzens condensation of α-ionone, 39, with either methyl or ethyl chloroacetate was successful, using the conditions previously employed for the oxaspirane syntheses. Alternative procedures proved disappointing.[46] The work-up presented problems of decomposition and separation of the products. These problems were overcome by using Girards T reagent[24] to remove any 39 and flash chromatography[47] followed by high vacuum distillation yielded the products as a mixture of the four diastereoisomers 44. These were found to decompose rapidly even when stored at below 0°C.

Also isolated were other unstable compounds, tentatively assigned as 45, and the major decomposition product 46. The former is expected by analogy to the previous oxaspirane preparations, but the latter is



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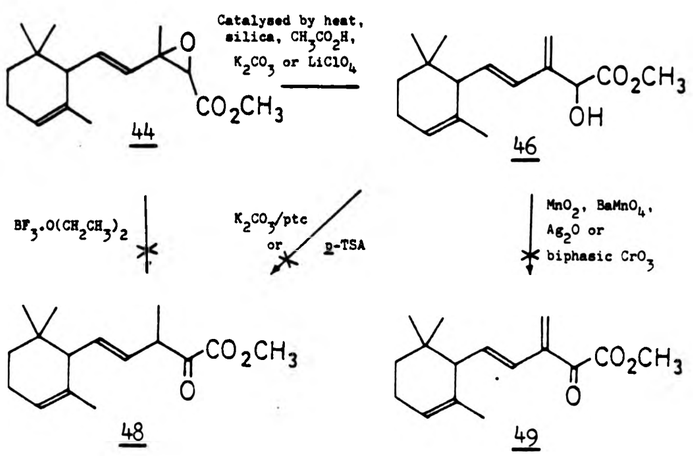


44 46 48 49

Diagram 15.

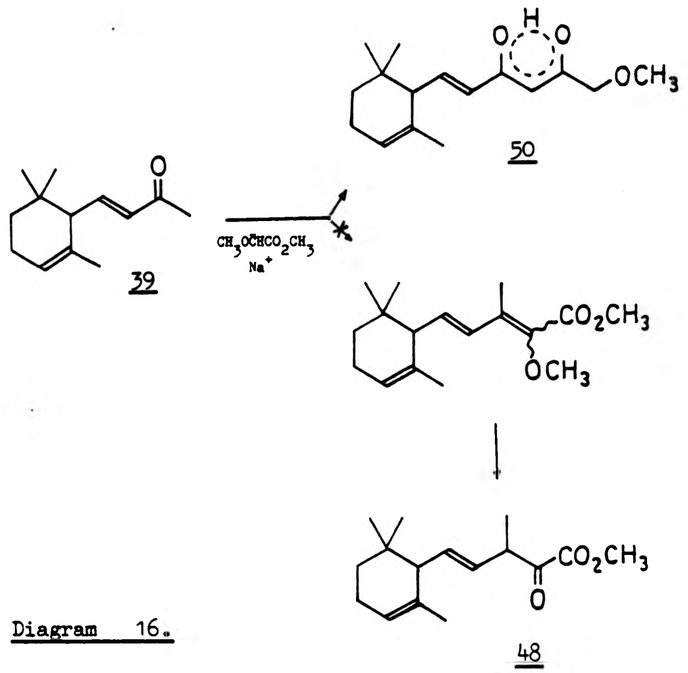
perhaps somewhat surprising in view of the previously described work of Davalian and Heathcock, shown in diagram 14. It would appear that the diallylic hydrogen of 44 is not as reactive as the allylic methyl hydrogens to the rearrangement process. Clearly, the higher acidity of the diallylic proton is not sufficient to overcome the steric restraints, which disfavour the abstraction of this proton.

In a similar fashion, the ethyl glycidates 47 were prepared. The major product isomers were thought to have trans stereochemistry[36] at the epoxide junction, but all four diastereoisomers appeared to be present from spectral and chromatographic data.

It has been demonstrated, then, that the Darzens condensation, under rigorously controlled conditions, will provide the glycidates 44 and 47.

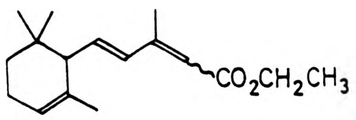
The aromas of 44 and 47 were indistinguishable and weak, slightly sweet and with mentholic and camphoraceous undertones in character, and were not detected at 1000 p.p.m. on a cigarette. The aroma quality reported here is in direct conflict with the odour of 47 reported by Arctander[6] as the "strikingly reminiscent ..... odour of dried figs, dates or dried bananas" and, of 44, as having a "distinct resemblance to the odour of dried figs, dates, prunes or similar fruit preserve". It was noted, however, that in the distillation residues of one reaction, a faint and vague dried fruit aspect could be perceived. Separation of the plethora of compounds, observed by thin layer chromatography, using silica chromatography provided no individual fraction with this aspect. Recombination also failed to reproduce the aroma, so it was surmised that the dried fruit smelling component was either decomposed or irreversibly bound during the separation. Attempts were made to prepare possible candidates for this compound.

The procedures shown in diagram 15 were used in an attempt to prepare the probably unstable 48 and 49, but were unsuccessful. Of



39 50 48

Diagram 16.



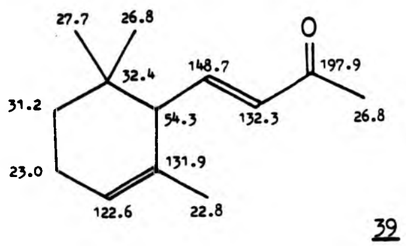
51

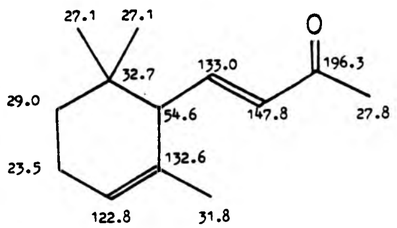
interest, the potassium carbonate phase transfer catalysed rearrangement of the allylic alcohol 46 gave, as the only detected volatile product, 39.[48] This rearrangement would explain the perseverence of α-ionone in the reaction mixture even when a large excess of the other reagents was employed. In essence, the starting material is also a product presumably due to 46 degradation in work-up.

In an alternative approach, the preparation of 48 was tried *via* the methyl enol ether, as shown in diagram 16, and precedented by Horner and Renth.[49] The product 50 is presumably formed by attack of the α-ionone enolate anion on the ester carbonyl of the methyl methoxyacetate followed by elimination of methoxide. The novel structure of 50 was determined by spectroscopy and is consistent with the positive result of an iron (3) chloride/pyridine ß-diketone spot test.[50] The diketone 50 was sweet, fruity and mentholic in odour, and was somewhat unstable.

The deoxygenated analogues of 47, 51[51,52] were prepared by a Wittig-Horner reaction with α-ionone. The products were isolated, slightly impure, and were sweet, fruity and estery in aroma.

The failure of this work to duplicate the claimed aroma characteristics of the above glycidates may be due to the subjective nature of aroma assessment. It seems more likely, however, that the compounds made in earlier work were not of sufficient purity to enable accurate assessment of their odour properties. Indeed, the structural assignments of some of the claimed compounds must be questioned in view of the instability demonstrated by these glycidates. It should also be stressed that the purity of the starting materials was rigorously checked. The α-ionone commercially available nowadays is very pure compared with the material available in the 1940s. Furthermore, it was distilled using spinning band apparatus and purified further by radial chromatography and stored at <4°C under nitrogen before use.





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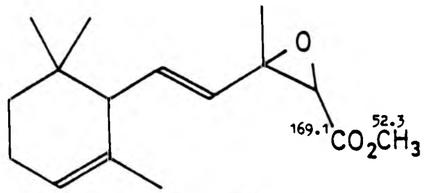

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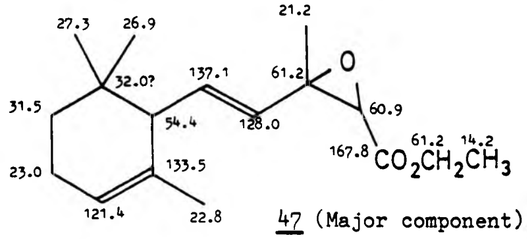


47 (Major component).

Table 1. ^{13}C n.m.r. data. For experimental details, see p. 89.

In view of the correlations of the spectra reported here and other literature data,[53] the ^{13}C n.m.r. spectrum of α-ionone 39 has been tentatively reassigned from the values quoted by Stothers,[39] as shown in table 1. Empirical parameter calculations[54] also support the proposed exchange of the C-3 and C-4 assignments given by Stothers.

With regard to ionone derived glycidates, spectral data in the literature are sparse for these or related compounds. As such, some n.m.r. assignments have to be made tentatively, especially in view of the inherent instability of these compounds. The glycidate 44 decomposed too rapidly for sensible assignment of the ^{13}C n.m.r. spectrum to be made, despite being run at low temperature. The ethyl esters 47, although showing considerable decomposition, did give a discernable ^{13}C n.m.r. spectrum, and most of the resonances were assigned as shown in table 1. The quarternary carbon signals were not easily distinguished from the signals of the decomposition products. The proton n.m.r. spectra of 44 and 47 were very similar and it was possible, in both, to see splitting of the olefinic signals presumably due to isomers. Reinforcement for this observation was shown for 47, because careful chromatography facilitated some isomeric separation between product fractions, as observed by n.m.r. spectroscopy. The infrared spectra of 44 and 47 were almost identical.

With specific regard to 47, the i.r. spectrum had glycidate carbonyl bands at ~1755 and ~1730 cm^{-1}. In the ^{1}H n.m.r. spectrum, the epoxide protons on C-2 were observed at 3.50δ, signals between 5.9-5.2δ corresponded to the alkene protons and the ethyl ester methylenes were seen as quartets at 4.2δ (J~7 Hz), for the major product isomers. The minor isomer components showed, as the most marked difference, the epoxide proton as a singlet at 3.38δ. The ^{13}C n.m.r. spectrum had a peak at ~60p.p.m. from carbon 2 and the other assignments were made by

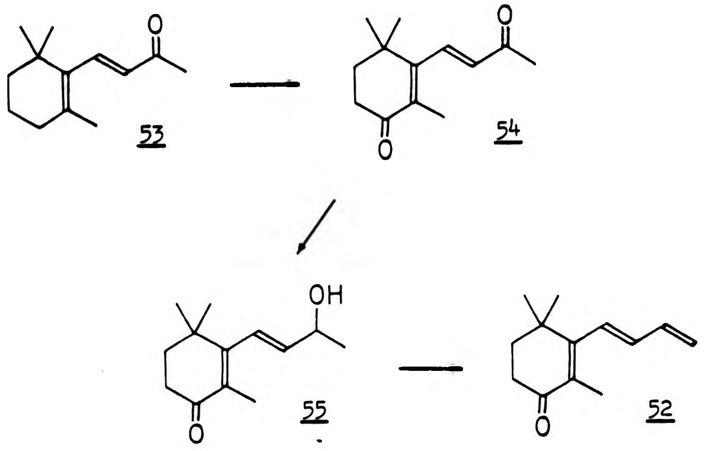
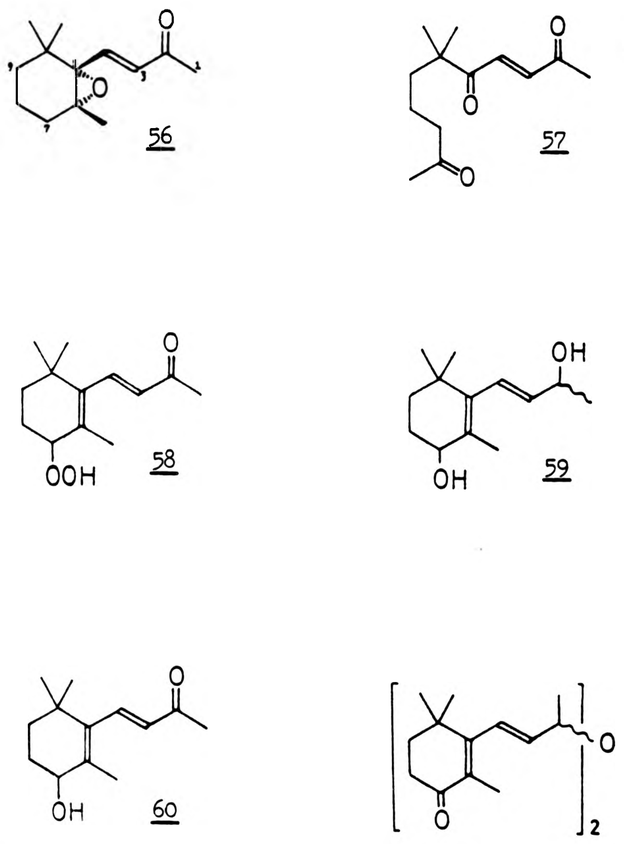


Diagram 17.



comparison with α-ionone, 39, and other compounds. Decomposition made assignment of the quaternary carbons provisional. The major isomers were believed to have a trans[36] configuration about the epoxide because of the comparative chemical shifts of the protons on carbons 4 and 5,[51] and the carbon resonance at 22.8 p.p.m. corresponding to the methyl carbon on carbon 3.[27,45] This assignment also has some tenuous literature precedent. Davalian and Heathcock[45] described a closely analogous compound giving full spectral data.

Compound 44 was assigned from i.r. and ^{1}H n.m.r. spectral evidence. Carbonyl stretching bands were seen at ~1760 and ~1735 cm^{-1} from the glycidate and the ^{1}H n.m.r. spectrum was almost identical to that of 47 except that the ester alkyl was seen as a set of singlets between 3.8 and 3.7δ.

The t-butyl ester 45 was tentatively assigned from its i.r. and n.m.r. spectra. The i.r. spectrum showed typical glycidate splitting of the carbonyl band at ~1750 and ~1725 cm^{-1}. The ^{1}H n.m.r. spectrum was very similar to that of 44 except that the methyl ester resonance was absent and a large singlet had appeared at 1.50δ, from the t-butyl group.

The hydroxyester 46 was identified from its spectral data. The i.r. spectrum showed an ester band at ~1745 cm^{-1} and a broad O-H absorption at 3600-3100 cm^{-1}. The ^{1}H n.m.r. spectrum showed the alkene region almost completely resolved, with the resonances at 6.01, 5.71 and 4.85δ corresponding to the protons attached to C-4, C-5 and C-2 respectively. The methyl ester protons resonated at 3.78δ and the geminal alkene protons were observed at 5.21δ.

The preparation of the megastigmatrienone 52,[1] a compound isolated from Osmanthus-absolute and synthesised by Kaiser and Lamparsky,[55] was repeated in order to verify the claimed dried fruit aspect in its aroma profile. The published synthesis of 52 is shown in diagram 17.

ß-Ionone, 53, was oxidised using tertiary butyl chromate, as described by Ina and Etoh.[56] The resulting dione 54 is a constituent of black tea, tobacco, Osmanthus-absolute,[57] and the tail scent gland of the red fox.[58] Selective reduction with sodium borohydride gave 55, again detected in Osmanthus-absolute, and treatment with p-toluene sulphonic acid gave the dehydration product 52.

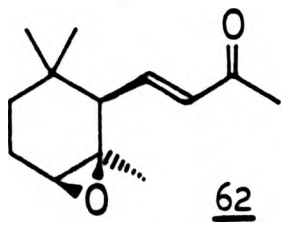
On repeating this work, tertiary butyl chromate was prepared using the method of Leo and Westheimer.[59] The oxidation of ß-ionone with this reagent gave the desired product 54 along with the byproducts 56 and 57. The well-known 56 has been identified as a component of various natural products.[57,60] The triketone 57 isolated impure, has been reported by Skorianetz and Ohloff.[61]

An alternative preparation of 54 has been patented by Jaedicke and Paust,[62] using sodium chlorate, sodium iodide and sulphuric acid. Repetition of this work did indeed give 54, but the major product was 56. Also isolated were the novel but unstable hydroperoxide 58, an unstable halogenated compound and an aldehyde of unknown structure. The hydroperoxide 58 was tentatively assigned from spectroscopic data and may be an intermediate in the oxidation process to 52.

Borohydride reduction of 54 provided the alcohol 55 as well as some of the diols 59, isolated slightly impure, which have been previously reported by Ide and Toki as ß-ionone metabolites extracted from rabbit urine.[63] Compound 55 slowly rearranged to 60,[64] unless stored at below $0^{o}C$. The (E) stereochemistry of the C-3 - C-4 double bond precludes an intramolecular rearrangement and so we presume this transition proceeds via an enol or intermolecular mechanism.

The acid-catalysed dehydration of 55 gave the products 52 and the novel dimers 61.

The aroma of 52 was assessed to be sweet, fruity and spicy, but did



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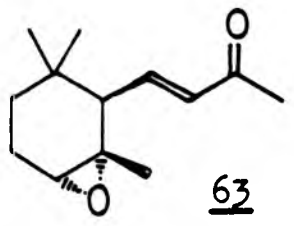

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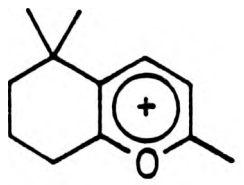

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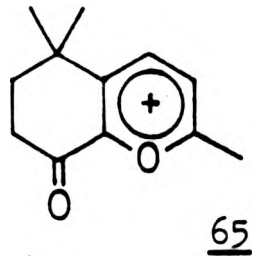

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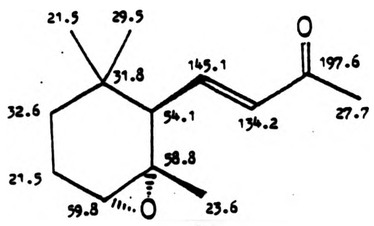

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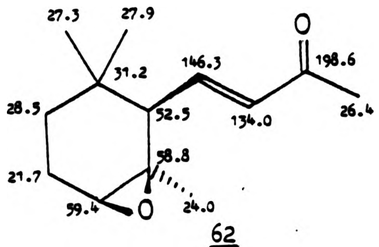

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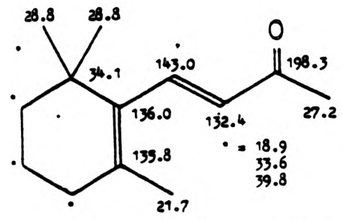

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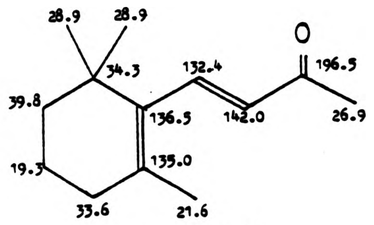

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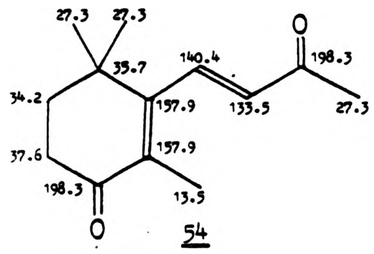

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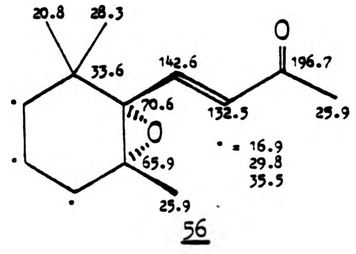

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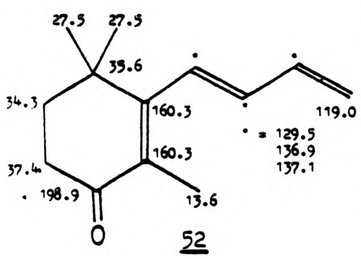

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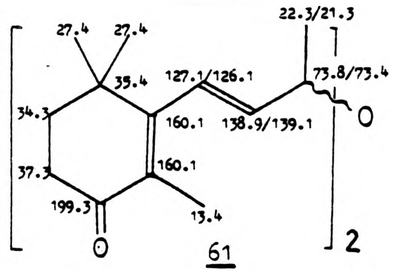


61

^{13}C n.m.r. data. For experimental details, see p. 89.

not have the dried fruit aspect that was desired. Its threshold on a cigarette was adjudged to be greater than 1000 p.p.m.

Also prepared were the epoxides 62 and 63, from the epoxidation of α-ionone 39 by m-chloroperoxybenzoic acid.[65,66] The epoxide 62 was separated from a mixture of 62 and 63 by radial chromatography. These compounds were made in order to assist the assignments of spectral and chromatographic data and were identified as impurities in commercial 39.

The ^{13}C n.m.r. spectrum of ß-ionone 53 has been tentatively reassigned from that given by Stothers.[39] The alkene signals have been reversed for the same reasons that α-ionone 39 was reassigned. Support for the revision came from empirical parameter calculations[54] and observed values for analogous compounds published in the literature.[65,67]

The dione 54, prepared by either route detailed above, was spectroscopically identical to literature data.[56] The m.s. base peak at m/e = 43 presumably arises from the cleavage of the acetyl group, which also accounts for the peak at m/e = 163. This is in accord with mass spectral work reported for ß-ionone.[68] Interestingly, the peak at m/e = 191 is of low intensity. The bicyclic cation 64 has been shown to be the fragment responsible for the ß-ionone 53 base ion, obtained by loss of $^{\bullet}CH_3$.[69] It is evident, from the m.s. of 54, that the analogous ion 65 is not a favoured fragmentation. The ^{13}C n.m.r. spectrum has not been previously reported.

The trione 57 was assigned by comparison of spectral data reported by Skorianetz and Ohloff.[61] Most of the mass spectral fragments, not previously published, can be deduced from simple cleavages.

The well known epoxide 56 was assigned by comparison with the mass spectrum published by Winter and Enggist,[70] the melting point reported by Karrer and Stürzinger,[71] and the data provided by Acemogly et al.

for the (5R,6S)-enantiomer.[67] The loss of the methyl on carbon 6 may lead to the mass spectral fragment at m/e = 193, which corresponded to $C_{12}H_{17}O_2^+$. The base peak at m/e = 123, measured accurately as $C_8H_{11}O^+$, may be due to the ion 66. The ^{13}C n.m.r. spectrum, published, but not assigned, by Frei et al.,[72] was assigned by comparison with β-ionone 53.

The hydroperoxide 58 was somewhat unstable. The ^1H n.m.r. spectrum was extremely similar to that of 60. The mass spectrum indicated a molecular formula of $C_{13}H_{20}O_3$ and had a base peak at m/e = 43 corresponding to the acetyl cation. The compound decomposed during the ^{13}C n.m.r. experiment. On this evidence, the compound was tentatively assigned the structure given.

The alcohol 55 was identified from its ^1H n.m.r. spectrum by comparison with that obtained by Kaiser and Lamparsky.[55] Further spectral analysis was not achieved before partial decomposition to the alcohol 60 had occurred, as identified by comparison with data given by Rosenberger et al.[64]

The diols 59 gave spectral values comparable to those of Ide and Toki,[63] whom also describe 54 and 55.

The products of dehydration were 52 and 61. Compound 52 was spectroscopically identical to the product described by Kaiser and Lamparsky.[55] Its ^{13}C n.m.r. spectrum, not previously reported, was assigned by comparison with other compounds described herein.

The other product 61 was isolated as a mixture of diastereoisomers, as reflected by the ^{13}C n.m.r. spectral assignments, made by comparison with similar compounds. The ^1H n.m.r. spectrum similarly showed doubling of peaks, and resembled that of 52. The doublet at 1.33δ (3H, ~6 Hz) coupled to the quintet at 4.10δ (1H, ~6 Hz), was consistent with a $R{=}CHCH(CH_3)OR'$ type structure, and the simplification of the alkene

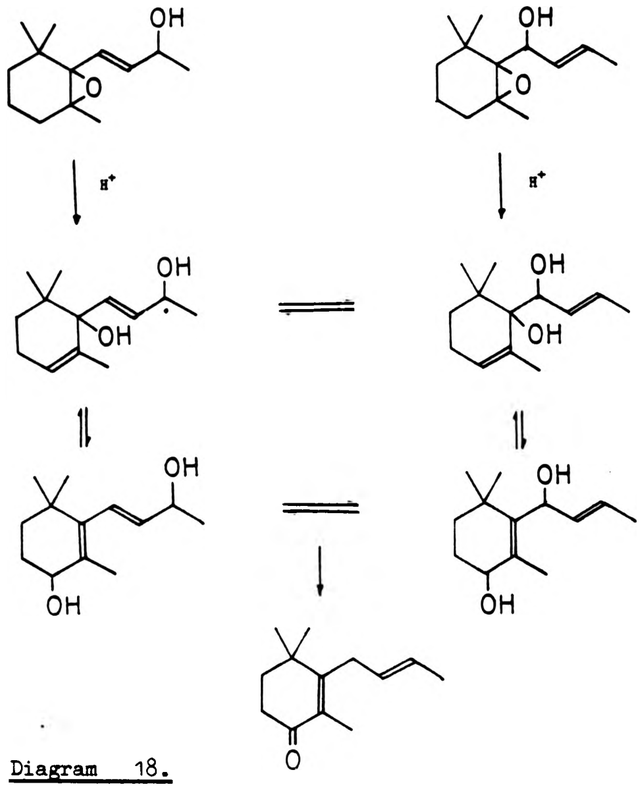


Diagram 18.

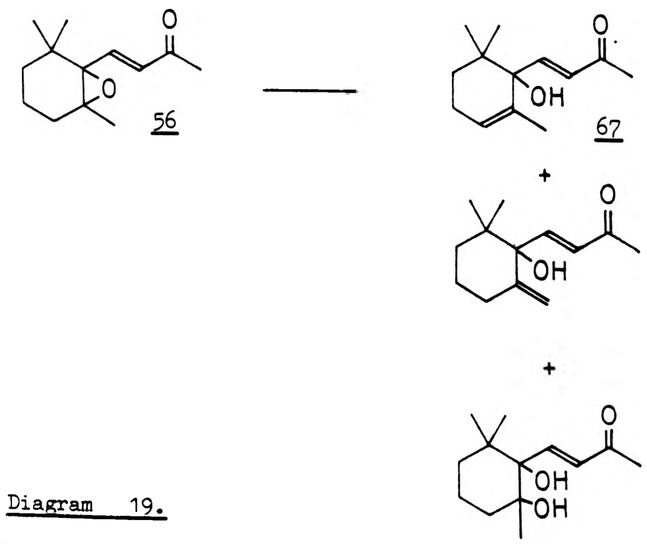
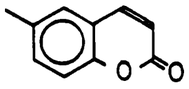


Diagram 19.

proton resonances were instrumental in assigning the structure. Likewise the i.r. and m.s. data were consistent with the structure given. The m.s. showed peaks generally explicable by simple fragmentations of the parent ion, which corresponded to $C_{26}H_{38}O_3$. Although not the most abundant, the sizeable fragments at m/e = 191 and 163 can be derived from cleavage of C-2 - O and C-2 - C-3 bonds respectively.

This work on megastigmanes was a more rigorous analysis of the literature work. Detection of compounds other than those reported by Kaiser and Lamparsky[55] provided a more complete picture of the reactions involved, but left several unanswered questions about the mechanism of the allylic oxidations. Interestingly, Ohloff[73] described the transformations shown in diagram 18. The analogous reaction with 56 gave as one product 67, as shown in diagram 19, according to Skorianetz and Ohloff,[61] but the rearranged product 60 was not reported. It would be interesting to react 67 with t-butyl chromate in the conditions used by us with ß-ionone 53 to discover if 54 is prepared. In the acidic sodium chlorate oxidation of ß-ionone 53, the hydroperoxide 58 was believed to have been isolated, which may lend support to the direct oxidation of the ring carbon, elimination of water from which, would provide 54. Evidently, further mechanistic work on these reactions could prove rewarding.

The aroma assessments of the megastigmane compounds, prepared by us, were uninteresting as dried fruit-like odorants. It should be noted that ß-ionone 53 has a threshold detection value reported by Ohloff[73] as 7×10^{-6} p.p.m. when assessed as an aqueous solution. Clearly a small amount of ß-ionone as an impurity in any of the megastigmane compounds prepared by us could contribute to the aroma characteristics of the test compound. In the event no violet or ionone-like notes were



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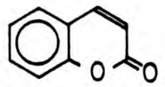

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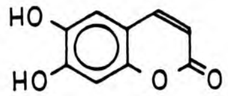

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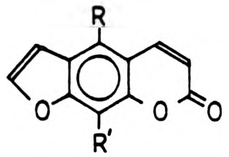
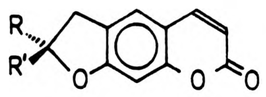
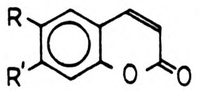
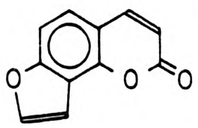
Table 2. Coumarin constituents of fig plants.









	R =	R' =
Bergapten	OCH_3	H
Imperatonin	H	$OCH_2CH_2\overset{CH_3}{C}CH_2$
Isoimperatonin	$OCH_2CH_2\overset{CH_3}{C}CH_2$	H
Oxypeucedanin hydrate	$OCH_2\overset{OH}{C}HC(CH_3)_2$	H
Psoralen	H	H
4',5'-Dihydropsoralen	H	H
Marmesin	$\overset{OH}{C}(CH_3)_2$	H
Nodakenetin	H	$\overset{OH}{C}(CH_3)_2$
Scopoletin 71	OCH_3	OH
Suberosin	$CH_2CHC(CH_3)_2$	OCH_3
Umbelliferone	H	OH
Angelicin	-	-

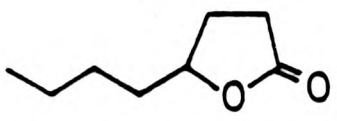
observed in the neat compounds, although they were reported in the taste of a laced cigarette when smoked, for those compounds where thresholds were quantified.

The lack of success with the glycidate and megastigmane derivatives detailed above led to the consideration of coumarins[74] as potential dried fruit aroma compounds. 6-Methyl coumarin 68 has been described as fig- or date-like in aroma.[6] The commercial material was assessed by us to have a coconut primary impact with a coumarinic warmth aspect. It seemed possible that the aroma of dried fruits themselves could be combinatory, in that the dried fruit aroma aspect could be a composite of a fruity aspect and a warmth aspect.

The fruity aspect could be due to the many acids, aldehydes, ketones, etc. known to be in dried fruit volatiles,[75,76] the relative quantities varying with the particular fruit and processing procedure.

The warmth aspect, which is intrinsic to dried and not fresh fruit, is somewhat more difficult to define, but is not unlike coumarin 69, itself a compound well known as a tobacco additive.[6] It is the warmth aspect that rolls around the back of the mouth and seems to stimulate the saliva glands. It seemed plausible that coumarins may be responsible for this warmth aspect for the reasons following.

The occurrence of at least twelve coumarins has been reported in fig plants,[77,78,79] as shown in table 2. Although coumarins have been observed in fig root volatiles,[80] they do not appear to have been looked for in dried fruit volatiles. The literature also documents that coumarins are able to survive the drying processes and that dried fruits are considered a good natural source of coumarinic materials.[81] In fresh fruit, it has been suggested, and seems likely, that coumarinic compounds are present as relatively involatile glycosides,[79] which might decompose, during the drying processes, to give volatile coumarinic



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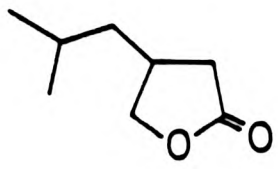
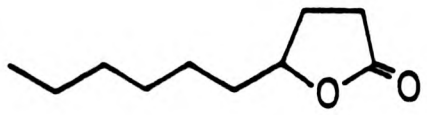
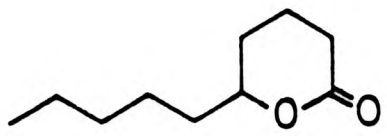


73



74



75

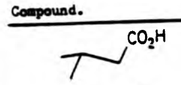
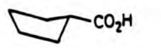
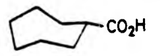
Compound.	Purity.	Odour Quality; Neat (on Smoking in a Cigarette).	Threshold.
 80	99%	Characteristic sweaty feet, cheesy, strong. (Weak, sweet).	20000 p.p.m.
	98.5%	(Costus, richer, sweeter taste).	1000 p.p.m.
	97%	Pungent, butyric acid, cheesy. (Cheesy, turkish tobacco flavour).	50 p.p.m.
 77	97%	Sweet, ester-like.	30 p.p.m.
	98%	Sweet, cheesy, turkish tobacco, burnt. (Slightly cheesy, turkish tobacco).	800 p.p.m.
 78	97%	Overipe fruit, sweet, heavy, chocky. (Pruny, figgy, dried fruity).	200 p.p.m.
 76	99%	Unlit pipe tobacco, honey, fruity, chocking, dried fruit. (Cheesy, "Friars Balsam" note).	800 p.p.m.
 79	99%	Fruity, sweet, dried fruit, cinnamon, Danish pastry aroma.	3000 p.p.m.
	99%	Buttery, butyric acid. (Cheesy, diluting to sour).	40 p.p.m.

Table 3.

substances.

Another interesting observation is that coumarins, such as esculetin 70 and scopoletin 71 have been identified in tobacco, both free and as glycosides.[82]

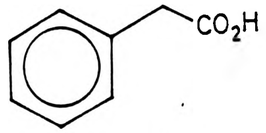
It was also noted that lactones[83] such as 72 and 73 have been described as coconut-like and 73 is alleged to be detectable at less than 1 p.p.m. Changing these structures slightly can cause the coconut aspect to be replaced by a fruity aspect. For example, 74 has been described as "peach, creamy and fruity" and 75 has a "heavy, fruity aspect" and a very low threshold at less than 0.1 p.p.m. All of these compounds have been reported by Arctander[6] and, as has been highlighted before, may not be accurately described. However, if this trend was demonstrable, a coumarin of suitable substitution might retain the warmth aspect of 68, replace the coconut note by a fruit note and hence give an overall dried fruit aroma.

This area of research was not investigated further due to the serendipitous discovery of the dried fruit smelling compound cyclohexylacetic acid.

During some assessment work by the Gallaher research group at Stirling, the serendipitous discovery of cyclohexylacetic acid 76 as an odorant compound with dried fruit aspects, provided the reference point necessary for structure-activity relationships to be derived.

The Gallaher workers had assessed the effect of ring size and ring to acid chain length changes[84] and the required odour quality proved to be best represented by cyclohexyl acetic acid 76. Reduction of the ring size to cyclopentyl acetic acid 77 gives an odorant of a "sweet, ester-like" nature with a threshold of ~30 p.p.m. Shortening the ring to acid chain gives cyclohexyl carboxylic acid 78, which was assessed as "overripe fruit, sweet, heavy, choky" in odour. When smoked on a

81



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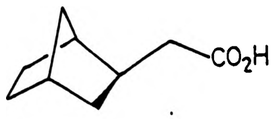


83

cigarette, 78 was somewhat dried fruit-like in flavour and of threshold ~200 p.p.m. A dried fruit note was also observed with 3-cyclohexyl propionic acid 79, but the most dried fruit reminiscent was cyclohexylacetic acid 76. More complete data are shown in Table 3, in which isovaleric acid 80 is included as one acyclic analogue of remarkably high threshold at 20,000 p.p.m. on a cigarette.

In order to extend the data base provided by the Gallaher work, several commercial items were tested by us.[85] 1-Adamantyl acetic acid 81 was weak, fruity and animal in odour and of high threshold. Phenylacetic acid 82 is well documented and was confirmed as having a sweet, heavy and honey-like aroma and a somewhat lower threshold of ~100 p.p.m. Of greatest interest was exo-2-norbornyl acetic acid 83, which was a low threshold odorant with a sweet, animal and cheesy aroma, but a strong dried fruit-note when applied to a cigarette. This later quality was also apparent when cyclohexylacetic acid, 76, was laced on a cigarette.

Other work by the Gallaher group[86] has shown that, in a series of n-alkyl carboxylic acids, the threshold detection concentrations varied with alkyl chain length. Increasing the alkyl chain length from hexanoic acid decreased the threshold to a minimum value corresponding to n-decanoic acid, before increasing again for longer chained acids. Furthermore, branching of the chain could cause a dramatic decrease in threshold value, as demonstrated for 4-isopropyloctanoic acid, which was of threshold value ~0.06p.p.m. when loaded on a cigarette.

The above results seemed to indicate that a suitably substituted cyclohexane acetic acid might retain and improve the dried fruit aspect, shown by 76, and lower the threshold detection concentration.

The initial target compounds required to extend the data base would be ring substituted and so it was proposed that the methyl substituted

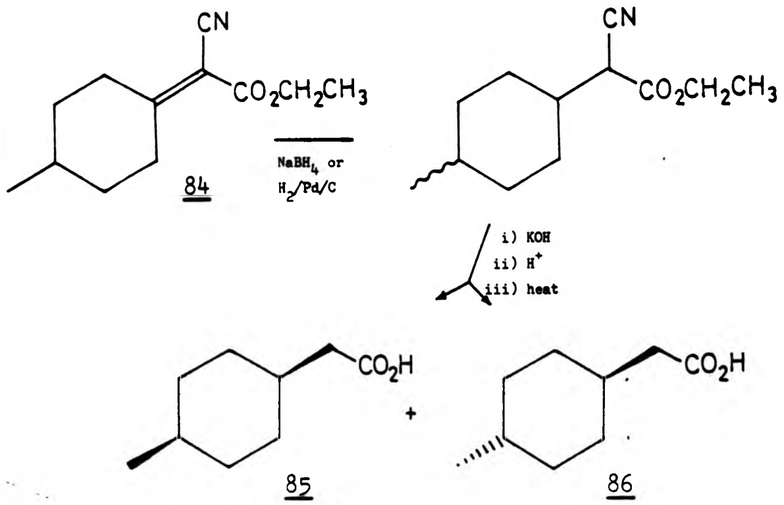


Diagram 20.

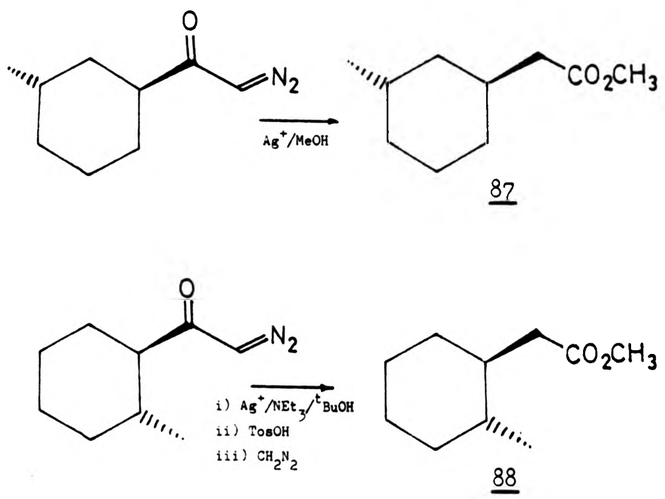


Diagram 21.

cyclohexane acetic acids should be prepared. The rationale was to investigate the steric requirements of the presumed hydrophobic binding site by specific steric alteration of 76, although it was realised that the change in hydrophobicity and other physical properties might also facilitate a change in threshold. It was assumed that, for threshold determination, the changes in the transfer coefficient, from the loaded cigarette to the smoke, and the aqueous/lipid partition coefficient could, at least in the initial analysis, be neglected. It was also realised that the chiral nature of most of the substituted acids would make resolution desirable, but only the racemic materials are considered here.

A search of the literature[87] revealed that the ring methyl substituted cyclohexylacetic acids had been prepared previously by a variety of routes, some of which will be summarised here.

The approach of Amsterdamsky et al.,[88] and later Nasipuri et al.,[89] shown in diagram 20, involved the initial preparation of 84. Reduction of the electron poor double bond, followed by base hydrolysis and decarboxylation gave the products as a mixture of isomers 85 and 86. When borohydride reduction was employed the proportion of the product which was cis, 85, was 60% and with hydrogenation it was 58%, presumed to be due to equatorial attack on the substrate 84. The borohydride reduction, followed by hydrolysis and esterification, was used by Iida and Sugawara[90] in their preparation of all of the regioisomeric n-propyl esters. These authors separated the diastereoisomers by repeated automated preparative g.l.c.

The trans-3-methyl and trans-2-methyl compounds have been reported as their methyl esters 87 and 88 by Agosta and Wolff[91] and House and Richey[92] respectively. These groups utilised Arndt-Eistert syntheses as shown in diagram 21. A diazoketone precursor was decomposed by a

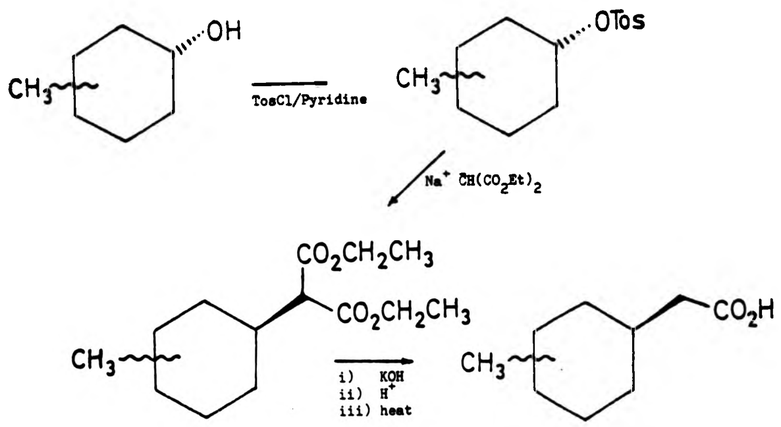
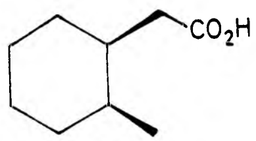


Diagram 22.



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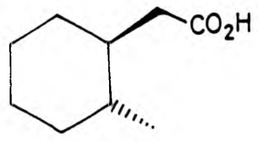

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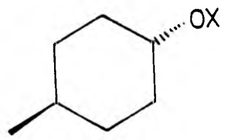
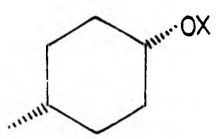
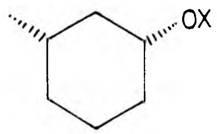


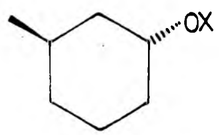
X=H 91
X=Tos 92



X=H 93
X=Tos 94



X=H 95
X=Tos 97



X=H 96
X=Tos 98

silver (1) salt to a carbene intermediate which rearranged to the ketene, and then reacted with solvent to give the ester. This approach requires the preparation of the diastereoisomerically pure methyl substituted cyclohexylcarboxylic acid chlorides, in order to prepare the corresponding diazoketones.

The most promising approach seemed to be that of Stork and White.[93] Their method is shown in general form in diagram 22 and involved the nucleophilic displacement of a tosylate group by malonate anion as the key step. In view of the commercial availability of most of the alcohol precursors as diastereoisomerically pure compounds, this synthetic pathway should enable pure isomers to be obtained. Stork and White described the synthesis of 85, 86, 89 and 90 by this route. Consideration of molecular models implied that the tosylate of trans-3-methylcyclohexanol might not react similarly due to the potential steric interaction of the attacking nucleophile with the methyl substituent.

We investigated the conditions reported by Stork and White,[93] but on a smaller scale. The reaction of the isomeric alcohols with p-toluenesulphonyl chloride in pyridine, proceeded smoothly and in good yield. The trans-4-methyl alcohol 91 gave 92 as a crystalline solid. Likewise, purified cis-4-methylcyclohexanol 93 gave 94. No isomerisation was evident from the products. A mixture of cis- and trans-3-methylcyclohexanol 95 and 96 gave the corresponding p-toluenesulphonates 97 and 98, but the products were not separated by conventional methods.

The 3-methylcyclohexanols, obtained commercially as a ~70:30 cis:trans mixture, could not be separated by column chromatography. Repeated spinning band distillation did separate a small amount of the pure cis alcohol 95. Separation was equally difficult by distillation

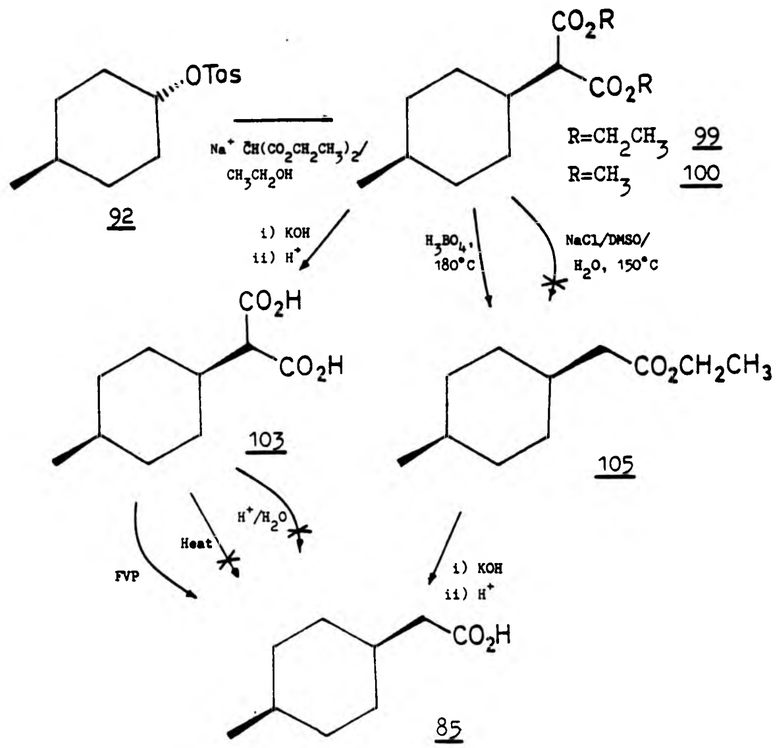
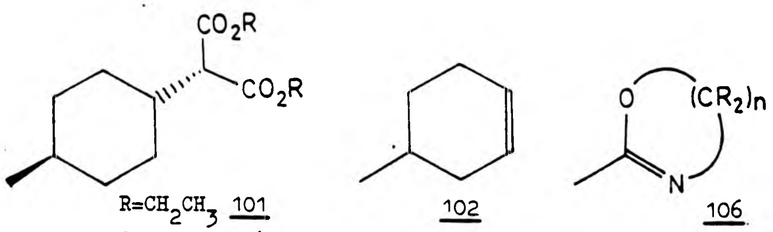


Diagram 23.



of the trimethylsilyl ethers prepared using trimethylsilyl chloride and triethyl amine.[94] The trans isomer 96 was not obtained pure.

Reaction of the trans-4-methyl tosylate 92, so prepared, with diethyl malonate and sodium ethoxide gave a 42% yield of the adduct 99, as shown in diagram 23. Using the lithium salt of diethyl or dimethyl malonate, with or without a complexing agent, failed to give the products 99 or 100. Likewise, the sodium salt of dimethyl malonate prepared by reaction with sodium hydride in tetrahydrofuran did not produce 100. The product 99 was pure by t.l.c. and possessed spectral characteristics consistent with the proposed structure, but the ^{13}C n.m.r. data showed general low intensity resonances not corresponding to the cis-malonate 99. The ^{1}H n.m.r. spectrum also contained peaks not due to 99, which added evidence to the belief that the trans isomer 101 was present as a by-product. The major product of the reaction was assumed to be the elimination product 4-methylcyclohexene 102, as reported by Stork and White.[93] This product was removed from the malonate products along with solvent during isolation.

Saponification of 99 gave the dicarboxylic acid 103 in good yield. The n.m.r. spectral data of the purified material again implied the possible presence of the trans isomer 104 as an impurity. Heating 103 at ~180°C for one hour did not yield the acid 85, but caused substantial charring. At lower temperatures, decarboxylation was not effected. Using refluxing aqueous mineral acid[95] also failed to effect decarboxylation. Flash vacuum pyrolysis gave a poor yield of the impure acid 85. G.c. and n.m.r. spectral data implied the presence of the trans isomer 86 in the ratio 83:17 85:86.

Alternative methods of removing one of the carboxylate functions were sought. Using the method of Krapcho and Lovey,[96] 99 and sodium chloride in aqueous dimethyl sulphoxide was heated to ~150°C, returning

the malonate 99 slightly impure. The method of Ho[97] using boric acid at elevated temperature was more successful in that 99 gave a low yield of mostly the monoester 105 which was obtained impure. Analysis of the saponification products showed, by g.c., that the acids 85 and 86 were present in the proportion 84:16. The method of Dehmlow and Kunesch,[98] in which the malonate is heated with a fatty acid and a phosphine salt, was reported after this work was completed.

The above results imply that the malonate anion attack on the tosylate 92 does not occur cleanly. Evidently, more mechanistic work is required before rigorous deductions can be made. Possibilities include internal return, a feature well known in the solvolysis of secondary sulphonates.[99] It must also be noted that Stork and White did not have the powerful analytical tools available nowadays, that are capable of detecting small quantitites of 100 in 99 or 86 in 85. The data published were presumably referring to 85 extensively purified by recrystallisation of 85 itself and/or its derivatives, although full details are not given. It should also be noted that in a paper written by Eliel and Manoharan,[100] 85 was prepared according to the method of Stork and White. No reference was made as to the preparation of 86 and yet some n.m.r. spectral data were presented.

In summary, the malonate displacement reaction was found to be somewhat inadequate in terms of both product selectivity and synthetic convenience. An alternative acetic acid carbanion equivalent was therefore sought, which would be more selective and might favour nucleophilic displacement over elimination.

The use of 1,3 oxygen-nitrogen heterocycles of general structure 106 as masked carboxylic acids was first reported by Wehrmeister[101] who described the use of some oxazolines in the synthesis of substituted cinnamic acids. It was mainly due to Meyers and coworkers[102] that

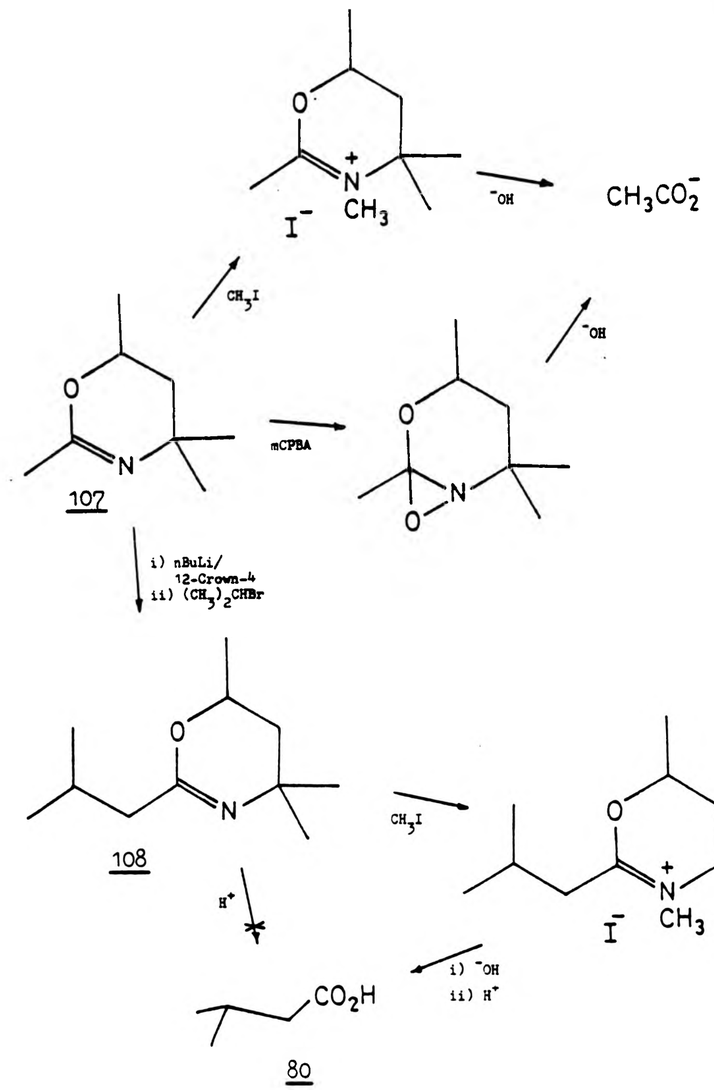
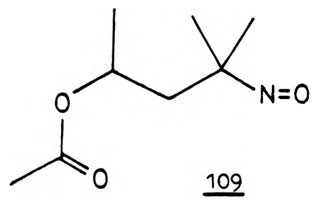


Diagram 24.



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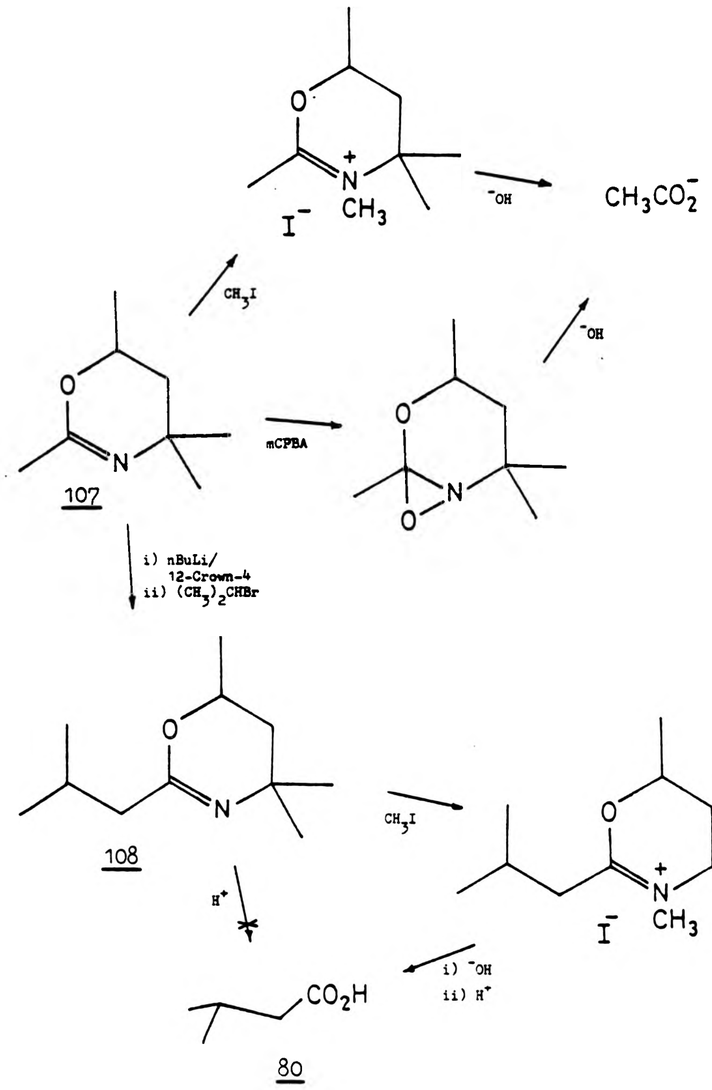
such heterocycles were developed as compounds of great synthetic utility for the preparation of both acids and aldehydes, and notably their potential for asymmetric induction.[103]

It seems viable that reagents of the 106 type structure would provide a synthetic route to the desired acids and offer the potential for asymmetric induction. In the first instance, a reagent was required that would not induce asymmetry.

The use of the oxazine 107 as its anion has been described as an acetic acid carbanion equivalent,[104,105,106] but such reagents require halide as the nucleofugal functionality. Meyers et al.[107] noted that anions such as this, preferentially attack the sulphur of tosylates. Interestingly, Meyers et al.[108] reported a low yield for the coupling of 107 with 2-bromobutane but a high yield with bromocyclopentane.

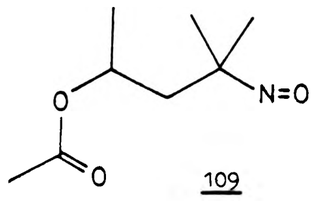
Model experiments with isopropyl bromide and 107, purified to ~99.5%, showed that the product 108 could be prepared, in good yield, using the modified conditions shown in diagram 24. The addition of the crown ether, as a chelating agent for the lithium cation, improved the yield from ~40% to ~85%. The hydrolysis of 108 was somewhat difficult. Using acid-catalysed ring opening conditions with aqueous mineral acid[109] proved inadequate, an observation reported before in similar systems.[110,111] This problem can be overcome by conversion of the oxazine product 108 into a moiety susceptible to base-induced hydrolysis.

Two methods were investigated. Quaternisation of the nitrogen with methyl iodide[112,113] followed by base hydrolysis proved a more efficient method than epoxidation of the imine double bond[114] followed by saponification of the resulting adduct. Utilising the methylation procedure, the oxazine 107 gave its methylated adduct in ~95% yield as pale yellow crystals which could be saponified in near quantitative



107 108 80

Diagram 24.



109

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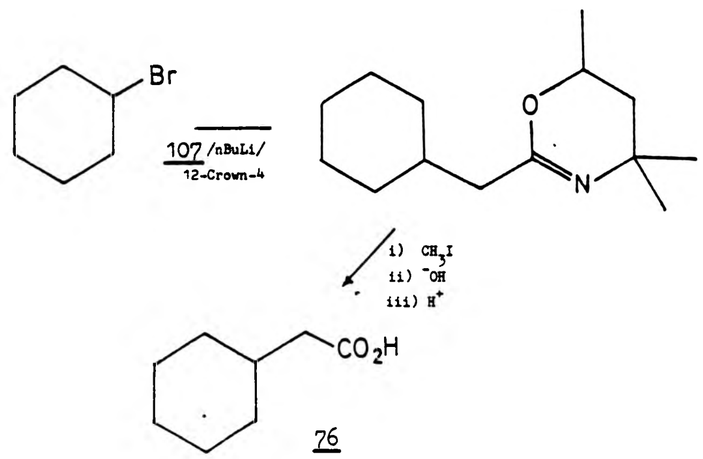

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Diagram 25.

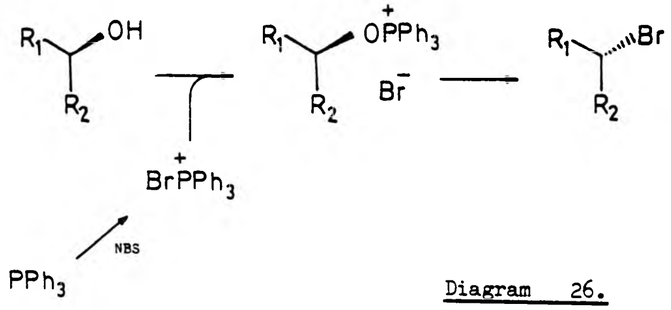

Diagram 26.

yield. The epoxidation procedure gave an intensely blue coloured solution, presumably due to a small amount of the nitroso ester 109, which was hydrolysed without isolation to give the acetate salt in ~40% yield overall.

Isovaleric acid 80 was similarly prepared from 2-bromo propane, using the methyl iodide system, in 46% yield overall. An interesting innovation was that the 12-crown-4 used in the initial step could be separated by distillation of the reaction mixture from lithium chloride.

A second, more appropriate, model system was examined. As shown in diagram 25, bromocyclohexane was converted into cyclohexylacetic acid 76, without extensive intermediate purification, in an overall yield of ~26%. The lower yield of product, compared to the isovaleric acid preparation, is a little curious when compared to the above mentioned preparations reported by Meyers et al., in which the cyclic bromide gave the better yield.

It seemed that the methyl substituted bromocyclohexanes would react similarly, the steric requirements of the oxazine anion assumed to be no greater than those of the malonate.[93] It was necessary, then, to prepare isomerically pure methyl bromocyclohexanes. It was envisaged that the isomerically pure alcohols could be converted into bromides whilst retaining the isomeric purity.

A search of the various synthetic methods available for the transformation of secondary alcohols into their bromides suggested a number of different approaches,[115] in which the stereochemical integrity of the product could be controlled.

One candidate system was the triphenyl phosphine/N-bromosuccinimide complex described by Bose and Lal.[116] The assumed mechanism of this reaction is shown, in a general form, in diagram 26. Using cyclohexanol, the bromide could be obtained via a modified procedure and

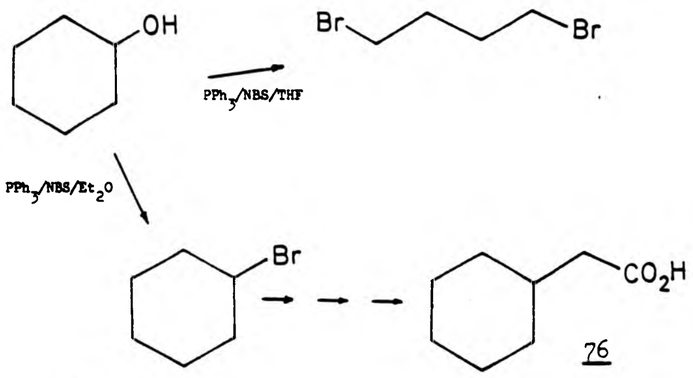

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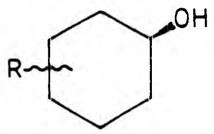
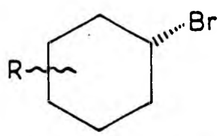
Diagram 27.



R=4CH3 cis 93
trans 91

R=3CH3 cis 116
trans 117

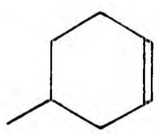
R=2CH3 trans 112



R=4CH3 cis 110
trans 118

R=3CH3 cis 114
trans 115

R=2CH3 cis 113



111

employed, without extensive purification, in the oxazine system to give cyclohexylacetic acid 76 in an overall yield of 7%, as shown in diagram 27. When tetrahydrofuran was used as solvent, the bromide material isolated was mostly 1,4-dibromobutane presumably from attack of the solvent. Substitution by diethyl ether at reflux temperature proved more acceptable. Other problems associated with the bromides in general were their apparently low vapour pressure and the lack of a suitable visualisation agent for thin layer chromatography. These problems required careful removal of solvents and the use of the Beilstein test[50] and gas chromatography (g.c.) as analytical tools.

With trans-4-methylcyclohexanol 91, the inverted bromide 110 was obtained in 34% yield in a slow reaction. The main product appeared to be the alkene 111, due to the competing elimination reaction. The stereochemical purity of the bromide product was excellent.

In similar conditions, the trans-2-methyl alcohol 112 gave the cis-2-methyl bromide 113, isolated slightly impure, in the poorer yield of 7%.

The 3-methyl bromides 114 and 115 were isolated in a yield of 16% from a 89:11 mixture of cis:trans isomers of the alcohols 116 and 117, as an isomeric mixture of 92:8 trans:cis bromides 115 and 114. Likewise a 92:8 mixture of cis:trans alcohols provided nearly exclusively the trans bromide 115.

The cis-4-methyl alcohol 93 did not produce any isolable amounts of the trans bromide 118. The major product was the elimination product 111. An alternative procedure based on recent work by Ho and Davies[117] using triphenyl phosphine/diethyl azodicarboxylate/zinc dibromide in tetrahydrofuran or diethyl ether, again failed to produce isolable amounts of the bromide 118, the alkene 111 being the major product, via elimination.

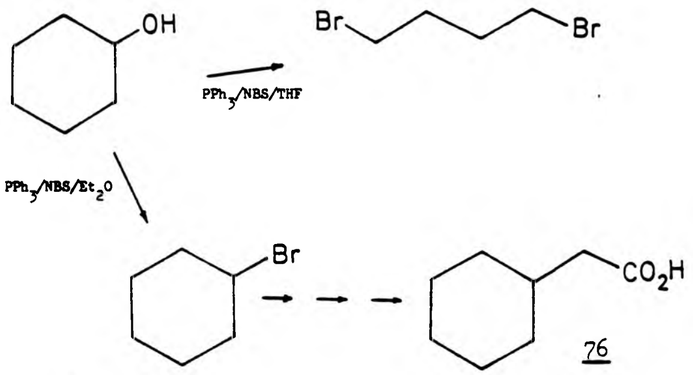


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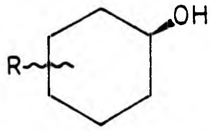
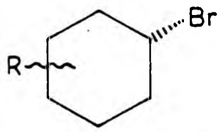
Diagram 27.





R=4CH$_3$ cis 93
trans 91

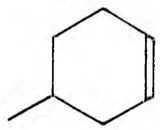
R=3CH$_3$ cis 116
trans 117

R=2CH$_3$ trans 112

R=4CH$_3$ cis 110
trans 118

R=3CH$_3$ cis 114
trans 115

R=2CH$_3$ cis 113



111

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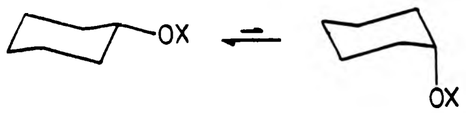
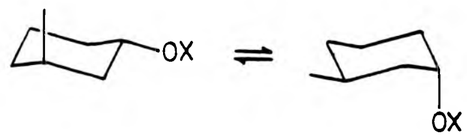
Diagram 28.



X=H cyclohexanol

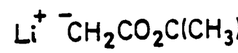


X=H 117

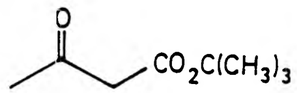
X=PPh_3 119

The bromides that were obtained were subjected to the oxazine displacement/methylation/saponification sequence used for bromocyclohexane. The major products in all cases were those of elimination. The reactions yielded a variety of low yield by-products, and it was later shown that one of the many g.c. peaks possessed an identical retention time to the desired acid in certain cases.

The above results can be rationalised as follows. As shown in diagram 28, the preferred conformation of cyclohexanol has the substituent equatorial.[118] The same is true for the activated phosphine complex. The approach of the nucleophilic bromide from the backside inverts the stereochemistry at the substituted carbon. With a methyl substituent on the ring, such that both substituents adopt equatorial positions, the bromination reaction still proceeds, although considerable competition from the elimination reaction occurs. When the substituents cannot both be equatorial in the chair conformation, a more or less evenly distributed equilibrium exists between axial methyl-equatorial hydroxyl and axial hydroxyl-equatorial methyl, as shown for trans-3-methyl cyclohexanol 117. The activated intermediate 119 possesses two features that favour elimination over substitution. Firstly, the -OX function is axial in a greater proportion than in cyclohexanol. This conformation facilitates the preferred mode of E_2 type elimination, in which the eliminated groups are anticoplanar orientated. Secondly, in the conformation with -OX equatorial, the axial methyl group sterically hinders the attacking nucleophile. This reasoning explains the kinetic resolution shown in the reaction of 116 and 117 mixtures. The latter argument does not hold for the 2- or 4-methyl substituted compounds. However, even in these compounds the effect of syndiaxial interactions in distorting the ring conformation, may indirectly hinder the S_N2 attack of bromide.



120



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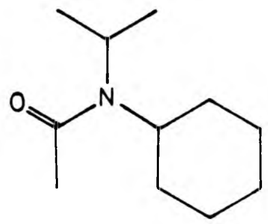

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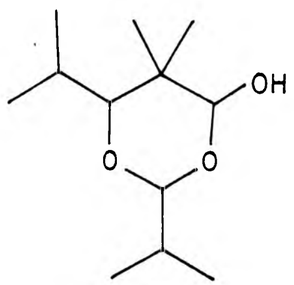

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As mentioned before, Meyers et al.[108] noted the yield of the S_N2 product of attack of the 107 anion on bromocyclopentane to be 88% whereas it is only 21% with 2-bromobutane. The conformational flexibility of the latter bromide would, from the above arguments, facilitate elimination, hence the lower yield of the substitution product.

Another acetic acid carbanion equivalent is 120, which can be obtained via deprotonation t-butyl acetate. The preparation using lithium diisopropylamide has been described by Bos and Pabon.[119] These authors used 120 for the nucleophilic displacement of primary halides. Using this method and 2-bromopropane as a model substrate, we found the major identified product to be 121, although, after treatment with acid, a trace amount of isovaleric acid was detected by g.c. and in the aroma of the product mixture. The production of 121, in certain reactions, was also noted by Bos and Pabon.

Using 120 prepared according to Rathke and Lindert[120] with lithium cyclohexyl(2-propyl)amide for the reaction with various primary halides, 2-bromopropane gave, in our hands, 122 as the only isolated product. Isovaleric acid was not observed after acid treatment of the reaction mixture.

When n-butyl lithium with 12-crown-4 was used as the base in the preparation of 120, the reagent so prepared was also unsuccessful at producing the desired product with 2-bromopropane.

Interestingly, a recent paper by Lawson et al.[121] reported the preparation of 120, anion formation being proved by trapping with a silyl halide. These authors also reported that the ethyl ester analogue of 120, when used with a complexing agent such as 12-crown-4, gave with isobutyraldehyde a significant amount of 123. The implication made by this work was that the complexing agents promote the deprotonation of the substrate. These results are seemingly at odds with the results found

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by us with the lithium salt of the oxazine reagent 107, which was found to react in a more nucleophilic manner with 12-crown-4.

Compounds 121 and 122 were isolated impure and their structures were assigned from their ^1H n.m.r. spectra. The ester 121 had singlets at 1.47, 2.25 and 3.35δ corresponding to the t-butyl, methyl and methylene protons respectively and gave a positive ferric chloride test for ß-dicarbonyl compounds.[50] The amide 122 is a known compound[122] and its structure was tentatively assigned from its spectral data.

Thus far, we had failed to develop a general methodology, using the specific diastereoisomer approaches reported above. We therefore reassessed the known ring methyl substituted cyclohexylacetic acid preparative techniques. As previously outlined in diagram 20, an α,ß-unsaturated system has been reduced to give a diastereoisomeric mixture of 85 and 86 using hydride or hydrogenation reduction in the key step. The double bond in this system is highly electron deficient and conjugate hydride reduction was thus facilitated. It seemed likely that this approach would be applicable to all the ring methyl substituted targets considered here. In addition, Amsterdamsky et al.[88] described the preparation of the 3-methyl substituted isomer 124 via a 1,4-conjugate addition of a Grignard reagent to the unsubstituted α,ß-unsaturated cyanoacetate followed by saponification and decarboxylation. It was anticipated, however, that the decarboxylation reaction of the last step would be equally difficult as previously described for the malonate displacement reaction sequence, when attempted on a small scale. An alternative procedure using a system which did not require this last step was considered preferable.

The double bond reduction of α,ß-unsaturated carbonyl compounds is well known. Reagents such as lithium tri-sec-butyl borohydride[123] or lithium aluminium hydride/copper (1) iodide[124,125] have been used to

125

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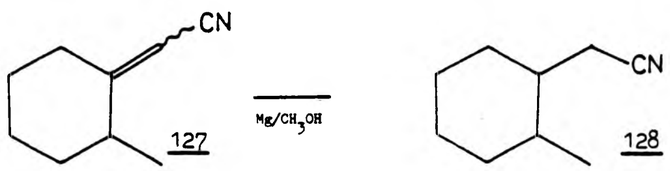
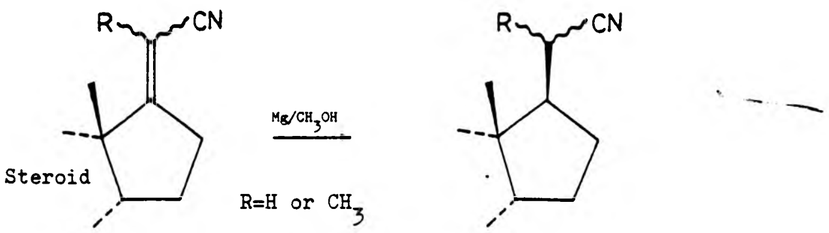
R=H, CH_3 or CH_2CH_3
R'=H or CH_3



127 128

Diagram 29.



Steroid

R=H or CH_3

Diagram 30.

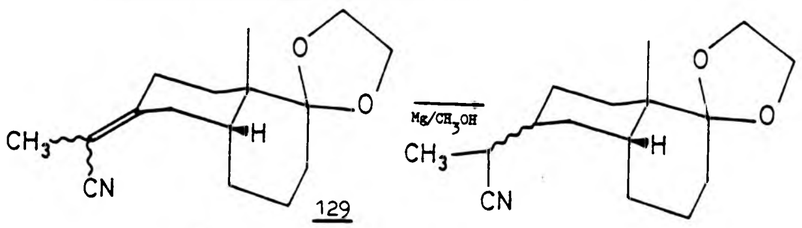


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Diagram 31.

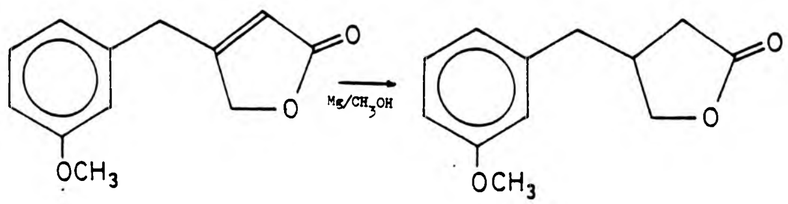


Diagram 32.

this end in various systems. If suitable systems[126] could be found, this methodology would provide a method of preparation of the ring methyl substituted saturated acids and also their α,β-unsaturated analogues, hence obtaining additional test compounds, and therefore information, from a given reaction sequence. Furthermore, different systems may provide different ratios of products.

With regard to the ester system 125, no references were found in a computerised literature search,[127] concerning its transformation into 126. However, Iida and Sugawara[90] and earlier Gerlach[128] have described this transformation (R = Et or H, R^1 = 6 Me), achieved by hydrogenation with supported palladium as a catalyst. Using their procedure, the products 126 were 66% trans. Lithium aluminium hydride preferentially reduced the ester to give the allylic alcohol[129] with substrates such as 125 and more selective hydride reagents such as lithium tri(sec-butyl)borohydride or lithium triethyl borohydride[130] failed to reduce the alkene or ester moiety.

In a related system, the nitriles 127 have been reduced to 128 using magnesium in methanol,[131] as shown in diagram 29. The stereochemistry of the starting material or product was not specified. From work on steroids,[132,133] the product selectivity can apparently be influenced by steric hindrance, as shown in diagram 30. Against this, the somewhat less hindered substrate 129 shows no stereochemical preference,[134] as shown in diagram 31. Magnesium in methanol has been used recently in the transformation shown in diagram 32, a reaction in which catalytic hydrogenation of the unsaturated lactone had failed.[135] No references were found for the application of this reagent to α,β-unsaturated esters or acids.

The α,β-unsaturated esters seemed likely to react with magnesium in methanol with reduction of the double bond, but to ascertain the

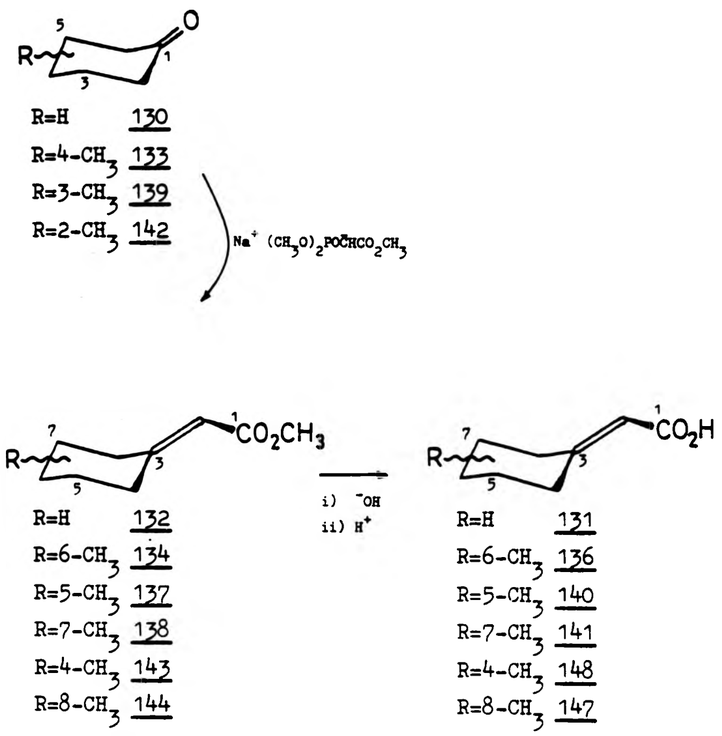
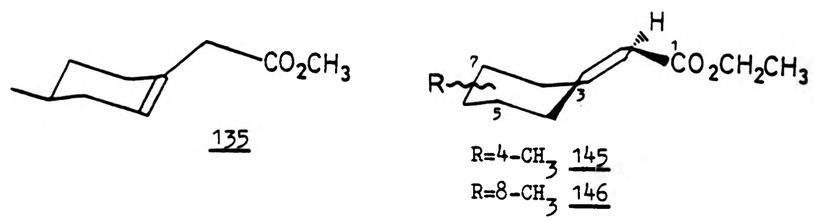


Diagram 33.



experimental outcome, these esters must be prepared. Probably the most convenient preparation of such compounds involves the Wittig or Peterson type condensation with the respective cyclohexanones. The Wittig-Horner procedure has been employed by Duraisamy and Walborsky[126] in their syntheses of 125 type compounds. The Peterson reaction might be expected to result in different product isomer ratios.[21] The unsaturated acid could be prepared by saponification of the esters. The geometric isomers, of either the esters or acids, were thought to be separable by conventional techniques.

The preparation of the α,ß-unsaturated esters by a Wittig-Horner procedure, similar to that of Wadsworth Jnr. and Emmons,[19] was achieved in acceptable yields.

Using cyclohexanone, 130, the acid 131 was obtained by reaction with trimethyl sodiophosphonoacetate followed by saponification of the resulting methyl ester 132, as shown in diagram 33.

Using 4-methylcyclohexanone 133, the ester 134 was isolated along with a small amount of the deconjugated compound 135, which were separated by radial chromatography. In the other condensations, the impurities were separated without characterisation. A novel compound, 135 was also prepared by reaction of 134 with lithium isopropyl-cyclohexylamide followed by quenching with methanol.[137] This kind of deconjugation has been recently achieved by ultraviolet irradiation.[138] The acid 136 was obtained by saponification of 134 with potassium hydroxide in aqueous methanol. The 5-methyl and 7-methyl esters 137 and 138 were prepared from 139 as a 54:46 mixture, in a similar fashion. This product ratio is, perhaps, peculiar in that a slight preference for the (E) product might be expected.[21] Partial separation of these isomers was achieved by extensive silica chromatography. The (Z) product 137 was thus enriched to 85% isomeric

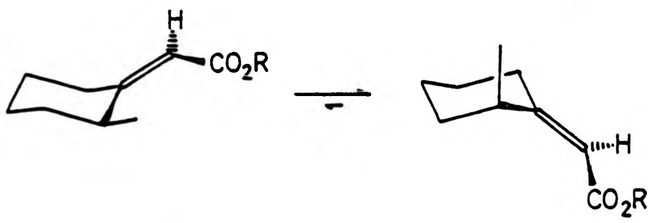


Diagram 34.

purity and the (E) product 138 to 80%. The corresponding acids 140 and 141 were obtained by saponification of these esters, and possessed similar isomeric proportions.

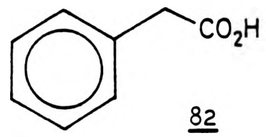
The 2-methyl ketone 142 was reacted with methyl diethylsodiophosphonoacetate in similar conditions. The products obtained appeared to be the methyl esters 143 and 144 together with the ethyl esters 145 and 146, presumably via alkoxyl exchange. The pure methyl esters were obtained by the saponification of the mixture and esterification of the resultant acids by acidic methanol. The methyl esters obtained in this way were subjected to extensive purification, which enriched the (E) ester 144. Saponification and further enriching purification provided the (E) acid 147 of 96% isomeric purity. The (Z) acid 148 was not readily obtained via this route. It proved much easier to isolate 148 using the Peterson condensation.[136] Ethyl trimethylsilylacetate was reacted with lithium isopropylcyclohexylamide to form a Peterson reagent which condensed with 142 to give, after purification, a 80:20 mixture of 145 and 146, slightly impure. This ratio is comparable with the reported Peterson syntheses of this mixture of compounds.[21,136] Enrichment by chromatography provided a 90% isomerically pure sample of the (Z) ester 145. Saponification provided the (Z) acid 148 of similar purity.

It should be noted that the α,β-unsaturated esters and acids described above all adopt a chair minimal energy conformation with the methyl substituent equatorial, except the 4-methyl systems 143, 145 and 148. As shown in diagram 34, the steric interaction between the methyl and the carboxylate forces the substituent axial, as reported by Hauth et al.[139] for the acid 148.

The α,β-unsaturated esters, that were obtained in a sufficiently pure form, were assessed as odorants. All the tested esters were

149

150



82

sweet, fruity and estery in aroma, both as neat compounds and when smoked on a cigarette.[140] Compound 132 had a threshold of ~50 p.p.m. on a cigarette. A 50:50 mixture of 137 and 138 had a threshold at ~500 p.p.m. whereas and 6-methyl ester 134 and its deconjugated isomer 135 were of higher thresholds.

The α,ß-unsaturated acids derived from the esters were likewise assessed. All of these acids were described as sweet, 131, 136 and 147 being described as animal-like also. A sweaty note was detected in 148 along with a fruity note which was also apparent in 140. The acid 141 possessed a mentholic note. Although all the acids were generally weakly odorous, this aspect was stressed for 140, 141 and 147. With regard to threshold detection concentrations, 131 and 136 were assessed as ~2000 p.p.m. All the other α,ß-unsaturated acids possessed higher thresholds. Acid 136 tasted sweet, cheesy and woody on a cigarette.

Using Wittig-Horner or Peterson condensations, the required unsaturated esters had been prepared in readiness for the reduction experiments. Two other unsaturated acids were also examined however. The previously mentioned lithium dialkylamide deconjugation procedure, used to prepare 135, was applied to the acid 131. The deconjugated acid 149 so prepared was found to possess a sweet, animal-like odour of threshold ~100 p.p.m. An impure sample of the isomeric acid 150, prepared *via* the method of Cambie *et al.*,[141] was a gift.[142] Purification provided 150 which was sweaty, sweet and animal-like in aroma and of threshold ~750 p.p.m. The acid 150 was sour, sweet and cheesy in taste, when smoked on a cigarette.

Also assessed was commercial phenyl acetic acid 82, which was sweet, heavy and honey-like in aroma. On a cigarette, the threshold was determined to be ~100 p.p.m.

Interestingly one member of each aroma panel, although not the same

one, indicated a vague dried fruit-like note in 131, 147 and 149. Otherwise, none of the unsaturated esters or acids described above had any dried fruit-like aspect mentioned.

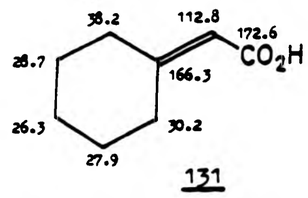
The structures of the α,β-unsaturated methyl esters, which could be assigned by comparison with literature data, were 132,[143] 134[144] and 145.[21,139] The other esters, which were assigned from their spectral data by comparison with related compounds, were 137, 138 and 144.[21,139] The most powerful tool for the structural assignment of the geometric isomers of these compounds was ^{13}C n.m.r. spectroscopy, and in particular, the chemical shifts of the C-4 and C-8 carbon resonances. It should be noted that the literature is devoid of ^{13}C n.m.r. spectral data of these ring methyl substituted α,β-unsaturated esters.

Common characteristic spectral features, in those compounds that were measured, were the following: i.r. - a carbonyl stretching band at ~1720 cm^{-1} and an alkene stretching band at ~1650 cm^{-1}; m.s. - the parent ion as the base ion and abundant fragments corresponding to the loss of $^{\cdot}CO_2Me$ and CH_3CO_2Me at m/e M^{+}-59 and M^{+}-74 respectively; ^{1}H n.m.r. - resonances at 3.7δ appearing as an unresolved multiplet from the equatorial proton on the allylic methylene C-4. Exceptions to the above features are the C-4 methylene proton resonance observed at 3.1-2.7δ in 132 and at 4.2-3.9δ in 145 for the C-4 methine proton. The value for 132 reflects the conformational flexibility of the monosubstituted ring. The ester deshielding effect is therefore averaged over the two C-4 methylene protons.

The structures of the compounds 137 and 138 were assigned from spectral data. The i.r. spectrum of a 50:50 mixture of 137 and 138 showed a carbonyl stretch at ~1720 cm^{-1} corresponding to a conjugated ester and an alkene band at ~1645 cm^{-1}. The m.s. of the enriched

compounds were virtually identical, differing only slightly in relative fragment abundance, with a parent ion as the base ion at m/e = 168. The ^{1}H n.m.r. spectrum of the enriched 137 showed the proton attached to C-2 at 5.60δ, the equatorial C-4 proton at 3.8-3.5δ as an unresolved multiplet, the methyl ester protons at 3.66δ as a sharp singlet and the ring methyl as a doublet at 0.96δ (~6 Hz). The ^{1}H n.m.r. spectrum of enriched 138 was virtually identical except that the ring methyl resonance appeared at 0.94δ (~6 Hz). The ^{13}C n.m.r. spectra were similar and were consistent with the assigned structures. The unsaturated ester function exerts a greater deshielding effect on C-8 than C-4,[27] as seen in 132 and 134. The additional deshielding influence of an adjacent methyl substituted carbon[36,54] either endorses or counteracts this differential deshielding. The compound in which endorsement was observed was 138 as reflected by C-4 and C-8 assignments at 34.6 and 46.1 p.p.m. respectively. These carbons resonated at 37.6 and 37.9 p.p.m. respectively in 137, thus enabling the geometric isomers to be assigned without ambiguity.

The ester 145 cannot be considered closely comparable with the other α,β-unsaturated esters. By analogy with the free acid,[139] the conformation preferred for 145 has the methyl ring substituent axially orientated with the ring in a chair conformation, due to the steric interaction with the carboxylate function. The ^{1}H n.m.r. peak at between 4.2-3.9δ in 145 reinforced this assignment of conformation. This chemical shift must correspond to a preferred equatorial orientation for this proton[145] and would be expected to be relatively deshielded because of its tertiary nature. The lack of closely analogous compounds makes specific assignments of the ^{13}C n.m.r. peaks somewhat difficult, with regard to the methylene carbon resonances. It might be expected, by analogy with the other α,β-unsaturated esters described here, that the



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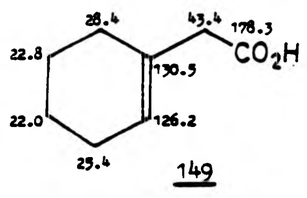

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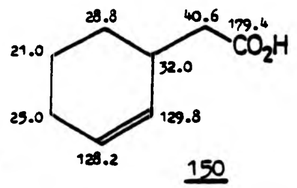

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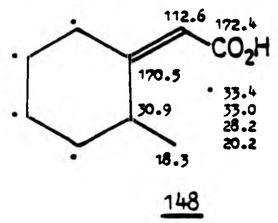

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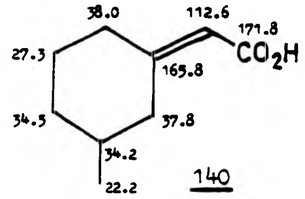

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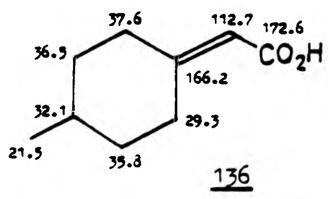

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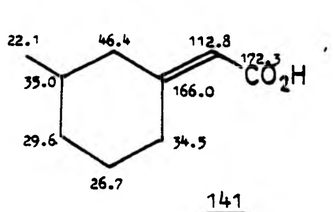

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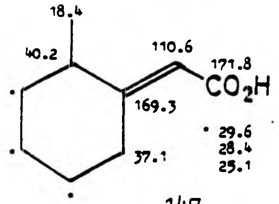


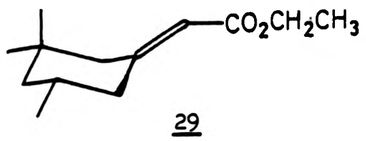
147

^{13}C n.m.r. data. For experimental details, see p. 89.

chemical shift for C-8 would be somewhat greater than 35 p.p.m., whereas it appeared at 33.2 p.p.m. or below.

The structure of the novel deconjugated ester 135 was assigned from its spectral characteristics. The m.s. base ion at m/e = 94 was derived from cleavage of CH_3CO_2Me and the parent ion corresponded to the formula $C_{10}H_{16}O_2$. The i.r. showed a carbonyl stretching band at ~1740 cm^{-1} which is consistent with a deconjugated ester. The ^1H n.m.r. contained the olefinic proton resonance at 5.55δ and the methyl ester signal at 3.68δ both as singlets. In addition, a singlet resonating at 2.96δ and integrating for two protons was assigned as the C-2 methylene. The ^{13}C n.m.r. spectrum showed this methylene resonating at 43.1 p.p.m. The C-3 and C-4 carbon chemical shifts and their off-resonance decoupled multiplicity reinforced the assignment of 135.

The unsaturated acids 131, 149 and 150 were assigned by comparison with literature spectral and physical properties.[129,141] All the methyl substituted α,β-unsaturated acids, 136, 140, 141, 147 and 148, could be similarly assigned.[129,139,146] The literature data was generally incomplete, especially with regard to ^{13}C n.m.r. spectra. The only published studies in this respect have been on specific enantiomers of 141 and 147.[136] The ^{13}C n.m.r. data obtained by us confirmed the literature assignments and was again most valuable in confirming assignments of geometric isomers. Notably, the acid 148 was considerably different in the ^{13}C n.m.r. and other spectra. The main ^{13}C n.m.r. spectral feature was the absence of an obvious C-8 assignment, in that a carbon resonance somewhat greater than 35 p.p.m. might be expected for C-8 by comparison with the isomeric acids, whereas it appeared at 33.4 p.p.m. or below. This observation is analogous to the α,β-unsaturated ester 145. Another interesting feature was apparent



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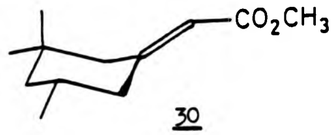

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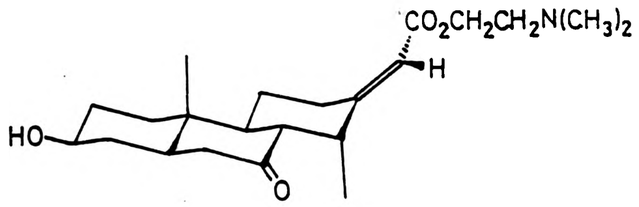
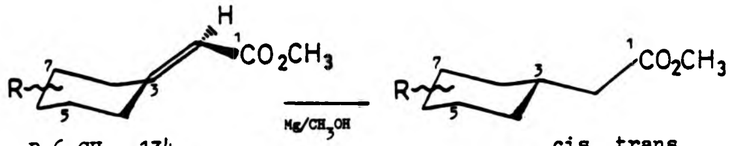


151

in the i.r. spectrum of 148, which displayed a strong band at ~1195 cm^{-1}, but no band at ~1220 cm^{-1}. In the other methyl substituted α,ß-unsaturated acids, a strong absorption invariably appeared at ~1220 cm^{-1}.

Other typical spectral features for the methyl substituted α,ß-unsaturated acids were as follows:- m.s. - parent ion as an abundant ion, often the base ion, a strong fragment at m/e = $M^{+\cdot}$-60, from loss of CH_3CO_2H and a fragment at m/e = 139 corresponding to a loss of $^{\cdot}CH_3$; i.r. - conjugated carbonyl bands at ~1690 and ~1640 cm^{-1}; ^1H n.m.r. - a singlet at ~5.6δ from the proton on C-2, a doublet centred between 1.1-0.9δ of coupling constant 6-7 Hz from the methyl substituent and a strongly deshielded proton, corresponding to the equatorial proton attached to C-4, resonating at ~3.7δ. The latter proton appeared at ~4.0δ in 148 as reported previously by Hauth et al.,[139] the additional deshielding probably being due to the tertiary nature of this proton.

The ^{13}C n.m.r. properties of these types of compounds is an area worthy of further study. The work on compounds such as 29 and 30 has shown that the signal corresponding to the cis methylene[147] appears as a definite AB quartet, even using an 80 MHz. n.m.r. spectrometer. This quartet reflects the considerable chemical shift difference between the attached protons, which can be seen on the ^1H n.m.r. spectra and was analysed using the S.F.O.R.D. technique. A study of similar systems would provide useful data in, for example, the structural and conformational assignment of natural products containing similar systems, such as the alkaloid cassain 151. It should be noted that the C-4 resonance of the ring methyl substituted cyclohexylideneacetic acids and esters prepared by us might also be expected to show quartet splitting of these signals, but long range coupling caused line broadening. These



		cis	trans
R=6-CH_3	134	152	153
R=5-CH_3	137	154	87
R=7-CH_3	138		
R=4-CH_3	143	155	88
R=8-CH_3	144		

Diagram 35.

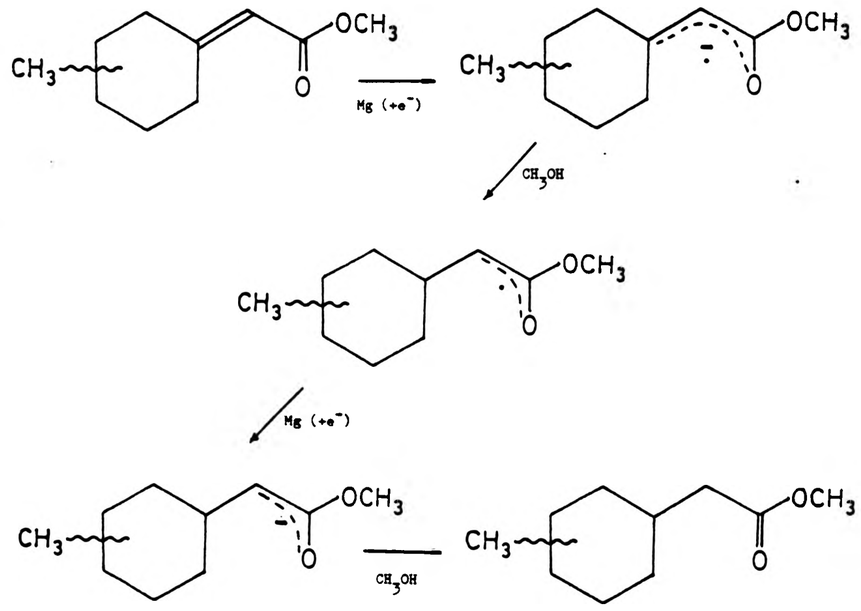


Diagram 36.

signals were therefore observed as poorly resolved triplets. A spectrometer of increased power would provide better resolved spectra, which might allow the individual splittings to be observed.

Having prepared and at least partially separated the isomeric methyl substituted α,ß-unsaturated esters using the Wittig-Horner or the Peterson methods, the starting materials for the dissolving magnesium reduction were available.

Using the conditions described by Profitt et al.,[131] forty equivalents of magnesium in methanol cleanly reduced 134 as shown in diagram 35. The g.c. showed no other products and no residual 134. The ratio of the isomeric product esters was 59:41, 152:153.

The mechanism of the reaction with esters or nitriles has not been proved, but presumably involves electron transfer to form a radical anion, removal of a proton from the solvent, followed by the same again, as shown in diagram 36. This is analogous to other dissolving metal reductions.[118] Coordination of magnesium to the ester function is undoubtedly important and the ability of a magnesium atom to donate two electrons opens the possibility of a single complex involved in the complete reduction sequence. In any case, the steric and electronic factors required by this reduction system gave a slight preference for the cis product 152. The product distribution should be compared with that obtained by Iida and Sugawara[90] and Gerlach,[128] as previously noted, for the hydrogenation of the ethyl ester analogue of 134, in which the products were 66% trans.

The analogous reaction with dissolving calcium in methanol was expected to give a different product ratio. Calcium was expected to be more reactive enabling the reaction to be undertaken, in principle, at lower temperature, which might affect the product ratio. The larger ionic radius of calcium, compared to magnesium, will effect greater

steric constraints on the reactive complex, again potentially affecting the product ratio. In the event, 134 reacted with forty equivalents of calcium in methanol to give a mixture of the starting material 134 and the desired products 152 and 153 in the ratio 49:51. Lowering the temperature of the reaction effectively stopped it.

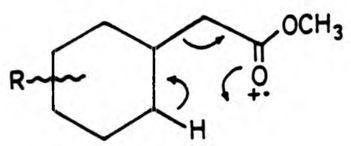
The dissolving calcium reaction was also employed with a mixture of 137 and 138 using up to 240 equivalents of the reagent, but still failed to go to completion. The products 154 and 87 were obtained in the ratio 49:51, respectively. Separation of the products from the starting materials was not facile. On the other hand, using magnesium dissolving in methanol, the reaction proceeded smoothly to completion giving 154 and 87 in the ratio 61:39.

The possibility that these reactions could be 1,4-hydride additions rather than electron transfer reactions was investigated. Calcium hydride failed to react with 134 in conditions akin to the metal reductions.

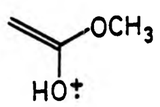
The synthetic utility of magnesium dissolving in methanol has been extended by Olah et al.[148] to the reduction of isolated double bonds, by the addition of palladium on carbon. It was thought that the complexation of palladium in these reductions might affect the product distribution, and could be applied to conjugated and non-conjugated systems.

Using 134 as the starting material, the products 152 and 153 were obtained in a slightly less clean reaction, but the product isomeric ratio was the same as in the reaction without the palladium catalyst. The procedure used was that described by Olah et al.[148]

Using 135 as the starting material, the reaction gave a plethora of products analysed by g.c. Unreacted 135 made up ~84% of the reaction mixture, but a small amount (<5%) of 152 and 153, in the ratio 63:37



m/e=170



m/e=74

Diagram 37.

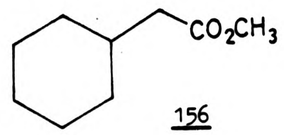
respectively, was also detected.

When a mixture of 143 and 144 was reacted with magnesium in methanol, the products 155 and 88 were obtained in a ratio of 62:38 respectively.

The yields of products from the magnesium reductions were excellent and the purification of the product material to the required standard was, generally, relatively facile. Separation of the starting materials from the products in the dissolving calcium system required extensive purification procedures. The separation of the isomeric product esters was not effected by standard techniques such as column chromatography or by distillation.

The saturated methyl esters were isolated as a mixture of cis and trans isomers and were assigned from their spectral properties and by comparison with literature values.[88,89,91,100]

Common spectral characteristics were as follows:- m.s. a parent ion at m/e = 170 corresponding to $C_{10}H_{18}O_2$ and a base ion at m/e = 74 corresponding to $[CH_3CO_2CH_3]^+$ which may arise via a six electron six centre cyclic transition state, as shown in diagram 37; i.r. - an ester carbonyl stretching band at ~1745 cm^{-1}; ^1H n.m.r. - The methyl ester protons as a sharp singlet at ~3.65δ. Two sets of doublets were observed in each case, centred at 0.87 and 0.92δ, except for the 155/88 mixture, which had one doublet at 0.86δ, corresponding to the ring methyl protons of each isomer. The coupling constants were 6-7 Hz. Only in the 154/87 mixture could the C-2 methylene protons be observed as discrete signals for each isomer. These resonances appeared as an unresolved multiplet centred at 2.26δ and a doublet of coupling constant ~7Hz centred at 2.19δ. By analogy to the assignments of the corresponding acids, which will be presented later, and of literature values,[91,100] the ^1H n.m.r. ring methyl proton resonances at 0.87δ



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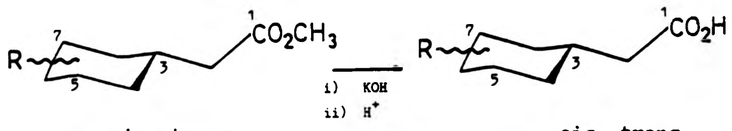


	cis	trans
R=6-CH_3	152	153
R=5-CH_3	154	87
R=4-CH_3	155	88

	cis	trans
R=6-CH_3	85	86
R=5-CH_3	157	158
R=4-CH_3	89	90

Diagram 38.

(J ~6 Hz) correspond to the diequatorial isomers. The ^{13}C n.m.r. data of the isomeric mixtures were likewise assigned.

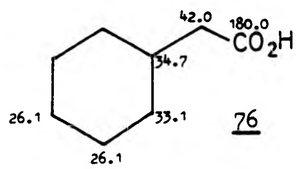
The aroma properties of the ester mixtures were assessed as sweet, fruity and ester-like for 152/153 and 154/87, but the fruity note was absent in the 155/88 mixture. The threshold concentrations on a cigarette were ~1000 p.p.m. for 152/153 and >1000 p.p.m. for 154/87. The mixture 155/88 was of somewhat lower threshold at ~100 p.p.m. and had a sour, dried fruit and cheesy taste on smoking.

For comparison, the aroma of commercial 156, purified by radial chromatography, was assessed as sweet, fruity and ester-like and had a threshold detection concentration of ~70 p.p.m. on a cigarette.

Saponification of the saturated methyl esters, prepared via the dissolving magnesium procedure, gave the saturated acids, as shown in diagram 38, which were generally purified to the required standard by radial chromatography and/or sublimation.

The saturated acids were obtained as isomeric mixtures, which reflected more or less the composition of the ester precursors. Thus, a 60:40 mixture of the esters 152 and 153 gave a 58:42 mixture of 85 and 86 in a yield of 94% after purification to >99.8%. Similarly, a 36:64 mixture of 157 and 158 and a 62:38 mixture of 89 and 90 were prepared. No other products were isolated from any of these reactions, and the isomeric acids could not be separated using column chromatography or distillation.

The mixture of 85/86 was sweaty and animal-like in odour, the mixture of 157/158 was weak, sweet and cheese-like in odour and the mixture of 89/90 was weak, sweet and fruity in odour. Interestingly, two of the six panelists described the 89/90 mixture as having a dried fruit note. The threshold detection concentrations were all greater than 2000 p.p.m., the 89/90 mixture being at ~2500 p.p.m., and



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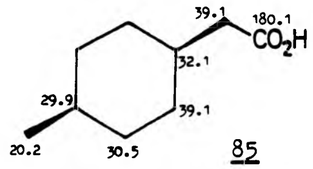
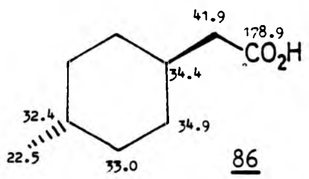
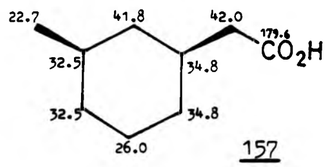


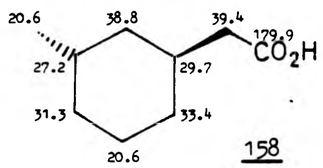
85



86



157



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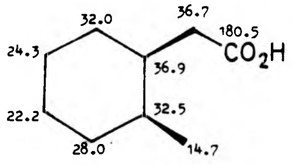

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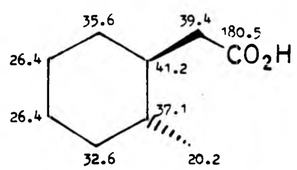


90

^{13}C n.m.r. data. For experimental details, see p. 89.

giving a sweet, cheesy taste.

Assignments were made by comparison with literature values[88,89,90,91,100] and from spectral data. Typical data were as follows:- m.s. - a parent ion at m/e = 156 corresponding to $C_9H_{16}O_2$ and a base ion at m/e = 97 corresponding to the loss of $^{\cdot}CH_2CO_2H$; i.r. - a broad carbonyl stretching band at ~1710 cm^{-1} and a very broad O-H stretching band at between 3600-2500 cm^{-1}; ^1H n.m.r. - comparison of the spectra of the acids made _via_ magnesium reductions and those prepared _via_ the copper (1) hydride procedure (see below) enabled peaks corresponding to the individual isomers of a mixture to be assigned. The methyl substituent appeared at 0.88δ as a doublet of coupling constant ~6 Hz if the ring was diequatorially substituted. If not, this signal appeared as a doublet at 0.92δ (~7 Hz), except in the case of 89, where the doublet was at 0.88δ (~7 Hz). In all cases, the C-2 methylene protons resonated between 2.5-2.0δ. For 85, this signal appeared as a doublet at 2.33δ (~7 Hz), whereas in 86 it appeared as a doublet at 2.21δ (~6 Hz). For 157, the protons on C-2 resonated as a doublet at 2.33δ (~7 Hz), whereas in the _trans_ isomer 158 this signal was an unresolved multiplet centred at 2.30δ; ^{13}C n.m.r. - the individual isomers of a mixture could be unequivocally assigned by comparison with the products of copper (1) hydride reduction (see below). The ^{13}C n.m.r. spectral data of 85 and 86 have been published by Eliel and Manoharan,[100] the assignment of signals being made by analogy and by using single frequency off-resonance decoupling techniques. The spectral data obtained by us for these compounds confirmed these assignments. The other spectra have not been previously reported. The ring methine carbons in all the acids were more deshielded in the diequatorial isomer than in the respective equatorial-axial isomer. The difference was in the range 5.5-2.5 p.p.m.

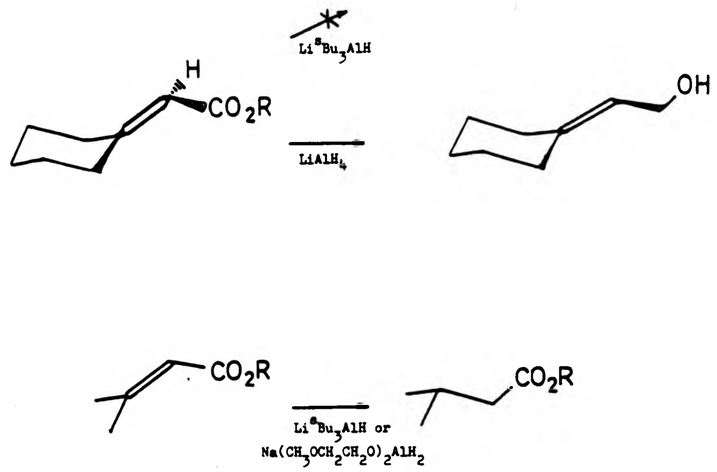


Diagram 39.

Likewise, the ring methyl and the C-2 methylene resonances of the diequatorial isomers appeared consistently downfield of their equatorial-axial orientated isomers.

Having demonstrated a convenient preparation of the saturated target acids as a roughly 60:40 mixture of isomers, we required a different ratio in order that some differentiation might be inferred about the individual isomers in a mixture. Clearly, the further away the ratio can be made from 60:40, the more accurate the information obtained would be. This information is of importance from both the odorant assessment and spectral assignment viewpoints.

It was thought that the conjugate addition of hydride to the α,ß-unsaturated esters would be one method whereby this aim might be achieved. It was noted previously that the specific transformations required by us have not been achieved by hydride additions. Indeed, reagents such as lithium aluminium hydride are known to attack the ester function preferentially,[129] in similar systems, to give the corresponding allylic alcohols, as shown in diagram 39. The more selective reagent lithium tri-sec-butylborohydride has been reported to undergo 1,4-additions to conjugated esters,[123] but was unsuccessful at reducing a cyclic system.[130]

Also shown in diagram 39 is the reduction of an acyclic system by copper (1) hydride which was prepared from copper (1) bromide and sodium bis-(2-methoxyethoxy)aluminium hydride.[149] Reductions using copper hydrides have been summarised in a review,[150] in which it was noted that such hydrides can be used for 1,2- or 1,4- reductions depending on how the reagent was prepared. This reactivity regulation is presumably mediated by the coordinating capacity of the medium. Thus, sodium bis-(2-methoxyethoxy) aluminium hydride can form complexes with the

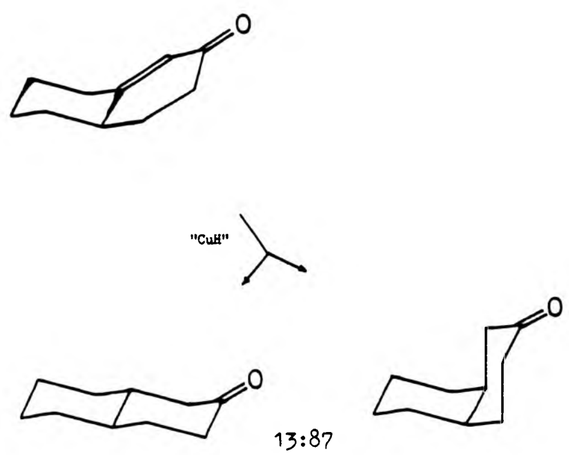


Diagram 40.

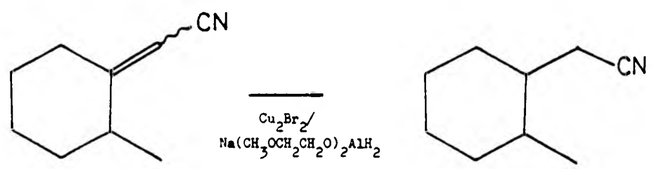


Diagram 41.

copper (1) bromide, in which the ethereal oxygens and/or the aluminium bind to the copper (1) hydride so formed. Copper (1) hydride coordinated in this fashion favours conjugate reductions.

The mechanism of copper (1) hydride reductions is not well understood. It may involve an electron transfer reaction or a formal Michael-type addition of hydride,[151] and possible involves copper (3) species.[152] Whatever the mechanism of the reaction, the reaction shown in diagram 40[153] illustrates that copper hydride reagents can be quite selective and potentially useful in cyclic systems. Perhaps the most relevant publication with regard to the reductions of interest herein, described the transformation shown in diagram 41.[151] This reaction has been effected by dissolving magnesium,[131] as previously noted, and, likewise, the stereochemistry of the starting materials and the products was not specified.

The reactions of copper (1) hydride described above show the following features. Firstly, the reductions involving the copper (1) bromide/sodium bis-(2-methoxyethoxy)aluminium hydride reagent has effected the conjugate reduction of α,β-unsaturated esters.[149] Secondly, the reagent has effected conjugate reduction of a cyclic α,β-unsaturated nitrile.[151] Thirdly, copper (1) hydride has been shown to be sensitive to steric constraints and, hence, can be selective.[153] It was therefore believed that the copper (1) bromide/sodium bis-(2-methoxyethoxy)aluminium hydride reagent would affect conjugate reduction of the cyclic α,β-unsaturated esters considered here, with some selectivity.

The copper (1) hydride reagent was prepared as described by Osborn et al.,[151] except that the sodium bis-(2-methoxyethoxy)aluminium hydride used was 3.4M in toluene instead of 3.5M in benzene. Using ten equivalents of this reagent, as suggested by these authors, with 134 did

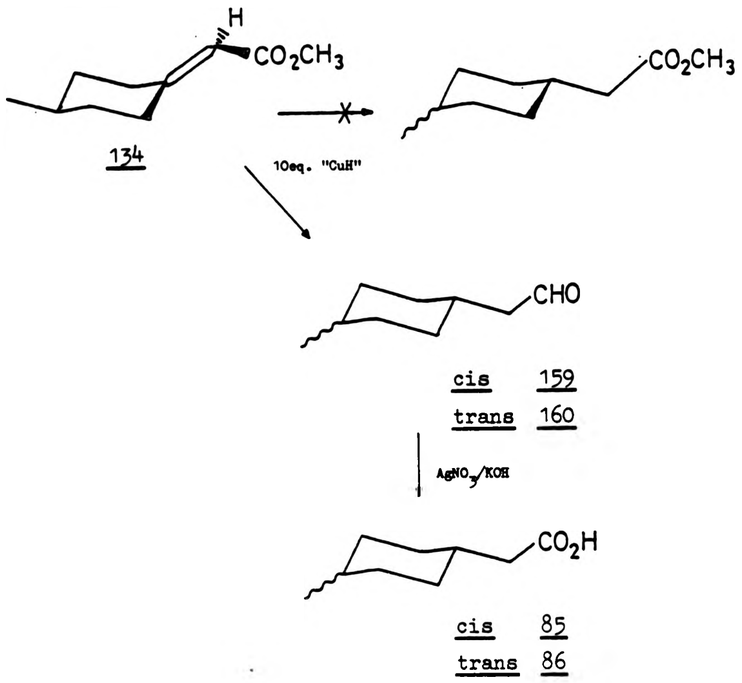


Diagram 42.

not give any detectable amount of the reduced esters 152 or 153. The only products characterised were the novel aldehydes 159 and 160, which were isolated impure and in low yield. The ratio of 159 to 160 was 85:15. The isolation of aldehydes is unprecedented and encouraging, in that the ratio of the products showed good selectivity. Furthermore, the aldehydes were oxidised using alkaline silver (1) oxide[154] to give the acids 85 and 86, as shown in diagram 42.

Various conditions were examined for the reaction. It was found that a stoichiometry of 2:1, reagent:substrate gave a mixture of the saturated aldehydes and esters as analysed by g.c. Other products with higher retention times were detected, but were not characterised in this instance. Further experiments, in which the ratio of the reagent:substrate was varied, showed that the reaction did not appear to provide any unsaturated aldehyde and that the reaction could not be stopped at the saturated ester stage. In the reactions where all the unsaturated ester was reacted, the aldehydes were always detected. Increasing the number of molar equivalents of 2-butanol decreased the reducing power of the reagent. Curiously, it was found that if, after exposing 134 to the reagent for three hours at -78°C, the temperature was rapidly increased to ~30°C, the reaction was cleaner and favoured aldehydic products, compared to using the same conditions but allowing the reaction mixture to reach room temperature slowly.

It was possible to separate the products into isomeric mixtures of esters and aldehydes, but it proved convenient not to do so. Although there were a variety of methods available for the oxidation of the aldehydes to their respective acids,[155,156,157] using the expensive reagent alkaline silver (1) oxide had two advantages. Firstly, the reagent was known to oxidise a cyclohexylacetaldehyde derivative to its acid[154] and indeed had successfully oxidised the partially purified

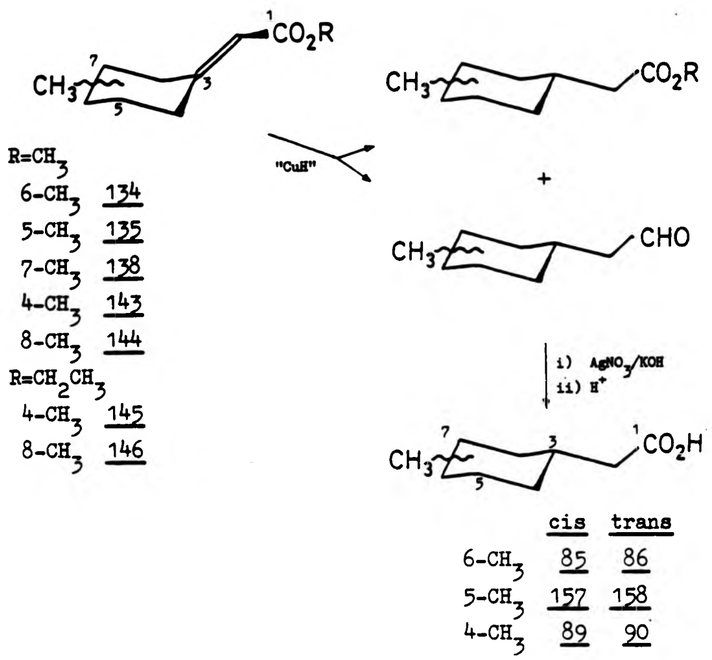


Diagram 43.

161

162

aldehyde mixture 159/160 to the acid mixture 85/86. Secondly, the alkaline nature of this reagent was expected to saponify the esters of the crude product. We reasoned that the products of the reaction with copper (1) hydride need not be separated and purified, so long as none of the unsaturated ester starting material remained unreacted.

Using this rationale, the unsaturated ester 134 was reacted with 2.5 equivalents of the copper (1) hydride reagent, the crude product of which was then isolated and further reacted with alkaline silver (1) oxide. The acids so obtained were isolated in an overal yield of 20%, after purification of the product acids to >99.8%. The product ratio was 79:21, 85:86.

Substitution of the sodium bis-(methoxyethoxy)aluminium hydride by lithium tri-sec-butylborohydride, when making the copper (1) hydride, gave a brown/black compound consistent with the colour of copper (1) hydride, 1.25 equivalents of which was reacted with the unsaturated ester 134 in similar conditions. Analysis of the reaction mixture, after a work-up in which metallic copper appeared to be produced, revealed none of the starting material or the saturated esters or aldehydes.

Lithium tris-(t-butoxy)aluminium hydride has been described as a selective reducing agent[126] and, in combination with a copper (1) salt, has been used to reduce epoxides, halides and sulphonates.[158] When this hydride was substituted for sodium bis-(methoxyethoxy)aluminium hydride in the preparation of copper (1) hydride, the resulting reagent failed to react with 134 in similar conditions.

The general procedure for these copper (1) hydride reductions was as summarised in diagram 43.

Using a mixture of 50:50 of the 5-methyl and 7-methyl unsaturated esters 137 and 138, the cis and trans 5-methyl substituted acids were likewise prepared. The isomeric ratio was 20:80 157:158, and the yield

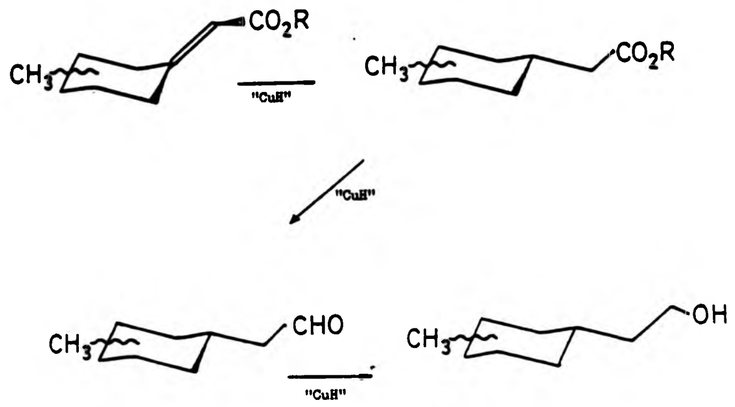
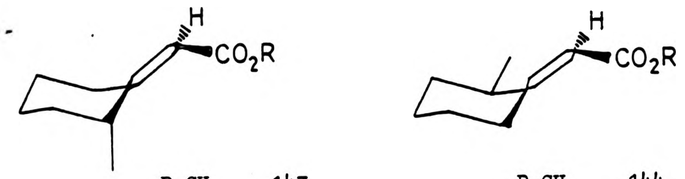


Diagram 44.



R=CH_3 143
R=CH_2CH_3 145

R=CH_3 144
R=CH_2CH_3 146

Diagram 45.

of pure material 31%. A by-product of this reaction was isolated and characterised as a mixture of the cis and trans isomeric alcohols 161 and 162, which were the impurities of high retention time on g.c. analysis of the copper (1) hydride reduction, which were not oxidised by the alkaline silver (1) oxide treatment. The detection of these saturated alcohols together with the saturated aldehydes and esters, without any trace of the allylic alcohols or aldehydes in these types of reactions leads us to propose a stepwise mechanism for the reaction, as shown in diagram 44. The ratio of the alcohols 161:162 was 20:80. Treatment with Jones' reagent[24] gave the corresponding acids.

Clearly, the first reduction step will determine the cis:trans ratio of the product acid, assuming no isomerisation takes place at C-3 during the later reductions. It was thought that this ratio could be affected in the preparation of the 4-methyl substituted acids 89 and 90, depending on whether the starting material was predominantly the (Z)- or (E)-ester. The axial methyl substituent in 143 or 145 was expected to hinder an equatorial attack, as shown in diagram 45, whereas 144 or 146 would be expected to give a similar ratio of equatorial:axial attack as in the 5-, 6- and 7-methyl systems. It should be noted, however, that if this hypothesis is correct, the postulated preferred mode of attack in each case gives rise to the same product, namely 89. If the product ratios observed experimentally were to be significantly different, it would provide strong support for the hypothesis.

In the event, the methyl ester mixture of 143 and 144 of ratio 4:96 gave the 4-methyl substituted acids 89 and 90 in the ratio of 87:13. The ethyl ester mixture of 145 and 146 of ratio 82:18 gave 89 and 90 in the same ratio of 87:13. The difference in the ester alkyls was considered unimportant to the product ratios. The purity of the 89/90 mixture was 99.5%. Evidently, if the copper (1) hydride reagent

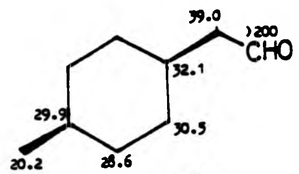
facilitates the isomerisation faster than the reduction of the double bond in a reversible fashion, the product ratios would be about the same with either substrate. Likewise, the preference for axial attack on a mixture rich in 143 could be about the same as that for equatorial attack on the 146 rich system, leading to similar product ratios by chance. In any case, no further work was done in this area.

The aromas of the acids prepared *via* the copper (1) hydride procedure were assessed and found to be almost identical with the acids prepared *via* the magnesium in methanol reduction method. Thus, a 79:21 mixture of 85:86 was sweaty and animal-like in odour, a 20:80 mixture of 157:158 was weak, sweet and cheesy in odour and a 87:13 mixture of 89:90 was weak, sweet, fruity and animal-like in odour. Interestingly, two of the six panelists described a dried fruit note in the mixture 157/158 and three of the six panelists described a similar note in the mixture 89/90. The threshold detection concentrations on a cigarette were greater than 2000 p.p.m. for all the mixtures except that of 87:13 89:90, which was assessed as ~750 p.p.m.

The alcohol mixture 161/162 was assessed to be weak and herbaceous in odour.

The saturated acids have been prepared previously, *via* the dissolving magnesium reduction, as a mixture of *cis* and *trans* isomers. The acid mixtures prepared using the copper (1) hydride procedure were of different isomeric ratios. The major product isomers were those resulting from equatorial attack. The ratio in the magnesium reduction was about 60:40 whereas in the copper (1) hydride system, it was about 80:20. This ratio difference and literature data enabled spectral assignments of individual isomers to be made unequivocally, and has been commented on already.

The novel aldehydes 159 and 160 were isolated impure, but were



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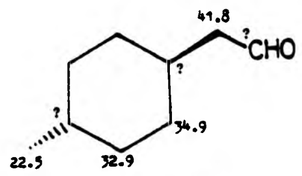

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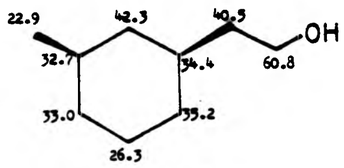

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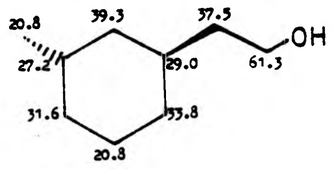

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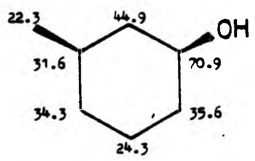


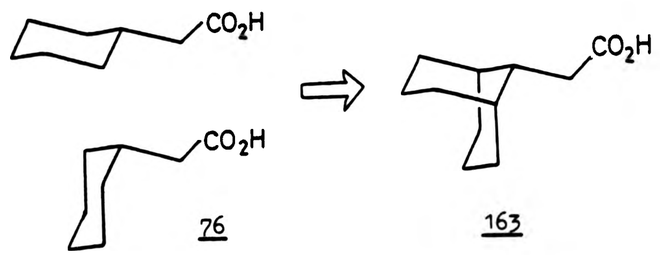
95

^{13}C n.m.r. data. For experimental details, see p. 89.

assigned from their n.m.r. spectra. The ^{1}H n.m.r. spectrum showed the aldehyde protons as a multiplet, which was not exchangeable on addition of deuterium oxide, at between 9.85 and 9.79δ, and the methyl substituent was observed between 1.0 and 0.8δ, the major isomer 159 appearing as a resolved doublet (J ~ 7 Hz) centred at 0.92δ. The ^{13}C n.m.r. spectrum was complicated by impurity signals but the major aldehyde 159 was easily discernible and assigned by comparison with the ^{13}C n.m.r. spectral data of its corresponding acid 85. The isomer 160 could only be partial assigned with confidence.

The alcohols 161 and 162 were obtained as a pure mixture. The m.s. did not give a parent ion. A fragment ion at m/e = 124 corresponds to the loss of water and the base ion at m/e = 95 was accurately measured as $C_7H_{11}^{+}$. The i.r. spectrum displayed a broad absorption between 3500–3000 cm^{-1} corresponding to the O–H stretching frequency. The ^{1}H n.m.r. showed, at 3.65δ, a triplet (J~7 Hz) due to the C-1 protons and pairs of doublets at 0.91δ (J ~ 7 Hz) and 0.87δ (J ~ 6 Hz), the former being from the major isomer, and corresponding to the ring methyl substituent. The ^{13}C n.m.r. spectrum was assigned by comparison with the spectra of their respective esters and acids and cis-3-methyl cyclohexanol 116.

The interestingly low threshold of exo-norbornylacetic acid 83 and the aroma properties of this compound and cyclohexylacetic acid 76 led to the preparation of various bicyclic compounds in order to assess their aroma properties. The increased rigidity of a bicyclic system to an analogous monocyclic system, makes the preparation of such systems attractive for structure-activity relationship assessment. The more rigid a system is, the smaller number of low energy conformers the system can adopt, when acting as a ligand in a receptor, and hence can be an



76 163

Diagram 46.

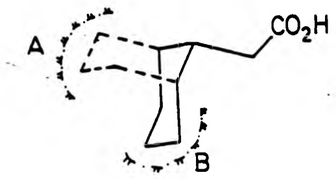


Diagram 47.

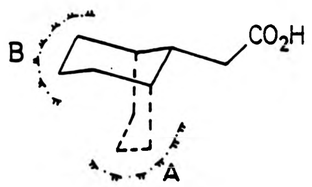
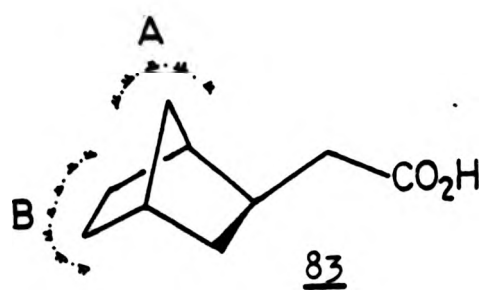


Diagram 48.

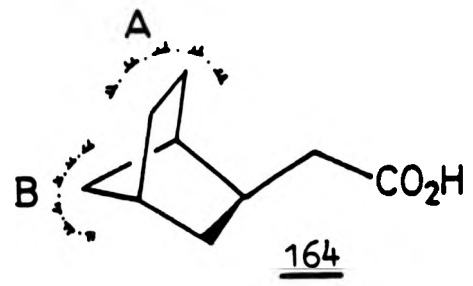
important source of receptor information. The lack of flexibility, however, decreases the likelihood of a given rigid system being able to interact with a receptor. These two factors make the logical design of a proposed bicyclic compound a necessity. We therefore designed two target compounds, which we believed were potentially interesting from both an aroma and a threshold viewpoint. In addition, intermediates in the syntheses of these compounds provided several analogous compounds of potential interest.

Cyclohexylacetic acid 76 has been described by us as having a dried fruit aroma aspect. The low energy conformations of 76, with respect to the ring, are the chair conformers with the substituent either axial or equatorial, as shown in diagram 46. The bicyclic compound 163 was designed to display both of these conformers simultaneously. Hence, the conformer of 76 with the substituent axial is mimicked as shown in diagram 47. There are several similarities and differences between the axial conformer of 76 and 163. Evidently, there is good overlap between the axial 76 conformer and 163, as highlighted in the diagram and implied from molecular models, and 163 adopts the overlapping conformation as its lowest energy conformation, with respect to the rings, due to the increased rigidity of the bicyclic structure. Evidence for the chair conformations of both rings, as the preferred conformation in 163, will be presented later. There are steric constraints in the area marked as 'A', but the area marked 'B' would be expected to mimic closely the possible receptor requirements shown by 76, if 76 were to interact with the receptor when adopting its axially substituted chair conformation. It should be noted that the steric constraints on the rotation about the C-1 - C-2 and C-2 - C-3 bonds were expected to be similar in both the axial substituted conformer of 76 and 163.

The bicyclic compound 163 also mimics the equatorial substituted

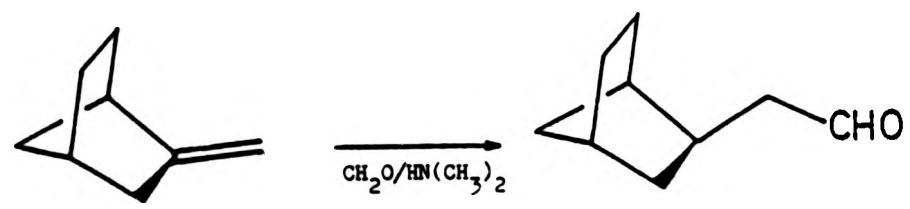


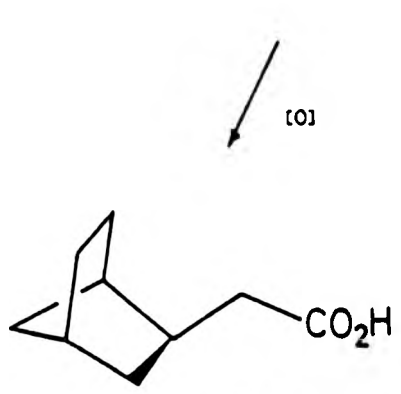
83



164

Diagram 49.





164

Diagram 50.

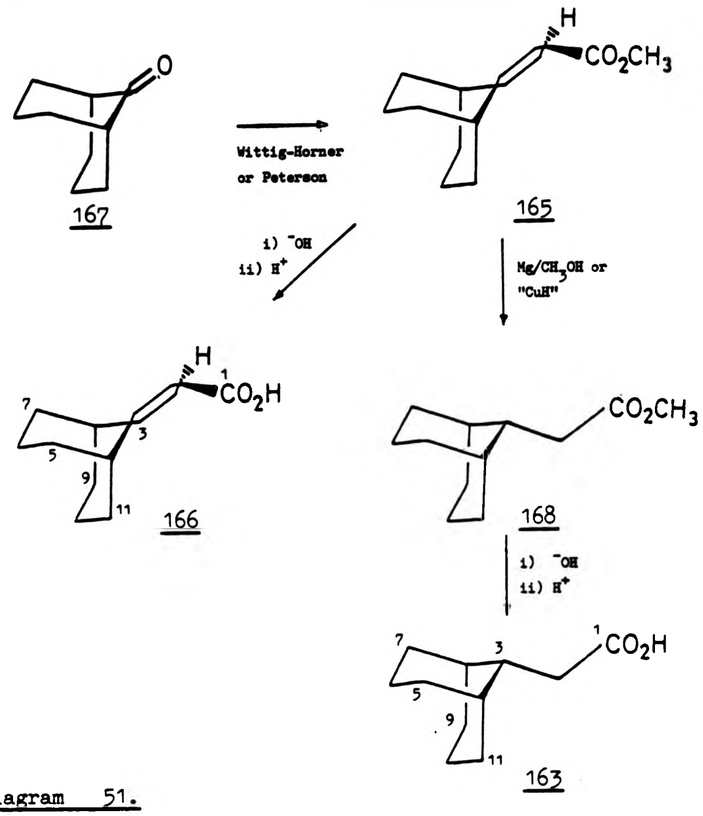
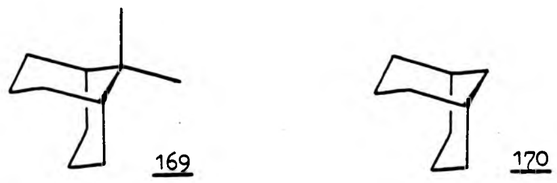


Diagram 51.


169

170

conformer of 76, as shown in diagram 48. Again, area 'A' represents increased steric requirements of the bicyclic compound 163 and area 'B' represents the area closely mimicking the possible receptor requirements shown by 76, if 76 were to interact with the receptor when adopting its equatorially substituted chair conformation. In this case, rotation about the C-2 - C-3 bond appeared, from models, to be somewhat sterically hindered in 163, but not in 76, which may be an important feature.

Another relevant difference between 163 and 76 is the change in the molecular mass resulting from the addition of the three carbon atom bridge. This change could be responsible for significant variation in parameters such as the partition coefficient and the transfer coefficient.

The bicyclic compound 164 has not such a large change in molecular mass and is comparable to the ring methyl substituted saturated acids. In view of the low threshold (~5 p.p.m.) of 83 and its interesting aroma qualities, the endo isomer was an obvious target compound. The physical parameters, such as the partition coefficient and transfer coefficient, of 164 were expected to be almost identical to 83, but there are subtle changes in the steric requirements of the ring system, as shown in diagram 49. A study of molecular models suggested that the area designated 'A' is slightly more crowded in 164, whereas area 'B' is more crowded in 83. It was expected that aroma information derived from 164 would be valuable, therefore, in a structure-activity relationship study.

A search of the chemical literature revealed that 163 has not been previously prepared. Compound 164 was claimed to be commercially available,[159] although we could not find a source at the time of this work.[160] Compound 164 has been previously prepared by Sodervall[161] using the route outlined in diagram 50.

The proposed synthesis of 163 is shown in diagram 51. The intrinsic symmetry of 163, and the other structures shown in the diagram,

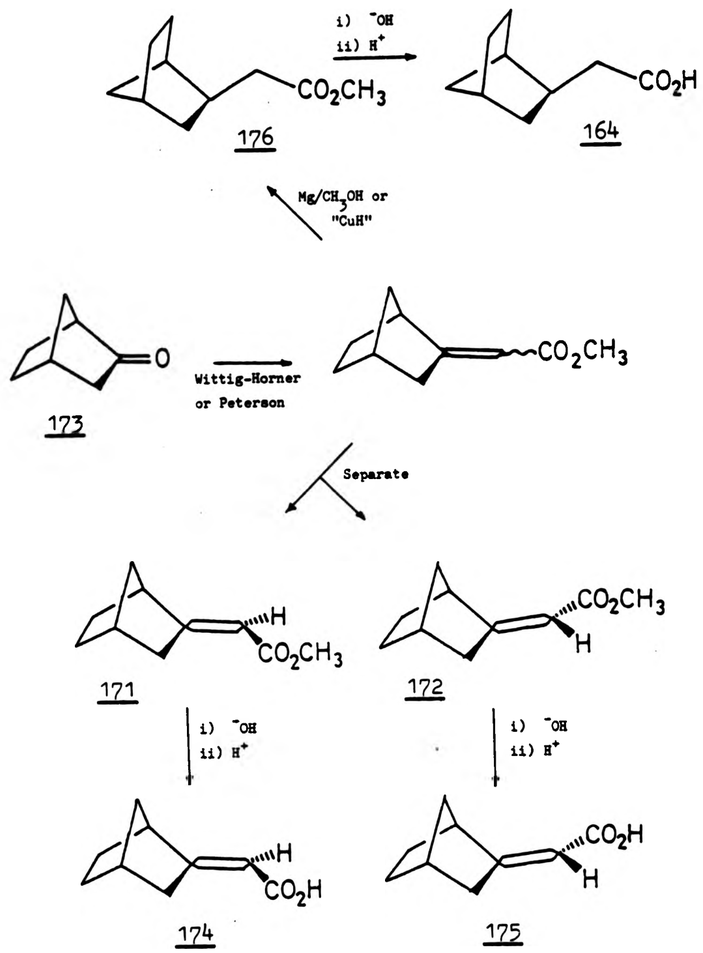


Diagram 52.

provides the distinct simplification of no isomeric products at any stage. The utilisation of the methodology, developed for the ring methyl substituted acids prepared previously, suggested that the Wittig-Horner or Peterson condensation product 165 could be reduced by dissolving magnesium or possibly copper (1) hydride. Saponification of 165 would yield the α,ß-unsaturated analogue of 163, 166 which is a potentially interesting compounds itself.

It should be noted that 167 is commercially available and 163, 165, 166 and 168 are all novel compounds. These compounds are all drawn with both rings in chair conformations. We will present later direct evidence in support of this assumption in the cases of 165 and 166. With regard to 163 and 168, there is literature precedent for a chair-chair structure. The compound 169 has been recently reported by Aranda et al.[162] Using ^{13}C n.m.r. spectroscopy, force field calculations and X-ray crystallography, several compounds of this type were shown to adopt a chair-chair conformation preferentially. Likewise, 170 and similar compounds have been extensively studied by Peters et al.,[163,164] and these authors showed that only when there was substitution on the rings, which resulted in serious steric interactions, did the bicyclic structure not adopt a chair-chair conformation. In addition, some further support from n.m.r. and i.r. spectral data will be presented later.

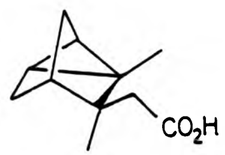
The proposed synthesis of 164 is shown in diagram 52. The α,ß-unsaturated esters 171 and 172 were to be obtained from the commercially available ketone 173 using either Wittig-Horner or Peterson conditions. If separation of these geometric isomers proved viable using techniques available to us, the isomeric α,ß-unsaturated acids 174 and 175 could be prepared via saponification. Reduction of 171 and 172 using dissolving magnesium or copper (1) hydride, in the conditions used

173

$LiAlH_4$ 11:89

$LiAl(OCH_3)_3H$ 2:98

Diagram 53.



177

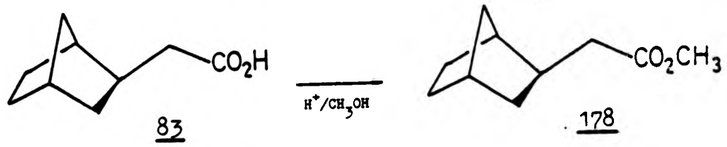
by us previously, was expected to provide the saturated ester with selectivity for the endo isomer 176, by analogy to the selectivity shown in the reduction of the ketone 173 by hydride reagents,[126] as detailed in diagram 53.

The acids 174 and 175 have been reported by Mariani et al.,[165] 175 being isolated pure. Their synthesis invoked base induced condensation of 173 with acetonitrile followed by treatment of the resultant α,ß-unsaturated nitrile with basic hydrogen peroxide and then acidic sodium nitrite.

Although these specific compounds are not expected to be naturally occurring in tobacco or dried fruit, it is interesting to note that structurally similar compounds are known as natural products. For example, the acid 177 has been identified in lavender oil.[166]

The synthesis of the bicyclic acid 163 was achieved using the scheme previously outlined in diagram 51. Thus, the Wittig-Horner condensation of the commercially available ketone 167 with 1.1 equivalents of trimethyl sodiophosphonoacetate reacted incompletely. On average, 79% of the ketone was returned unreacted. The yield of 165 was on average 81%, based on reacted ketone, of the 90% pure compound which was further purified to 99.8% by g.c. in a yield of 26%, by radial chromatography. Saponification of 165 gave the corresponding acid 166 as a crystalline compound in 98% yield. Reduction of 165 using magnesium dissolving in methanol provided the saturated ester 168 in 76% yield after purification. The product ester was then saponified by potassium hydroxide in aqueous methanol to give the target compound 163 in 70% yield after purification. The unsaturated acid 166 was converted back to its ester 165 in 92% yield by treatment with refluxing acidic methanol.

The aroma of the unsaturated ester 165 was weak and paraffin oil-like and had a detection threshold concentration of ~700 p.p.m. on



83 178

Diagram 54.

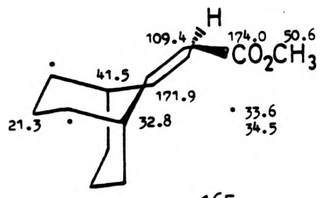
being smoked on a cigarette. The saturated ester 168 was also weak smelling and had a eucalyptus or mentholic note and a threshold value of ~200 p.p.m. The corresponding acids 163 and 166 were both weak and animal-like in aroma, 163 being also described as horse-like, and both compounds had threshold values greater than 2000 p.p.m.

The synthesis of the bicyclic compound 164 was achieved using the scheme previously outlined in diagram 52. The Wittig-Horner reaction of 173 with 1.1 equivalents of trimethyl sodiophosphonoacetate was not efficient. The ketone 173 was returned unreacted in about 75% yield and the yield of the adduct mixture 171/172 was about 39% based on the amount of ketone reacted. The isomeric mixture was 64:36 171:172. Extensive radial chromatography separated enriched samples of the individual geometric isomers. Hence, a small sample of 171 of 95% isomeric purity was separated. Likewise a small sample of 172 was separated which was of 91% isomeric purity. Saponification with potassium hydroxide in aqueous methanol provided the respective acids 174 and 175 from these enriched ester samples. The isomeric purity of these acids was akin to the respective esters. The reduction of a mixture of 171 and 172 was achieved with magnesium dissolving in methanol. On a micromolar trial scale, the reduction appeared to give one isomer as the product by g.c. and t.l.c. analysis. Upscaling the reaction, a mixture of 62:38 171:172 again appeared to give one product by g.c. and t.l.c. analysis but the ^{13}C n.m.r. spectrum showed the product to be a 91:9 mixture of 176 and 178. The ester 178 was prepared by refluxing the commercial acid in acidic methanol, as shown in diagram 54, in order to confirm this finding. G.c. analysis of the two ester isomers showed the peaks to be poorly resolved. Saponification of the ester mixture rich in 176 yielded a mixture of the corresponding acids 164 and 83 again in a 91:9 ratio.

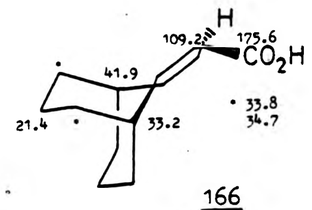
The aromas of all the esters 171, 172, 176 and 178 were adjudged to be sweet, fruity and ester-like. The α,β-unsaturated esters 171 and 172 tasted sweet, sickly and cheesy on smoking in a cigarette and the threshold detection concentration was determined to be ~150 p.p.m. for the enriched 171 and ~100 p.p.m. for the enriched 172. The saturated esters 176 and 178 were of slightly lower threshold at ~35 p.p.m. with a taste described as cheesy with a dried fruit aspect.

The aroma assessments of the corresponding acids were more varied. The enriched unsaturated acid 174 was sweet, fruity and horse-like in odour, which became a weak cheese-like effect on smoking in a cigarette, with a threshold determined as ~2000 p.p.m. The isomeric enriched acid 175 was of higher threshold and had a sweet, fruity and animal-like odour. The odour of the 91% endo acid 164 was likewise sweet, fruity and animal-like, but this material gave a strong dried fruit aroma, when laced on a cigarette before smoking, and had an interesting threshold detection value of ~70 p.p.m.

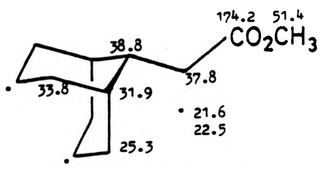
The novel [3,3,1] bicyclic compounds, prepared by us, possessed spectral characteristics consistent with the structures shown. Thus, the unsaturated ester 165 had an m.s. with a base ion equivalent to the molecular ion at m/e = 194 and a fragment of equal intensity at m/e = 91. The i.r. spectrum showed a carbonyl absorption at ~1705 cm^{-1}, which is quite a low value for an α,β-unsaturated ester.[167] A band of weak to moderate intensity was also observed at ~1690 cm^{-1}, which is characteristic of methylene scissoring absorption of chair-chair [3,3,1] bicyclic systems.[168] The ^{1}H n.m.r. spectrum showed a singlet at 5.68δ corresponding to the proton attached to C-2 and a singlet at 3.70δ corresponding to the methyl ester protons. Two broad unresolved multiplets appeared at 4.2-3.9δ and 2.5-2.2δ corresponding to the protons on C-4 and C-5 respectively. The remaining protons resonated in an



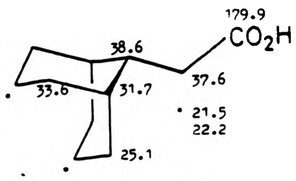
165



166



168



163

^{13}C n.m.r. data. For experimental details, see p. 89.

envelope in the region 2.2-1.3δ. The ^{13}C n.m.r. spectrum showed peaks which could be assigned by analogy to the methyl substituted cyclohexylideneacetic acid esters, previously prepared by us, and also literature compounds.[39,162] The simplicity of the nine line broad band decoupled spectrum implied that there was a plane of symmetry on which lay C-1, C-2, C-3, C-4 and C-8. The two rings would therefore be in similar conformations, i.e. either both chair or both boat, or possibly in rapidly converting twist conformations. Chemical shift information and literature precedents[162,168] firmly support the chair-chair conformation.

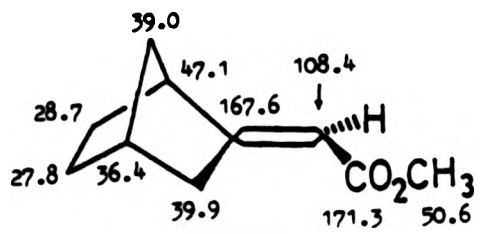
Notably, the chemical shift of the proton on C-4 in 165 was observed at ~4.0δ, which is similar to the C-4 proton in ethyl 4-methylcyclohexylideneacetate 145. In 165, the C-4 proton must be equatorial with respect to either of the rings and hence the chemical shift similarity lends weight to the claim that the methyl substituent in 145, and similar compounds, is axially orientated.[139] This information also supports the assumption that an equatorial C-4 proton in certain cyclohexylideneacetate systems is more strongly deshielded than an axially orientated C-4 proton. This argument can be extended to the α,ß-unsaturated acids.

The unsaturated acid 166 was isolated as a crystalline solid, which melted sharply at 166°C. Its m.s. showed the molecular ion as the base ion at m/e = 180. Loss of CH_3CO_2H gave rise to an abundant fragment at m/e = 120. The i.r. spectrum showed a carbonyl stretching band at ~1685 cm^{-1}, which is within the normal range for α,ß-unsaturated acids.[167] A broad O-H stretching band was also observed at 3400-2500 cm^{-1}. A weak band due to methylene scissoring in the [3,3,1] bicyclic system was observed at ~1490 cm^{-1}, implying a chair-chair conformation.[168] The ^{1}H n.m.r. spectrum was similar to

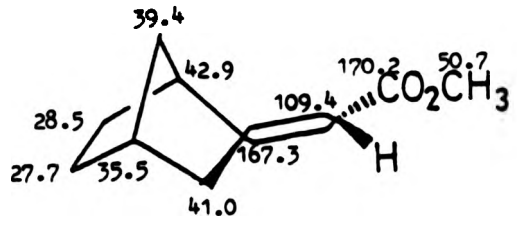
the ester 165 with unresolved multiplets at 4.2-3.9δ and 2.6-2.3δ corresponding to the protons on C-4 and C-8, and a singlet at 5.68δ from the proton on C-2. The ^{13}C n.m.r. spectrum of 166 was very similar to the ester 165 and was consistent with the proposed structure.

The saturated ester 168 had its base ion at m/e = 74 corresponding to the fragment $[CH_3CO_2Me]^+$. The parent ion at m/e 196 was accurately measured as $C_{12}H_{20}O_2$. An abundant fragment at m/e = 136 corresponded to the loss of HCO_2Me from the molecular ion. The i.r. spectrum showed a carbonyl stretching band at ~1740 cm^{-1} and a methylene scissoring band at ~1490 cm^{-1}, which implies a chair-chair conformation for the compound. The ^1H n.m.r. spectrum showed the methyl ester protons as a singlet at 3.68δ and the protons on C-2 at 2.49δ as a doublet of coupling constant ~7 Hz. The remaining protons were observed as an unresolved envelope at 2.2-1.4δ. The ^{13}C n.m.r. spectrum contained nine resonances implying a plane of symmetry containing C-1, C-2, C-3, C-6 and C-10. The spectral data and literature precedent for similar compounds[162,168] led to the structure of 168 as drawn, in which the rings preferentially adopt the chair-chair conformation.

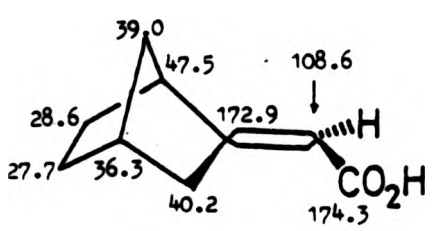
The acid 163 derived from 168 had an m.s. with a base peak at m/e = 136 due to loss of HCO_2H from an abundant molecular ion, measured accurately as $C_{11}H_{18}O_2$, at m/e = 182. Curiously, the expected fragments at m/e = 122 and m/e = 123 from the cleavage of CH_3CO_2H and $^{\cdot}CH_2CO_2H$ from the parent ion, were not particularly abundant. ß-Cleavage of the acid was a favourable process in the other saturated acids prepared by us and reported herein. The i.r. spectrum contained the carboxyl stretching band at ~1710 cm^{-1} and a weak band at ~1490 cm^{-1} characteristic of a chair-chair conformation.[168] A broad O-H stretching band at 3400-2500 cm^{-1} was also observed. The ^1H n.m.r.



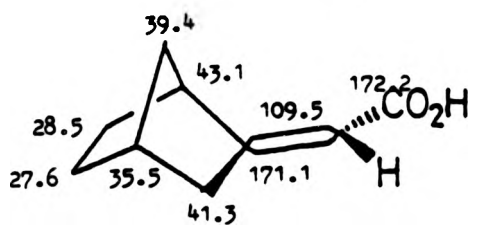
171



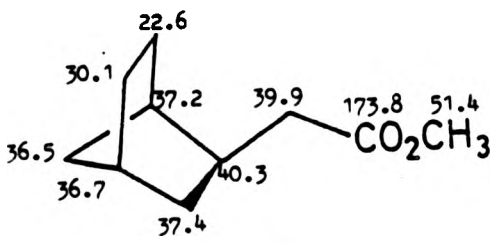
172



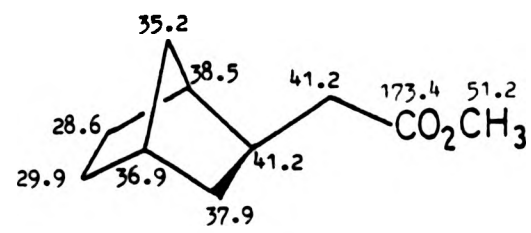
174



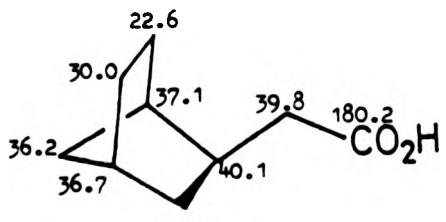
175



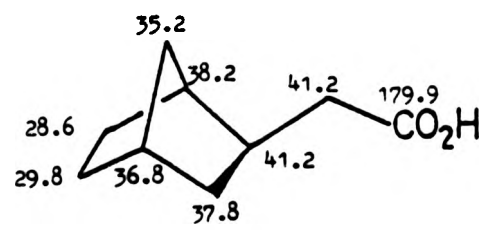
176



178



164



83

^{13}C n.m.r. data. For experimental details, see p. 89.

Odor Value = $\frac{\text{Concentration of Flavor}}{\text{Threshold Concentration}}$

Diagram 55.

$$\frac{1}{T_{1,2..n}} = \sum_{x=1}^{n} \frac{K_x}{T_x}$$

where $T_{1,2..n}$ = Threshold of a mixture of n components

T_x = Threshold of pure component x

K_x = Proportion of x in mixture

Diagram 56.

$$\frac{1}{T_{1,2}} = \frac{K_1}{T_1} + \frac{K_2}{T_2}$$

such that $K_1 + K_2 = 1$

$$\Rightarrow \quad T_{1,2} = \frac{T_1 . T_2}{K_1(T_2 - T_1) + T_1}$$

Diagram 57.

spectrum contained a doublet at 2.50δ with the coupling constant equal to ~7 Hz and integrating for two protons. The remaining non-exchanging protons resonated in an unresolved envelope at 2.2-1.3δ.

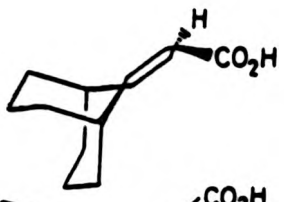
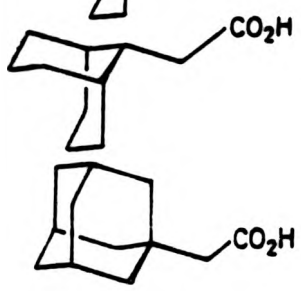
The ^{13}C n.m.r. spectrum of 163 was very similar to 168 and contained eight non-equivalent resonances. This data implied that 163 preferentially adopts a similar conformation to 168 and that this conformation probably has the rings preferentially chair-chair, by analogy to 168. The melting point of 163 was 160-1°C.

We have prepared a number of compounds analogous to cyclohexylacetic acid 76. We have attempted to ensure that the purity of the test materials are as close to 100% as we could purify them, given the facilities available to us. Any compounds that were not of sufficient purity were not tested. The importance of trace compounds in odorous materials has been adequately reviewed by Ohloff,[73,169] with his concept of "odor value" an important development. This value is defined as the ratio of the concentration of a constituent to its threshold concentration, as shown in diagram 55.

We shall analyse our results using the notion of goodness of fit between an odorant ligand and its receptor, as discussed by Dodd,[170] and is quintessential to structure-activity analysis. This notion implies that there are stereoelectronic criteria that, if fulfilled, induce maximal response from a receptor and that odorant ligands fit, more or less, these criteria. Hence, an odorant ligand that fits the criteria for response well, will induce a response greater than one that fits badly.

We have attempted, as previously stated, to prepare only pure materials for testing. However, we have prepared a number of compounds as mixtures of isomers that we were unable to separate. The mixtures *per se* were of sufficient purity, but mixtures are by definition not

Table 3 cont.

Structure.	Label and Ratio. (Z):(E) cis:trans endo:exo	Odour Quality; Neat (on Smoking in Cigarette).	Threshold. (p.p.m.)
	164:83 91:9	Sweet, fruity, animal. (Cheesy, sour, sweet).	70
	164:83 0:100	Sweet, animal, cheesy. (Cheesy, balsamic).	5
	166	Weak, animal.	>2000
	163	Weak, animal, horse-like.	>2000
	(Commercial >98%)	Weak, fruity, animal.	>3000
	161:162 78:22	Weak, herbaceous.	-

* For experimental details, see p. 89.

The Odour Quality on Smoking in Cigarette is only given if different from Odour Quality Neat.

Table 3 cont.-

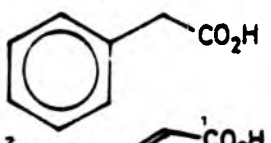
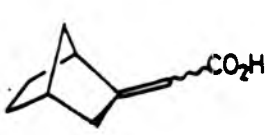
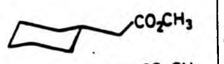
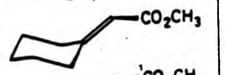
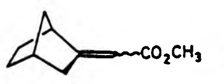
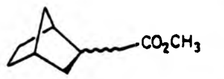
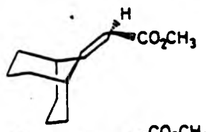
Structure.	Label and Ratio. (Z):(E) cis:trans endo:exo		Odour Quality; Neat (on Smoking in Cigarette).	Threshold. (p.p.m.)
	76		Sweet, honey, dried fruit. (Cheesy, "Friars Balsam" note).	800
	131		Sweet, animal. (Weak, flattening).	2000
	149		Sweet, animal. (Honey, fruity, animal).	100
	150		Sweaty, sweet, animal. (Sour, sweet, cheesy).	750
	82		Sweet, heavy, honey. (Sweet, honey, animal).	100
 6-CH_3	136		Sweet, animal. (Sweet, cheesy, woody).	2000
5-,7-CH_3	140:141	81:19	Sweet, fruity, weak.	>2000
	140:141	13:87	Sweet, mentholic, weak.	>2000
4-,8-CH_3	148:147	91:9	Sweet, sweaty, fruity.	>2000
	148:147	4:96	Sweet, animal, weak.	>2000
 6-CH_3	85:86	58:42	Sweaty, animal.	>2000
	85:86	79:21	Sweaty, animal.	>2000
5-CH_3	157:158	36:64	Weak, sweet, cheesy.	>2000
	157:158	80:20	Weak, sweet, cheesy.	>2000
4-CH_3	89:90	62:38	Weak, sweet, fruity. (Sweet, cheesy).	2500
	89:90	87:13	Weak, sweet, fruity, animal. (Sweet, cheesy).	750
	175:174	91:9	Sweet, fruity, animal.	>2000
	175:174	4:96	Sweet, fruity, horse-like. (Weak, cheesy).	2000

Table 3.*

Structure.	Label and Ratio. (Z):(E) cis:trans endo:exo		Odour Quality; Neat (on Smoking in Cigarette).	Threshold. (p.p.m.)
	156		Sweet, fruity, ester-like. (Dried fruit, cheesy).	70
	132		Sweet, fruity, ester-like. (Cheesy, sweet, fruity).	50
 6-CH_3	134		Sweet, fruity, ester-like.	> 1000
5-,7-CH_3	137:138	85:15	Sweet, fruity, ester-like.	-
	137:138	20:80	Sweet, fruity, ester-like.	-
	137:138	50:50	Sweet, fruity, ester-like.	500
	135		Sweet, fruity, ester-like.	> 500
 6-CH_3	152:153	60:40	Sweet, fruity, ester-like.	1000
5-CH_3	154:87	39:61	Sweet, fruity, ester-like.	>1000
4-CH_3	155:88	62:38	Sweet, fruity, ester-like. (Sour, dried fruit, cheesy).	100
	172:171	91:9	Sweet, fruity, ester-like. (Sweet, sickly, cheesy).	150
	172:171	5:95	Sweet, fruity, ester-like. (Sweet, sickly, cheesy).	100
	176:178	91:9	Sweet, fruity, ester-like. (Cheesy, hint of dried fruit).	35
	176:178	0:100	Sweet, fruity, ester-like. (Cheesy, diluting to dried fruit).	35
	165		Weak, paraffin oil-like. (Eucalyptus, mentholic, sweet, camphoraceous).	700
	168		Weak, eucalyptus or mentholic note. (Weak, woody, eucalyptus).	200

single compounds.

With respect to any conclusions that we could infer from the assessments of these compound mixtures, we required initially a mathematical method that enabled us to extrapolate to approximate values for the threshold detection concentrations of pure components, in the mixture of two isomeric compounds.

For our purposes, it was assumed that in a mixture of compounds, the threshold can be considered to be related to the thresholds of the individual components by the expression shown in diagram 56. The expression is essentially a linear relationship in which components are assumed to affect thresholds as a sum. For a two component mixture of '1' and '2', the equation simplifies to that shown in diagram 57. The assumptions made by this treatise are that the compounds concerned are interacting with, and only with, the same receptor site, that the compounds have no significant interaction with each other and that a linear relationship is appropriate. As a first approximation, the equation can provide estimated threshold values from mixtures of compounds, for their individual components. In the case of a two component mixture, the individual thresholds can only be extrapolated if at least two different combinations are assessed, so that the simultaneous equations obtained can be solved. The accuracy of the result depends on the certainty of the individual values. Also, it is clear that the larger the range of values to which a line is fitted and the broader the spread of the different ratios, the more accurate the extrapolation will be.

The acids that we have prepared have all proved to be of higher threshold than the potent commercially available acid 83, when tested on a cigarette. Table 3 shows the threshold values obtained for the products, experimentally prepared either pure or as mixtures of

$$\frac{1}{T_{1,2}} = \frac{K_1}{T_1} + \frac{K_2}{T_2}$$

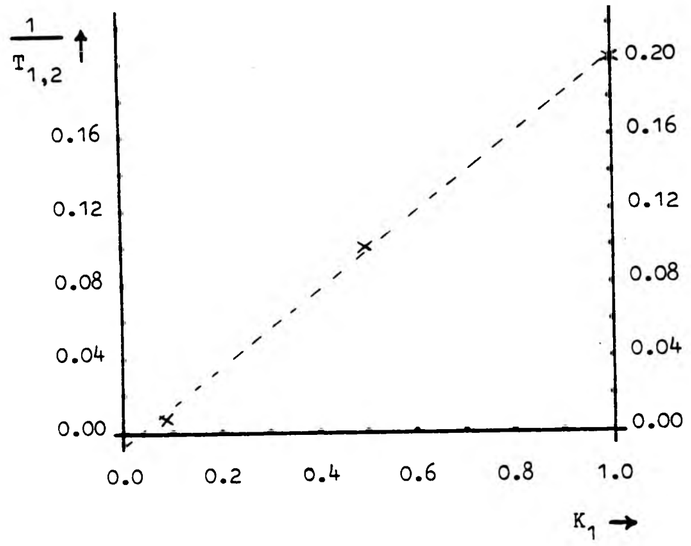
Let component 1 be 83 and 2 be 164.

When K_1=0.09 and K_2=0.91, $T_{1,2}$=70p.p.m.

and when K_1=1.00 and K_2=0.00, $T_{1,2}=T_1$=5p.p.m.

$\Rightarrow \quad T_2 \div -245$p.p.m.

Diagram 58.



Least squares fit ⇒

$$\frac{1}{T_{1,2}} = 0.204K_1 - 0.003$$

Diagram 59.

isomers. The threshold values for individual components in a mixture was inferred by the linear analysis detailed previously and shown in diagram 57. We have not considered enantiomers as separate compounds, although this would be appropriate if separation had been effected.

One result that was seemingly not consistent with this simplistic approach was the observed threshold values for the 2-norbornylacetic acids. The exo-isomer 83 had a threshold value determined as about 5 p.p.m. whereas that the of 91% endo-isomer 164 was about 70 p.p.m. This result is clearly at odds with the linear approach for if the contribution from the endo-isomer was negligible, the threshold value of the 91:9 endo:exo mixture would be expected to be about 50 p.p.m. The value of 70 p.p.m. implies a threshold value for the pure endo-isomer of ~-245 p.p.m., as can be seen in diagram 58. If these results are accurate, then the endo-isomer must be an antagonist of exo-isomer-receptor binding. However, the error margins on threshold value assessments are probably at least ±20%. Taking the closest value limit within this range, T_1 = 6 p.p.m. and $T_{1,2}$ = 56 p.p.m., which implies a T_2 value, which is that of the endo-isomer 164, of ~320 p.p.m. We also assessed the threshold value of a 50:50 mixture of 83:164, which was 10 p.p.m. With three points, we derived the threshold of the pure endo-isomer 164 using least squares fitting of a straight line to the points. The system can be considered graphically, as shown in diagram 59. The intersect of the 'y' axis provides the value of T_1^{-1}, which proved to be negative once again, implying a threshold greater than infinity, which is absurd. In practice, it is probably appropriate to say that the threshold value of 164 is >70 p.p.m. and that 164 is possibly an antagonist of 83 binding.

The other pair of compound mixtures of specific interest are those made up of 89 and 90. Solving the simultaneous equations, after

substitution of the known variables by the two sets of data, gives threshold values for pure 89 and 90 equal to about 550 p.p.m. and -520 p.p.m. respectively. For the threshold value of 90 to attain a positive value, using the error limits approach, would imply a ~40% deviation from the experimentally observed values. An alternative interpretation is that there is some antagonistic effect of 90 on the receptor binding of 89.

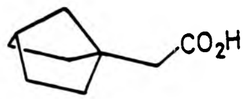
With regard to the other acid mixtures assessed by us, thresholds for these were invariably >2000 p.p.m. Based on these values and assuming a linear relationship without antagonism, the thresholds of the individual components must be >800 p.p.m.

If the observed antagonism is a real effect and not an artifact from experimental errors, how could this effect be rationalised in the receptor-ligand system? A factor that must initially be considered is whether or not the ratio of the mixture of compounds at the receptor is equivalent to the ratio loaded onto a cigarette. Clearly, if there is considerable variation in factors, such as partition coefficients, transfer coefficients or rates of metabolism, between the components of the mixture, then there could be a significant change in the effective component ratio at the receptor and, hence in the threshold perception. Also, it is assumed that the perception of an aroma effect reflects the binding of the components odorant ligands at the receptor site. Evidently, if ligand binding can occur without effect perception, antagonism can result. This is similar to the reversible inhibition concept utilised in enzymology,[171] and is an extension of the response-binding and no response-binding concept noted by Dodd.[170] Further experiments are needed in order to clarify the actual situation.

There are certain general trends that can be extracted from the threshold detection data of the monocyclic systems. Ring substitution

of methyl in the 5- or 6- position causes a probable increase in threshold values compared to that of the unsubstituted acid 76. The α,ß-unsaturated acids with ring methyl substitution or no substitution increased the threshold. The threshold was decreased slightly by the introduction of unsaturation in the 3,4- or 4,5- position, as shown by the values for 149 and 150. Likewise, a fully unsaturated ring gave a ten fold decrease in threshold as seen in 82. The cis-4-methyl substituted acid 152 had a decreased threshold inferred to be in the range 500-700 p.p.m. It would seem that 6-, 5- or trans-4- ring methyl substitution and α,ß-unsaturation are disfavoured from a threshold point of view in the cyclohexylacetic acid analogues. Ring unsaturation, on the other hand, apparently lowers the threshold value.

It is somewhat difficult to draw conclusions from the threshold trends of the monocyclic acids, which seem to imply that the receptor system prefers a limited size in the hydrocarbon part of the molecule, tolerating and possibly preferring cis-4-substitution or ring unsaturation. The problem is complicated by the fact that the quality of the aromas of the acids was not consistent. These molecules seem to be capable of causing, more or less, stimulation of receptor systems interpreted centrally as sweet, sweaty, animal, fruity, honey and/or cheesy. It may be that some of these interpretations are related, such as the sweet and honey aspects, or that the aromas do not fit neatly into a definite descriptive class, but the most significant observation is that only in 76 was a dried fruit-like aroma definitely observed. Also the tastes on smoking were not consistent, although a cheesy note was generally observed. Again, a dried fruit-like taste was not observed, except in cyclohexyl acetic acid, 76, itself. A dried fruit note was observed in the aroma of the certain compounds, when assessed neat, by a minority of the flavour panel. Three out of the six panelists noted a



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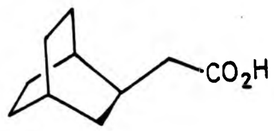

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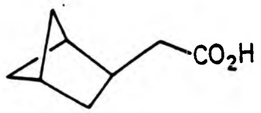

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dried fruit aspect in the profile of the 87:13 mixture of 89:90. Two out of six panelists described the same aspect in the 62:38 mixture of 89:90 and the 20:80 mixture of 157:158. One of the panelists remarked on a similar aspect in enriched 131, 147 and 149.

The multicyclic acids were generally of threshold >2000 p.p.m. The exceptions, as highlighted above, were the 2-norbornylacetic acids. It would seem that from the quite low threshold value of the exo-isomer 83, that this material has a high affinity for some kind of cheesy odorant receptor, but evidently more work is required to establish this. Potentially interesting compounds in this respect would be the bicyclic acids 179, 180 and 181.

One interesting result from the cigarette testing was the dried fruit-like aroma produced by certain compounds when loaded onto a cigarette. The effect was noted with 76, the mixtures of 89/90 and the bicyclic acids 83 and enriched 164 and was particularly powerful for the latter two compounds. Further investigation of this effect could possibly provide useful flavourings for tobacco, with compounds such as 179, 180 and 181 a logical choice in a structure-activity study.

Against the rather varied quality profiles of the acids tested by us, all the methyl esters of these acids, with the exception of the [3,3,1] bicyclic esters 165 and 168, were sweet, estery and generally fruity in aroma. Unfortunately, full data on all the structural isomers was not forthcoming, usually due to difficulties in preparing the esters of sufficient purity. We can summarise, from the data we do have that 6-methyl substitution of the saturated, α,β- or β,γ-unsaturated methyl esters 134, 135 and 152/153 appeared to substantially increase the threshold detection values compared to the unsubstituted esters 132 and 156. The same can be inferred for the 137/138 and 87/154 mixtures. The 4-methyl substituted mixture 88/155 was of comparable threshold, as

were the enriched α,ß-unsaturated bicyclic compounds 171 and 172. Using the linear relationship used in diagram 57, the extrapolated thresholds for pure compounds 171 and 172 are 152 p.p.m. and 97 p.p.m. respectively. The saturated analogues of these, 176 and 178 were of the low threshold 35 p.p.m. The data support a fairly non-specific receptor showing preference for compounds without steric demands from substitution on the C-5, C-6 or C-7 carbons, as reflected by threshold values. Further data would be required before more specific receptor requirements could be deduced. It is interesting to note that the claimed threshold detection value of ethyl acrylate, using more conventional assessment techniques, was reported as 3×10^{-4} p.p.m.[172]

On smoking, the taste of those esters whose threshold values were determined, was generally sweet and cheesy. The cheesy effect may imply that the cheese-like receptor, postulated for the acids previously, may not be functionality specific. Another aroma aspect associated with these compounds was a dried fruit-like aspect. Compounds 88/155, 156, 178 and enriched 176 all possessed this aspect and it should be noted that these compounds are of relatively low threshold, and that the dried fruit note seemed to become more important at lower concentrations. It would seem that if a flavourant is desired that gives a dried fruit aspect to the taste of tobacco smoke, an ester analogous to these might prove appropriate. Evidently, more research is required in order to fully investigate the potential of such compounds.

EXPERIMENTAL

General

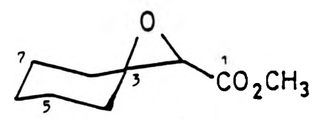
Commercial reagents were used as supplied, unless otherwise stated. Suppliers were well known companies such as the Aldrich Chemical Company, Fluka A.G. and B.D.H. Ltd. Trivial names of reagents are used, if well known. Otherwise I.U.P.A.C. nomenclature[36] is employed. All solvents were purified by conventional procedures[174] prior to use. Solutions were dried using dried magnesium sulphate unless otherwise stated. The silica used for columns and filtrations was Merck type 9385. Stirring of reactions was achieved by using teflon coated magnetic fleas and an external magnetic stirrer. All reactions were performed under a positive pressure of dry nitrogen. The work up of reactions was achieved at ambient temperature in a laboratory atmosphere, unless otherwise stated. Solvent evaporations were accomplished using a Büchi rotary evaporator at reduced pressure, unless otherwise indicated. Product yields are given based on starting material, not isolated unreacted in the work up, as giving 100% yield. When samples of crude material were purified, the yield given has been extrapolated to the total amount of crude material. Relative proportions of mixtures are taken from gas chromatographic data unless otherwise stated.

Melting points (m.p.) were determined on a Koeffler hot-stage apparatus and are uncorrected. Bulb to bulb distillations were achieved by use of a Büchi G.K.R.-50, suitably equipped. Spinning band distillations were effected with a Nester Faust apparatus. Column chromatography was achieved with glass columns packed with commercial Merck type 9385 silica gel and by using the flash method.[47] Radial chromatography was achieved with commercial Merck type 7749 (PF254)

coated glass plates on a Chromatotron model 7924 obtained from Harrison Research, California, U.S.A. Loading was either by loop injection, involving the use of variable sized loops on a type 50 Rheodyne teflon sample injection type rotary valve with minimal dead volume, or by using the in-line dry loading technique. The solvent was a mixture of methanol, ethyl acetate and hexane of appropriate polarity and was generally gravity fed. Thin layer chromatography (t.l.c.) utilised commercially obtained Merck type 5735 silica plates and relative mobility information is given as R_F values. Visualisation was generally achieved by use of ultraviolet irradiation at 254 nm, by exposure to iodine and/or by spraying the plate with a ceric ammonium sulphate/sulphuric acid mixture followed by treatment with heat. Other visualisation reagents are mentioned by name. T.l.c. data are presented in the format R_F (solvent system) = value. Certain compounds, such as the methyl substituted bromocyclohexanes and the cyclohexylacetic acid esters, were not observed by t.l.c. using various visualisation reagents.[174] Gas chromatographic (g.c.) analysis was achieved by use of a Dani HR6800 gas chromatograph fitted with a 25m. bonded phase capillary column of type OVI, SE54 or SP1000. The injection port and flame ionisation detector temperatures were set at 150°C and the carrier gas was either nitrogen or helium. No calibration was attempted. Retention times (R_T) are given in minutes after injection. Integration values were assessed by a Pye Unicam PU4810 computing integrator or by taking relative mass measurements of the cut out peaks. Samples were injected as solutions, the solvent peak being ignored in the analysis. G.c. data are presented in the format R_T (column type, solvent, column temperature) = retention time. Abbreviations used for solvents, not corresponding to formulae, are E.A. (ethyl acetate) and P.E. (hexane). Sublimations were accomplished

using the appropriate apparatus on a Büchi G.K.R.-50. For sublimations noted as 'onto a cold finger', a dry ice/acetone or liquid nitrogen cold finger was employed, enabling liquids to be sublimed. Mass spectra (m.s.) were obtained by use of a Jeol J.M.S.-D100 instrument.[175] The eight most abundant fragments are listed in order of mass. Certain ions were accurately measured and presented in the format $M^{+\cdot}$ (molecular mass) = formula. Infrared spectra (i.r.) were obtained on a Perkin-Elmer 577 grating i.r. spectrometer as thin films between polished sodium chloride plates or as solutions in sodium chloride cells against a reference cell. The 1603 cm^{-1} polystyrene peak was used to calibrate the instrument and i.r. values are given to the nearest 5 cm^{-1}. The eight most absorbant bands are listed in order of wave number. M.s. or i.r. peaks of diagnostic interest, but not apparent in these listings, are presented in brackets afterwards. Nuclear magnetic resonance (n.m.r.) spectra were obtained from samples dissolved in deuterochloroform, using tetramethylsilane as an internal standard, and were measured at ambient temperature unless otherwise stated. The ^{1}H n.m.r. spectra were obtained on a Perkin-Elmer R32 instrument running at 90 MHz, unless otherwise stated. The ^{13}C n.m.r. spectra[175] were obtained on a Bruker WP80 Fourier transform spectrometer running at 20.15 MHz. The data are presented as the chemical shifts from the broad band spectra and the multiplicity observed in the off-resonance decoupled spectra. To avoid confusion, ^{1}H n.m.r. sata are presented using δ units, whereas ^{13}C n.m.r. data are presented using p.p.m. units, both downfield from tetramethylsilane.

Odour assessments were made using a panel of generally six people. Compounds were assessed neat and the descriptions utilised were noted by a majority of the panel. Descriptions were generalised where appropriate, such that "old socks" or "sweaty feet" would be classed as



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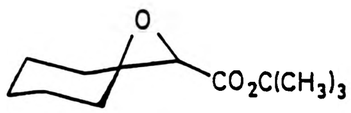

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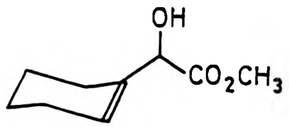


8

sweaty. The thresholds and the tastes of test compounds, when smoked on a cigarette, were assessed by Dr. R.C. Anderson. Cigarettes, which were evenly loaded with a sample of the test compound dissolved in ethanol, were provided with a control, which was laced with neat ethanol. The assessor did not know which was the control before testing the cigarettes. Before testing, the cigarettes were allowed to equilibrate in separate and sealed glass vessels for at least twelve hours. The cigarettes used were fresh Silk Cut king size[176] and pairs of cigarettes always came from the same packet. The detection threshold values obtained assume that such a cigarette contains about one gram of tobacco and the values are given as parts per million (p.p.m.), on a weight to weight basis. One milligram of test compound loaded on a cigarette would therefore correspond to one thousand p.p.m. It should be noted that this value does not represent a molar value. Also, the assumption that a cigarette contains one gram of tobacco is inaccurate; the average mass is closer to eight hundred milligrams. The threshold assessments by Dr. Anderson ignored nose effects. If a compound could be detected on a cigarette prior to lighting, the lit cigarettes would be mixed up in order to ensure unbiased assessment. Test cigarettes were assessed by drawing smoke into the mouth, without inhalation. The purity of the compounds synthesised was considered most important for reasons already discussed. We attempted to produce material that was pure by t.l.c., gave no sign of impurity in the spectral data and was >99.8% pure by g.c.

Preparation of methyl 2-(1-oxaspiro[2,5]octyl) carboxylate, 6.[31,32]

a) Using potassium t-butoxide in tetrahydrofuran

Cyclohexanone (7.25g., 74 mmol.) and methyl chloroacetate (9.10g., 84 mmol.) were dissolved in tetrahydrofuran (40ml.) at 0°C. Potassium t-butoxide (8.20g., 80 mmol.) was added as a solution in tetrahydrofuran

(50 ml.) dropwise over 1h. The reaction mixture was stirred overnight at ambient temperature. The solvent was evaporated. Water (30ml.) and chloroform (90ml.) were added and the separated organic phase was washed with water (2 x 30ml.), saturated aqueous sodium chloride (30ml.) and saturated aqueous sodium carbonate (30ml.). Drying, filtration and evaporation of the solvent gave the impure product (12.0g., 96%). Bulb to bulb distillation and silica chromatography of a sample of this material gave a low yield of the product, which after a further distillation, was obtained as a pure colourless oil, 6 (200mg., 12%).

R_F (E.A. 0.1, P.E. 0.9) = 0.52.

i.r. (film):- 2940, 2860, 1760, 1735, 1445, 1205, 1190, 670 cm^{-1}.

^1H n.m.r.[177]:- 3.70 (3H, s), 3.25 (1H, s), 2.0-1.3 (10H, m)δ.

A similar purification procedure gave t-butyl 2-(1-oxaspiro-[2,5]-octyl)-carboxylate, 7, contaminated heavily with 6, but suggesting that the t-butyl ester was present in the volatile product fraction to the extent of about 5%.

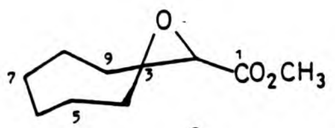
R_F (E.A. 0.1, P.E. 0.9) = 0.55.

^1H n.m.r.:- 3.08 (1H, s), 2.0-1.1 (10H,m), 1.45 (9H,s)δ.

Likewise a sample of 8 contaminated with 6 was isolated. The spectral properties for 8 were the same as those obtained from a sample of 8 derived in the following fashion.[13] A sample of the crude methyl glycidate 6 (1.0g., 5.9mmol.) was stirred in dry toluene (3ml.) at ambient temperature. Lithium perchlorate trihydrate (160mg., 1.0mmol.) was added and the reaction mixture was heated at 90-100°C for 1h. Evaporation of the solvent and distillation provided methyl hydroxy-(1-cyclohexenyl)acetate 8 (1.0g., 100%), slightly impure, as an oil.

R_F (E.A. 0.1, P.E. 0.9) = 0.24.

i.r. (film):- 2940, 1740, 1440, 1260, 1215, 1140, 1090, 1070 cm^{-1} (3600-3100 cm^{-1}).



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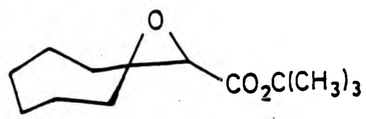

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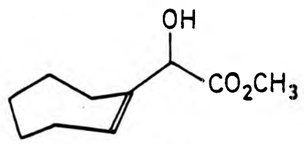

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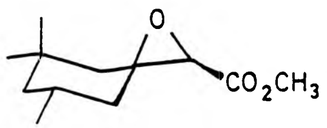

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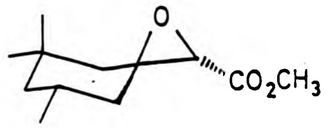

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^{1}H n.m.r.:- 5.75 (1H, m), 4.43 (1H, s), 3.72 (3H,s), 2.3-1.4 (8H, m)δ.

On storage, 6 slowly transformed into 8 at ambient temperature. Refluxing in tetrahydrofuran made the rate of rearrangement somewhat faster, but not nearly as rapid as the lithium perchlorate catalysed reaction.

b) Using potassium t-butoxide in t-butanol.

Cyclohexanone (7.25g., 74 mmol.) and methyl chloroacetate (9.10g., 84 mmol.) were mixed together. Potassium t-butoxide (8.30g., 74 mmol.) dissolved in t-butanol (62.5ml.) was added dropwise over 1.25h. at below 10°C. In order to retain mobility, diethyl ether (15ml.) was added and the system was stirred for 2h. at ambient temperature. The solvents were evaporated and diethyl ether (500ml.) and water (200ml.) were added. The separated ethereal phase was washed with saturated aqueous sodium chloride, dried, filtered and the solvent evaporated. Bulb to bulb distillation yielded the crude product (7.1g., 53%) contaminated with 7 and 8.

Preparation of methyl 2-(1-oxaspiro[2,6]nonyl)carboxylate, 9.[32]

Cycloheptanone (16.80g., 150mmol.) and methyl chloroacetate (16.27g., 150mmol.) were dissolved in tetrahydrofuran (80ml.). Potassium t-butoxide (18.5g., 165mmol.) in tetrahydrofuran (100ml.) was added dropwise over 1.5h at 0°C. The system was allowed to reach ambient temperature and was stirred for 80h. The solvent was evaporated from the dark brown solution and chloroform (150ml.) and water (150ml.) was added. The separated organic phase was washed with water (2x150ml.), saturated aqueous sodium chloride (150ml.) and saturated aqueous sodium carbonate (150ml.). After drying and filtering, the solvent was evaporated to give an orange oil. Bulb to bulb distillation gave the crude product 9 (23.80g., 86%). Silica chromatography of a

portion (3.0g.) of this crude material yielded a sample of 9 which, after distillation, was a colourless oil (2.07g., 60%).

R_F (E.A. 0.1, P.E. 0.9) = 0.32.

i.r.(film):- 2940, 2860, 1760, 1735, 1445, 1210, 1025, 670 cm^{-1}.

^1H n.m.r.:- 3.82 (3H, s), 3.38 (1H, s), 2.1-1.2 (12H, m)δ.

Similarly, a slightly impure sample of t-butyl 2-(1-oxaspiro-[2,6]nonyl)carboxylate, 10, was obtained as a colourless oil (180mg., 4%).

R_F (E.A. 0.1, P.E. 0.9) = 0.37.

i.r.(film):- 2980, 2930, 1750, 1725, 1370, 1225, 1160 cm^{-1}.

^1H n.m.r.[177]:- 3.20 (1H, s), 2.3-1.3 (12H,m), 1.50 (9H, s)δ.

Similarly, a slightly impure sample of methyl hydroxy(1-cycloheptenyl)acetate, 11, was obtained as an oil (40mg., 1%).

R_F (E.A. 0.1, P.E. 0.9) = 0.16.

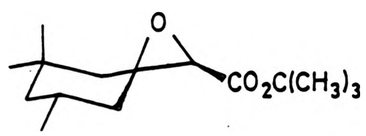
i.r.(film):- 2930, 2860, 1740, 1445, 1260, 1215, 1105, 1085 cm^{-1} (3600-3100 cm^{-1}).

^1H n.m.r.[177]:- 6.00 (1H, t, 7 Hz.), 4.52 (1H, s), 3.81 (3H, s), 2.4-2.0 (4H,m), 2.0-1.2 (6H, m)δ.

On storage at ambient temperature, 9 was found to rearrange slowly to 11. This rearrangement was catalysed by lithium perchlorate.[13] Thus, a sample of 9 (500mg., 2.7mmol.) was stirred at 90°C in toluene (3ml.) with lithium perchlorate trihydrate (50mg., 0.31mmol.) for 12h. The product (480mg., 96%) was obtained by evaporation of the solvent, followed by bulb to bulb distillation and was identical to the sample of 11 isolated previously.

Preparation of methyl (2R*, 3R*, 7S*)-5,5,7-trimethyl-2-(1-oxaspiro[2,5]-octyl)carboxylate, 25, and methyl (2R*, 3S*, 7R*)-5,5,7-trimethyl-2-(1-oxaspiro[2.5]octyl)carboxylate, 24.

3.3.5-Trimethylcyclohexanone (21.00g., 150mmol.) and methyl



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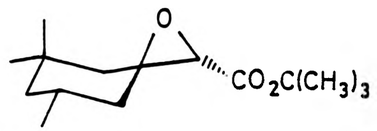

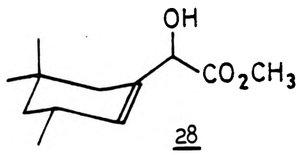

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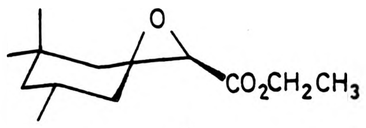

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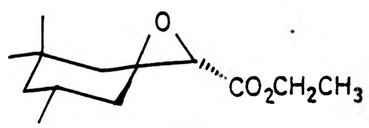

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chloroacetate (16.19g., 150mmol.) was dissolved in tetrahydrofuran (80ml.). Potassium t-butoxide (16.8g., 150mmol.) was added dropwise over 1h. at 0°C. After stirring overnight at ambient temperature, the solvent was evaporated from the brown solution. Chloroform (150ml.) and water (150ml.) were added and the separated organic phase was washed with water (2x150ml.), saturated aqueous sodium chloride (3x150ml.) and saturated aqueous sodium carbonate (3x150ml.). After drying and filtering, the solvent was evaporated to give the crude product as a yellow oil (33g.). A portion was purified by bulb to bulb distillation and silica chromatography. A sample of 24 and 25 (2.12g., 60%) was thus obtained as a colourless oil and of product ratio 50:50.

R_F (E.A. 0.1, P.E. 0.9) = 0.38.

R_T (OV1, Et_2O, 150°C) = 4.1 (50%), 3.9 (50%)m.

Likewise, t-butyl 5,5,7-trimethyl-2-(1-oxaspiro[2,5]octyl)-carboxylate, 18,[16] was isolated pure as a colourless oil (250mg., 7%) and of isomeric ratio 50:50, (2R*, 3S*, 7R*):(2R*, 3R*, 7S*).

R_F (E.A. 0.1, P.E. 0.9) = 0.54.

R_T (OV1, Et_2O, 150°C) = 6.6 (50%), 6.2 (50%)m.

i.r.(film):- 2980, 2960, 2940, 2920, 1750, 1370, 1160 cm^{-1}. (1725 cm^{-1}).

^1H n.m.r.[38,177]:- 3.08 (1H, s), 2.2-1.2 (7H, m), 1.50 (9H, s), 1.02 (3H, s), 0.92 (3H, s), 0.86 (3H, d, 6Hz.) δ.

^{13}C n.m.r.:- (2R*, 3R*, 7S*)167.3 (s), 81.9 (s), 62.9 (s), 57.3 (d), 48.1 (t), 42.5 (t), 39.5 (t), 33.0 (q), 32.4 (s), 26.3 (d), 26.1 (q), 22.3 (q), 13.5 (q) p.p.m.

(2R*, 3S*, 7R*) 167.3 (s), 81.9 (s), 62.9 (s), 57.5 (d), 48.1 (t), 46.1 (t), 35.9 (t), 33.0 (q), 33.0 (s), 26.3 (d), 26.1 (q), 22.3 (q), 13.5 (q) p.p.m.

Similarly, methyl hydroxy(3,5,5-trimethyl-1-cyclohexenyl) acetate,

28, was isolated pure as a mixture of isomers and as a colourless oil (110mg., 3%).

R_F (E.A. 0.1, P.E. 0.9) = 0.23.

i.r. film:- 2960, 2905, 2870, 1740, 1460, 1260, 1215, 1080 cm^{-1}. (3600-3100 cm^{-1}).

^1H n.m.r.[177]:- 5.53 (1H, d, 10Hz.), 4.47 (1H, s), 3.77 (3H, s), 2.4-1.2 (5H, m), 1.1-0.8 (9H, m)δ.

The remainder of the crude product was subjected to bulb distillation, spinning band distillation and radial chromatography. In this fashion, a pure sample of each isomer was obtained from the 24/25 mixture. Thus, 24 was isolated as a colourless oil (260 mg.).

R_F (E.A. 0.1, P.E. 0.9) = 0.38.

R_T (OV1, Et_2O, 150°C) = 4.1m.

i.r. (film):- 2960, 2930, 2910, 1760, 1740, 1445, 1210, 1190 cm^{-1}.

m.s.:- m/e = 155 (100%), 123 (37), 109 (43), 83 (78), 81 (43), 67 (53), 55 (52), 41 (84), M^+(212) = $C_{12}H_{20}O_3$. (m/e = 153 (32%)).

^1H n.m.r.[177]:- 3.78 (3H, s), 3.22 (1H, s), 2.1-1.2 (7H, m), 1.03 (3H, s), 0.95 (3H, s), 0.90 (3H, d, 6Hz.)δ.

^{13}C n.m.r.:- 168.8 (s), 63.5 (s), 57.1 (d), 52.0 (q), 47.9 (t), 46.0 (t), 36.1 (t), 32.8 (q), 32.8 (s), 26.3 (d), 25.9 (q), 22.3 (q) p.p.m.

Likewise, a sample of 25 was obtained as a colourless oil (250mg).

R_F (E.A. 0.1, P.E. 0.9) = 0.38.

R_T (OV1, Et_2O, 150°C) = 3.9m.

i.r. (film):- 2960, 2910, 1760, 1740, 1460, 1445, 1210, 1190 cm^{-1}.

m.s.:- m/e = 155 (100%), 123 (29), 109 (25), 83 (52), 81 (25), 67 (27), 55 (29), 41 (34). M^+(212) = $C_{12}H_{20}O_3$. (m/e = 153 (21%)).

^{1}H n.m.r.:- 3.78 (3H, s), 3.20 (1H, s), 2.1-1.2 (7H, m), 1.07 (3H, s), 0.96 (3H, s), 0.90 (3H, d, 6 Hz.)δ.

^{13}C n.m.r.:- 168.7 (s), 63.4 (s), 56.9 (d), 51.9 (q), 48.0 (t), 42.4 (t), 39.8 (t), 32.9 (q), 32.4 (s), 26.4 (d), 26.0 (q), 22.3 (q) p.p.m.

Also obtained was a sample of (2R*, 3S*, 7S*) enriched 18, which enabled the unequivocal assignment of ^{13}C n.m.r. data.

Preparation of ethyl (2R*, 3R*, 7S*)-5,5,7-trimethyl-2-(1-oxaspiro[2,5]octyl)carboxylate, 15, and enriched ethyl (2R*, 3S*, 7R*)-5,5,7-trimethyl-2-(1-oxaspiro[2,5]octyl)-carboxylate, 14.[33]

3,3,5-Trimethylcyclohexanone (21.0g., 150mmol.) and ethyl chloroacetate (18.4g., 150mmol.) were dissolved in tetrahydrofuran (80ml.). Potassium t-butoxide (16.8g., 150mmol.) in tetrahydrofuran (100ml.) was added dropwise over 1.5h. at 0°C, and then the reaction mixture was stirred overnight at ambient temperature. The solvent was evaporated to give an orange oil. Chloroform (150ml.) and water (150ml.) was added. The separated organic phase was washed with water (2x150ml.), saturated aqueous sodium chloride (3x150ml.) and saturated aqueous sodium carbonate (3x150ml.). Drying and filtering gave a solution which, on evaporation of the solvent, provided an orange oil of the crude product 14/15 (28.15g., 83%). Repeated bulb to bulb distillation and silica chromatography of a portion provided pure 14/15 as a colourless oil (1.56g., 69%).

R_F (E.A. 0.1, P.E. 0.9) = 0.48.

R_T (OV1, Et_2O, 150°C) = 5.2 (50%), 4.9 (50%)m.

Likewise, a sample of ethyl hydroxy-(3,5,5-trimethyl-1-cyclohexyl)acetate, 19. was obtained as a colourless oil (50mg., 2%).

R_F (E.A. 0.1, P.E. 0.9) = 0.28

i.r. (film):- 2960, 2930, 2870, 1735, 1265, 1200, 1130, 1080 cm^{-1}. (3600-3200 cm^{-1}.).

^1H n.m.r.:- 5.56 (1H, d, 10Hz.), 4.46 (1H, s), 4.25 (2H, q, 7Hz.), 2.4-1.2 (5H, m), 1.27 (3H, t, 7Hz.), 1.1-0.8 (9H, m)δ.

Although not isolated, t-butyl 5,5,7-trimethyl-2-(1-oxaspiro[2,5]octyl)carboxylate, 18, was also detected in an isomeric ratio 50:50 via g.c. analysis by comparison with pure material (vide supra).

Bulb to bulb distillation and spinning band distillation of the crude material previously obtained, provided samples which, after radial chromatography, were >99.8% pure and isomerically enriched. Hence, 15 was obtained chemically and isomerically pure as a colourless oil (40mg.).

R_F (E.A. 0.1, P.E. 0.9) = 0.48.

R_T (SE54, Et_2O, 150°C) = 10.3m.

R_T (OV1, Et_2O, 150°C) = 4.9m.

i.r. (film):- 2960, 2910, 2870, 1755, 1730, 1455, 1195, 1035 cm^{-1}.

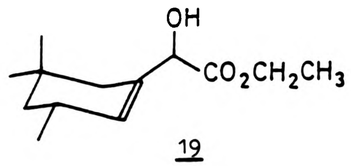
m.s.:- m/e = 169 (100%), 123 (38), 109 (44), 95 (38), 83 (79), 67 (40), 55 (40), 41 (58). M^{+}(226) = $C_{13}H_{22}O_3$. (m/e = 153 (30%)).

^1H n.m.r.:- 4.23 (2H, q, 7Hz.), 3.19 (1H, s), 2.3-1.2 (7H,m), 1.30 (3H, t, 7Hz.), 1.08 (3H, s), 0.94 (3H, s), 0.91 (3H, d, 6Hz.)δ.

^{13}C n.m.r.:- 168.1 (s), 63.4 (s), 61.3 (t), 56.9 (d), 47.9 (t), 42.3 (t), 39.6 (t), 32.9 (q), 32.3 (s), 26.3 (d), 26.1 (q), 22.3 (q), 14.3 (q) p.p.m.

Similarly, an enriched sample of 14 was obtained as a colourless oil (470mg.) containing 17% 15.

R_F (E.A. 0.1, P.E. 0.9) = 0.48.



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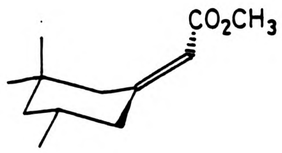

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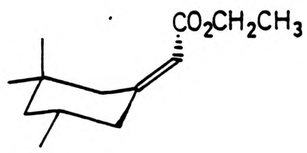


R_T (SE54, Et_2O, 150°C) = 11.2 (83%), 10.3 (17%)m.

R_T (OV1, Et_2O, 150°C) = 5.2 (83%), 4.9 (17%)m.

i.r. (film):- 2960, 2910, 2870, 1755, 1730, 1280, 1195, 1035 cm^{-1}.

m.s.:- m/e = 169 (100%), 123 (36), 109 (37), 95 (35), 83 (70), 67 (33), 55 (35), 41 (49). M^{+}(226) = $C_{13}H_{22}O_3$. (m/e = 153 (30%)).

^1H n.m.r.:- 4.23 (2H, q, 7Hz.), 3.19 (1H, s), 2.2-1.2 (7H, m), 1.29 (3H, t, 7Hz.), 1.04 (3H, s), 0.94 (3H, s), 0.91 (3H, d, 6Hz.)δ.

^{13}C n.m.r.:- (14) 168.1 (s), 63.3 (s), 61.1 (t), 57.1 (d), 47.8 (t), 45.8 (t), 35.9 (t), 32.8 (q), 32.8 (s), 26.3 (d), 25.9 (q), 22.3 (q), 14.3 (q) p.p.m.

The hydroxy esters 19 were also obtained by the lithium perchlorate catalysed rearrangement[13] of 14/15. Hence, a 50:50 mixture of 14 and 15 (160mg., 0.7mmol.) and lithium perchlorate trihydrate (30mg., 0.02mmol.) were heated in toluene (3ml.) overnight at 90°C. Evaporation of the solvent and bulb to bulb distillation gave a slightly impure sample of 19 identified by comparison of chromatographic and spectral data with the sample isolated previously.

Preparation of a mixture of (Z)-and (E)- isomers of methyl(3,3,5-trimethyl cyclohexylidene) acetate, 30, and ethyl (3,3,5-trimethyl cyclohexylidene)acetate, 29.[20]

a) Using methyl diethylphosphonoacetate.[19]

Sodium hydride as a 60% dispersion in mineral oil (3.20g., 80mmol.) was added to toluene (80ml.) with stirring. Methyl diethyl-phosphonoacetate (14.0g., 67mmol.) in toluene (10ml.) was added dropwise over 30m. causing evolution of a gas. The reaction mixture was kept below 40°C by cooling in a water bath. After 30m., 3,3,5-trimethylcyclohexanone (9.34h., 67mmol.) in toluene (10ml.) was

added dropwise over 30m. The reaction was stirred overnight at ambient temperature. After heating at 65°C for 15m., water (100ml.) was added and the organic phase was separated, dried over calcium chloride and filtered. Evaporation of the solvent provided the crude product (13.33g.) as a mixture of three components by t.l.c. and g.c. Spinning band distillation of a sample (10g.) provided unreacted 3,3,5-trimethyl cyclohexanone (3.76g.) and separated the esters (3.64g., 79%), but not their geometric isomers. Column chromatography enabled a 99.8% pure sample of the methyl esters 30 (1.39g., 30%) to be obtained as an approximately 65:35 mixture of (E):(Z) isomers and as a colourless oil.

R_F (E.A. 0.1, P.E. 0.9) = 0.78.

R_T (OV1, Et_2O, 150°C) = 2.9m.

i.r. (film):- 2960, 2930, 2910, 1725, 1650, 1440, 1215, 1160 cm^{-1}.

^1H n.m.r.:- 5.66(s) and 5.55(s) (1H), 4.0-3.6 (1H,m), 3.62 (3H, s), 2.3-1.1 (6H, m), 1.1-0.7 (9H, m)δ.

^{13}C n.m.r.:- ((E)-isomer) 167.0 (s), 161.5 (s), 114.2 (d), 50.8 (q), 50.7 (t), 48.5 (t), 37.8 (q), 34.2 (s), 32.4 (q), 30.0 (d), 25.3 (q), 22.7 (q) p.p.m.

((Z)-isomer) 167.0 (s), 161.5 (s), 114.4 (d), 50.8 (q), 48.7 (t), 46.2 (t), 42.1 (q), 34.3 (s), 32.4 (q), 30.8 (d), 25.0 (q), 22.7 (q) p.p.m.

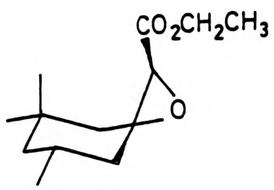
The results of an S.F.O.R.D. experiment are shown in the Results and Discussion section.

Likewise, a >99.8% pure sample of the ethyl esters 29 (1.05g., 23%) were obtained as an approximately 65:35 mixture of (E):(Z) isomers and as a colourless oil.

R_F (E.A. 0.1, P.E. 0.9) = 0.80.

R_T (OV1, Et_2O, 150°C) = 3.6m.

i.r. (film):- 2960, 2930, 2910, 1720, 1650, 1215, 1155, 1040 cm^{-1}.



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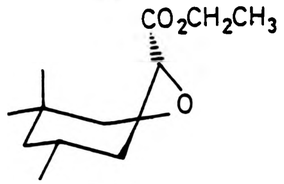

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^{1}H n.m.r.:- 5.66 (s) and 5.55 (s) (1H), 4.12 (2H, q, 7Hz.), 4.0-3.6 (1H, m), 2.3-1.1 (6H, m), 1.27 (3H, t, 7Hz.), 1.1-0.7 (9H, m)δ.

^{13}C n.m.r.:- ((E)-isomer) 166.5 (s), 161.1 (s), 114.8 (d), 59.4 (t), 50.8 (t), 48.5 (t), 37.8 (q), 34.2 (s), 32.4 (q), 29.9 (d), 25.3 (q), 22.7 (q), 14.4 (q) p.p.m. ((Z)-isomer) 166.5 (s), 161.1 (s), 114.6 (d), 59.4 (t), 48.7 (t), 46.2 (t), 42.1 (q), 34.3 (s), 32.4 (q), 30.8 (d), 24.9 (q), 22.7 (q), 14.4 (q) p.p.m.

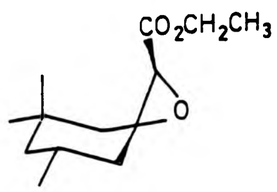
b) Using triethyl phosphonoacetate.[19]

Sodium hydride as a 60% dispersion in mineral oil (3.20g., 80mmol.) was added to toluene (80ml.). Triethylphosphonate (14.96g., 67mmol.) in toluene (10ml.) was added dropwise over 30m. A gas was evolved and the temperature of the reaction mixture controlled by cooling in a cold water bath. After stirring for 30m., 3,3,5-trimethylcyclohexanone (9.34g., 67mmol.) in toluene (10ml.) was added dropwise over 30m. After stirring for 2h., the reaction was heated to 65°C for 15m. After the reaction mixture had cooled to ambient temperature, water (150ml.) was added and the separated organic phase was dried over calcium chloride. After filtering, the solvent was evaporated. The impure product, so obtained, was vacuum distilled using a five inch Vigreaux column. After the 3,3,5-trimethylcyclohexanone (5.14g.) had distilled over, a slightly impure sample (5.75g., 91%) of the ethyl esters 29 was obtained. Its chromatographic and spectral characteristics were identical to the material obtained via method a).

Preparation of a mixture of the (2R*, 3S*, 7S*) and (2R*, 3R*, 7R*) isomers of ethyl 5,5,7-trimethyl-2-(1-oxaspiro[2,5]octyl)carboxylate, 16 and 17.

a) Using alkaline hydrogen peroxide.[24]

A sample (0.6ml.) of a solution of sodium carbonate (200mg.)



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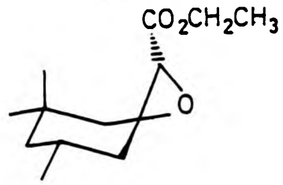

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^1H n.m.r.:- 5.66 (s) and 5.55 (s) (1H), 4.12 (2H, q, 7Hz.), 4.0-3.6 (1H, m), 2.3-1.1 (6H, m), 1.27 (3H, t, 7Hz.), 1.1-0.7 (9H, m)δ.

^{13}C n.m.r.:- ((E)-isomer) 166.5 (s), 161.1 (s), 114.8 (d), 59.4 (t), 50.8 (t), 48.5 (t), 37.8 (q), 34.2 (s), 32.4 (q), 29.9 (d), 25.3 (q), 22.7 (q), 14.4 (q) p.p.m. ((Z)-isomer) 166.5 (s), 161.1 (s), 114.6 (d), 59.4 (t), 48.7 (t), 46.2 (t), 42.1 (q), 34.3 (s), 32.4 (q), 30.8 (d), 24.9 (q), 22.7 (q), 14.4 (q) p.p.m.

b) Using triethyl phosphonoacetate.[19]

Sodium hydride as a 60% dispersion in mineral oil (3.20g., 80mmol.) was added to toluene (80ml.). Triethylphosphonate (14.96g., 67mmol.) in toluene (10ml.) was added dropwise over 30m. A gas was evolved and the temperature of the reaction mixture controlled by cooling in a cold water bath. After stirring for 30m., 3,3,5-trimethylcyclohexanone (9.34g., 67mmol.) in toluene (10ml.) was added dropwise over 30m. After stirring for 2h., the reaction was heated to 65°C for 15m. After the reaction mixture had cooled to ambient temperature, water (150ml.) was added and the separated organic phase was dried over calcium chloride. After filtering, the solvent was evaporated. The impure product, so obtained, was vacuum distilled using a five inch Vigreaux column. After the 3,3,5-trimethylcyclohexanone (5.14g.) had distilled over, a slightly impure sample (5.75g., 91%) of the ethyl esters 29 was obtained. Its chromatographic and spectral characteristics were identical to the material obtained via method a).

Preparation of a mixture of the (2R*, 3S*, 7S*) and (2R*, 3R*, 7R*) isomers of ethyl 5,5,7-trimethyl-2-(1-oxaspiro[2,5]octyl)carboxylate, 16 and 17.

a) Using alkaline hydrogen peroxide.[24]

A sample (0.6ml.) of a solution of sodium carbonate (200mg.)

dissolved in water (5ml.) was mixed with 30% hydrogen peroxide (0.2ml., 1.7mmol.). The ethyl ester mixture 29 (210mg., 1.0mmol.) in ethanol (2ml.) was added and the reaction mixture was stirred at ambient temperature overnight. T.l.c. analysis indicated no reaction. After heating at 70°C for 3h., t.l.c. and g.c. analysis of an extracted acidified sample also indicated no reaction.

b) Using hydrogen peroxide and sodium tungstate catalysis.[25]

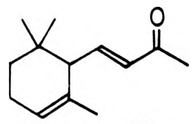
A sample (0.6ml.) of a solution of sodium carbonate (200mg.) dissolved in water (5ml.) was mixed with 30% hydrogen peroxide (0.2ml., 1.7mmol.). Sodium tungstate (30mg., 0.1mmol.) was added, followed by the ethyl ester mixture 29 (210mg., 1.0mmol.) in ethanol (2ml.). After 15d. at ambient temperature, t.l.c. and g.c. analysis of a sample, that had been acidified and extracted into an organic solvent, indicated no reaction.

c) Using m-chloroperoxybenzoic acid.[24]

A sample of the ethyl ester mixture 29 (10mg., 0.05mmol.) was stirred with m-chloroperoxybenzoic acid (10mg., 0.06mmol.) in dichloromethane (1ml.) for 10d. Analysis by t.l.c. implied no reaction.

d) Using p-nitroperoxybenzoic acid.[24]

A sample of the ethyl ester mixture 29 (1.0g., 4.8mmol.) and 85% p-nitroperoxybenzoic acid (1.15g., 5.3mmol.) in dichloromethane (50ml.) was stirred for 24h. at ambient temperature. After filtering, the reaction mixture was washed with saturated aqueous sodium carbonate (3 x 50ml.), water (3x50ml.) and saturated aqueous sodium chloride (3x50ml.). After drying and filtering, the solvent was evaporated to give an oil (980mg.). Radial chromatography separated the components. Thus, a sample of the α,β-unsaturated ester 29 (280mg.) was isolated unreacted. From the glycidate products (460mg., 59%), a slightly impure sample of the oxaspirane esters 14 and 15 (60mg., 8%) was isolated.



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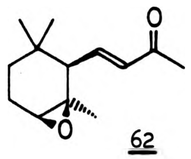

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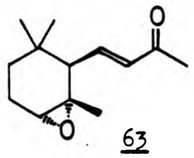

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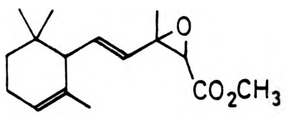

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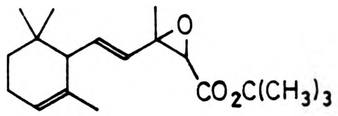

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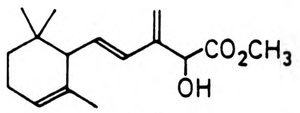


46

These materials were identified by chromatographic and spectral comparison with samples of the genuine material (vide supra). Next to be eluted was a mixture of the oxaspirane esters 16 and 17 (200mg.) of isomeric ratio 50:50. This material was purified by further radial chromatography. The resultant colourless oil (100mg., 12%) was pure.

R_F (E.A. 0.1, P.E. 0.9) = 0.44.

R_T (SE54, Et_2O, 150°C) = 11.5 (62%), 10.0 (38%)m.

i.r. (film):- 2960, 2930, 2905, 1760, 1730, 1200, 1155, 1030 cm^{-1}.

m.s.:- m/e = 169 (100%), 123 (41), 109 (46), 83 (76), 67 (40), 55 (46), 41 (60), 29 (41). M^+(226) = $C_{13}H_{20}O_3$. (m/e = 153 (33%)).

^1H n.m.r.:- 4.26 (q, 7Hz.) and 4.22 (q, 7Hz.) (2H), 3.33 (s) and 3.31 (s) (1H), 2.1-1.2 (7H, m), 1.28 (3H, t, 7Hz.), 1.1-0.7 (9H, m)δ.

^{13}C n.m.r.:- (16) 168.4 (s), 63.3 (s), 61.2 (t), 60.3 (d), 47.7 (t), 47.0 (t), 37.0 (t), 33.4 (s), 32.4 (q), 27.9 (d), 25.5 (q), 22.3 (q), 14.3 (q) p.p.m.

(17) 168.4 (s), 64.0 (s), 61.2 (t), 60.3 (d), 47.7 (t), 43.4 (t), 40.2 (t), 32.9 (s), 32.4 (q), 27.9 (d), 25.2 (q), 22.3 (q), 14.3 (q) p.p.m.

Purification of megastigma-4,7(E)-dien-9-one (α-ionone), 39.

Repeated radial chromatography of small amounts of commercial α-ionone 39, that had been distilled to ~98% purity, gave pure material. It proved more convenient to purify a large amount by spinning band distillation and to store the >99.8% pure material under nitrogen at <0°C.

R_F (E.A. 0.1, P.E. 0.9) = 0.45.

R_T (OV1, Et_2O, 150°C) = 3.9m.

i.r. (film):- 2960, 2920, 2870, 1700, 1680, 1620, 1370, 1255 cm^{-1}.

^{1}H n.m.r.:- 6.68 (1H, Ab q, 9Hz., 16Hz.), 6.09 (1H, d, 16Hz.), 5.57 (1H,m), 2.30 (3H, s), 2.2-1.1 (8H, m), 0.97 (3H, s), 0.88 (3H, s)δ.

^{13}C n.m.r.:- 197.9 (s), 148.7 (d), 132.3 (d), 131.9 (s), 122.6 (d), 54.3 (d), 32.4 (s), 31.2 (t), 27.7 (q), 26.8 (q), 23.0 (t), 22.8 (q) p.p.m.

An impurity at R_T(OV1, Et_2O, 150°C) = 3.7m. had an identical retention time to the epoxides 62 and 63, as confirmed by running a mixture. These epoxides were synthesised by us (vide infra).

Preparation of methyl (4E)-2,3-epoxy-3-methyl-5-(2,6,6-trimethylcyclohex-2-enyl)-4-pentenoate, 44.

a) Using potassium t-butoxide in tetrahydrofuran.

α-Ionone 39 (9.60g., 50mmol.) of purity >99.8% and methyl chloroacetate (6.0g., 55mmol.) in tetrahydrofuran (50ml.) at -78°C was stirred. Potassium t-butoxide (6.2g., 55mmol.) was added in portions over 0.5h. After stirring at ambient temperature overnight, diethyl ether (250ml.) and water (200ml.) were added. The separated organic layer was washed with saturated aqueous sodium chloride solution (150ml.) and dried. Filtration and solvent evaporation gave the impure product. The product 44 could not be easily separated from contaminating α-ionone 39 by silica chromatography. Girards T reagent[24] (2.52g., 15mmol.) in acetic acid (1.5ml.) and methanol (90ml.) was added and the mixture was stirred for 2h. Evaporation to half volume followed by the addition of diethyl ether (250ml.) and water (100ml.) provided an organic phase, which was separated. After washing with saturated aqueous sodium carbonate (100ml.) and saturated aqueous sodium chloride (100ml.), the system was dried. The filtered solution had the solvent evaporated and the resultant product was purified by

repeated flash chromatography. A small scale, rapid and high vacuum distillation[178] provided pure 44 (40mg.) as a colourless oil.

R_F (E.A. 0.1, P.E. 0.9) = 0.44.

R_F (E.A. 0.2, P.E. 0.8) = 0.68.

i.r. (film):- 2960, 2930, 2870, 1760, 1735, 1440, 1290, 1205 cm^{-1}.

^1H n.m.r.:- 5.7-5.0 (3H, m), 3.8-3.7 (3H, m), 3.6-3.3 (1H, m), 2.5-2.2 (1H, m), 2.2-1.2 (10H, m), 1.1-0.7 (6H, m)δ.

A ^{13}C n.m.r. spectrum was obtained at 0°C but proved too complex to interpret completely. Diagnostically, the carbonyl C-1 was observed at 169.1 p.p.m. and the methyl ester was observed at 52.3 p.p.m. More importantly, a multitude of resonances were observed between 63 and 57 p.p.m. corresponding to the epoxide carbons C-1 and C-3. It should be noted that the spectrum contained sixty eight discrete peaks from the four isomers of 44 and their decomposition products.

This compound decomposed rapidly, but spectra could be obtained of this material if kept cool and run rapidly. Analysis on silver nitrate treated t.l.c. plates[179] separated the product into four spots. Note also that flash chromatography gave another fraction of slightly different isomeric ratio, hence enabling the spectral data to be more easily interpreted. Another fraction was further purified to give an impure sample of the tentatively assigned t-butyl (4E)-2,3-epoxy-3-methyl-5-(2,6,6-trimethyl-2-cyclohexenyl)-4-pentenoate 45 (10mg.) as a colourless oil.

R_F (E.A. 0.1, P.E. 0.9) = 0.54.

i.r. (film):- 2960, 2930, 2860, 1750, 1725, 1370, 1260, 1160 cm^{-1}.

^1H n.m.r.:- 5.7-5.0 (3H, m), 3.5-3.0 (1H, m), 2.5-1.2 (11H, m), 1.50 (9H, s), 1.2-0.8 (6H, m)δ.

Likewise, the hydroxy ester 46 was identified, but not isolated pure, in the product mixture (vide infra).

In another preparation, the ∝-ionone 39 used was commercial 90% grade that had been distilled once, b.p. 88-92°C at 0.5mmHg. A sample of this ionone (9.6g., 50mmol.) in tetrahydrofuran (50ml.) at 0°C was mixed with methyl chloroacetate (5.43g., 50mmol.). Potassium t-butoxide (6.0g., 54mmol.) in tetrahydrofuran (50ml.) was added dropwise over 1h., and then the reaction mixture was stirred overnight at ambient temperature. Analysis by t.l.c. showed many components. The solvent was evaporated and chloroform (250ml.) and water (250ml.) were added. The separated organic layer was washed with water (250ml.), saturated aqueous sodium chloride (2x250ml.) and saturated aqueous sodium carbonate (2x250ml.). Drying and filtering, followed by solvent evaporation, gave the crude product. Bulb to bulb distillation gave a fraction at ~135°C, which contained at least ten components by t.l.c. This material possessed a vague dried fruit and raspberry aroma. Spinning band distillation gave a sample which was of similar aroma. Silica chromatography of this sample provided various fractions, none of which retained the original aroma quality. The major component was identified as methyl (4E)-2-hydroxy-3-methylene-5-(2,6,6-trimethyl-2-cyclohexenyl)-4-pentenoate 46.

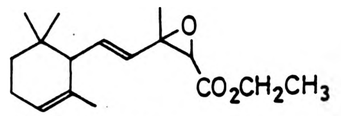
R_F (E.A. 0.1, P.E. 0.9) = 0.23.

i.r. (film):- 2960, 2920, 2870, 1745, 1445, 1260, 1220, 1100 cm^{-1}. (3600-3100 cm^{-1}).

^1H n.m.r.:- 6.01 (1H, d, 16Hz.), 5.71 (1H, ABq, 8Hz., 16Hz.), 5.42 (1H, m), 5.21 (2H, m), 4.85 (1H, s), 3.78 (3H, s), 2.3-1.0 (8H, m), 0.90 (3H, s), 0.82 (3H, s)δ.

b) Using sodium methoxide in pyridine.

∝-Ionone 39 (5.85g., 30mmol.) and methyl chloroacetate (4.76g., 44mmol.) were mixed together at -15°C. ~95% Pure sodium methoxide



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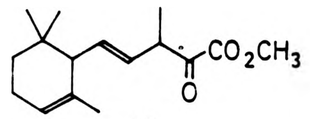

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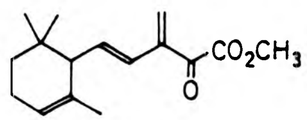

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(3.35g., 59mmol.) in pyridine, (30ml.) and just enough methanol to dissolve the solid, was added dropwise over 0.5h. After stirring overnight, the reaction mixture was evaporated to half volume and then chloroform (150ml.) and water (150ml.) were added. The separated organic phase was washed with water (150ml.) and saturated aqueous sodium chloride. Drying, filtering, solvent evaporation and bulb to bulb distillation provided impure α-ionone 39 (5.23g.), identified from its chromatographic and spectral properties.

Preparation of ethyl (4E)-2,3-epoxy-3-methyl-5-(2,6,6-trimethylcyclohex-2-enyl)-4-pentenoate, 47.

A sample of α-ionone 39 (9.60g., 50mmol.) was dissolved in tetrahydrofuran (50ml.) and ethyl chloroacetate (6.80g., 56mmol.) was added. The mixture was cooled to -78°C and then potassium t-butoxide (6.20g., 55mmol.) was added, with rapid stirring, in portions over 30m. The reaction mixture was stirred for 8h. at ambient temperature. The addition of tetrahydrofuran (30ml.) was required during this time in order to retain mobility. The solution was evaporated to half volume. Diethyl ether (250ml.) and water (200ml.) were added. The separated ethereal layer was washed with saturated aqueous sodium chloride (150ml.), dried, filtered and then the solvent was evaporated. Girards T reagent[24] (2.94g., 18mmol.) in acetic acid (1.75ml.) and methanol (105ml.) was added and the reaction mixture was stirred for 3h. The system was evaporated to half volume and then diethyl ether (250ml.) and water (150ml.) were added. The separated ethereal phase was washed with saturated aqueous sodium carbonate (100ml.) and saturated aqueous sodium chloride and was then dried. The solution was filtered and the solvent was evaporated. The crude product (11.46g.) was purified by column chromatography to yield two fractions containing 47. The second fraction of the two to be eluted from the column, was distilled using the

rapid, high vacuum technique[178] to yield the pure product (210mg., 2%).

R_F (E.A. 0.1, P.E. 0.9) = 0.57.

i.r. (film):- 2960, 2920, 1755, 1730, 1280, 1195, 1080, 1040 cm^{-1}.

^1H n.m.r.:- 5.73 (1H, ABq, 9Hz., 16Hz.), 5.45 (1H, d, 16Hz.), 5.41 (1H, m), 4.22 (2H, q, 7Hz.), 3.50 (1H, s), 2.3-1.1 (5H, m), 1.54 (6H, s), 1.28 (3H, t, 7Hz.), 0.88 (3H, s), 0.78 (3H, s)δ.

^{13}C n.m.r.:- (major isomer) 167.8 (s), 137.1 (d), 133.5 (s), 128.0 (d), 121.4 (d), 61.2 (t), 61.2 (s), 60.9 (d), 54.4 (d), 32.0 (s), 31.5 (t), 27.3 (q), 26.9 (q), 23.0 (t), 22.8 (q), 21.2 (q), 14.2 (q) p.p.m.

The ^{13}C n.m.r. spectrum showed doubling of some of the peaks (difference <0.5 p.p.m.) implying that the product is a mixture of two similar isomers. The quaternary carbon assignments are very tenetative. The spectrum was obtained at 0°C.

The fraction which was eluted first was likewise distilled to yield a product (150mg., 6%), which showed two poorly resolved spots on t.l.c. analysis at R_F (E.A. 0.1, P.E. 0.9) = 0.59 and 0.57, one of which corresponded to the second fraction material. Spectral data implied that the major component was that obtained from the second fraction. The minor component could be partially identified in the n.m.r. spectra. The i.r. spectrum was virtually identical to that of the second fraction. Data specific to the minor component included:

R_F (E.A. 0.1, P.E. 0.9) = 0.59.

^1H n.m.r.:- 5.69 (1H, ABq, 9Hz., 16Hz.), 5.41 (1H, m), 5.30 (1H, d, 16Hz.), 4.28 (2H, q, 7Hz.), 3.39 (1H, s), 2.3-1.1 (5H, m), 1.54 (3H, s), 1.50 (3H, s), 1.31 (3H, t, 7Hz.), 0.90 (3H, s), 0.84 (3H, s)δ.

^{13}C n.m.r.:- (Resolved peaks only) 135.3 (d), 131.5 (d), 54.0 (d), 15.4 (q) p.p.m.

The ^{13}C n.m.r. data excludes peaks which were poorly resolved from the resonances attributed to the major component.

Decomposition of methyl (4E)-2,3-epoxy-3-methyl-5-(2,6,6-trimethylcyclohex-2-enyl-4-pentenoate, 44.

T.l.c. scale experiments were conducted as follows. The glycidate 44 (10mg.) was dissolved in tetrahydrofuran (0.1ml.) and a sample of a test compound (10mg.). The decomposition was monitored by t.l.c. Using silica or potassium carbonate accelerated the decomposition compared to a control, but not to such a great extent as acetic acid or lithium perchlorate trihydrate. Thus, with lithium perchlorate trihydrate, the glycidate was completely decomposed after 10h. at ambient temperature. The control system was completely decomposed after 4d. The major decomposition product in each case had identical t.l.c. properties to 46.

Attempted preparation of methyl (4E)-3-methyl-2-oxo-5-(2,6,6-trimethylcyclohex-2-enyl)-pent-4-enoate, 48.

a) Using boron trifluoride:diethyl ether complex and 44.[180].

The glycidate 44 (50mg.) was dissolved in diethyl ether (3ml.) at ambient temperature. Redistilled boron trifluoride:diethyl ether complex (100mg.) in toluene (3ml.) was added and the reaction was monitored by t.l.c. After 2h., the reaction was virtually complete. Diethyl ether (18ml.) was added and this solution was washed with water (10ml.) and saturated aqueous sodium carbonate (10ml.), dried and filtered. The solvent was evaporated to give a yellow oil (20mg.). This material had at least ten components by t.l.c. and did not have an aroma of dried fruit. No attempt was made to separate or characterise the mixture. None of the components reacted with a 2,4-dinitro-

phenyl hydrazine visualisation reagent.[174]

b) Using potassium carbonate and 46.[13]

A sample of slightly impure 46 (110mg.) was dissolved in tetrahydrofuran (3ml.). Potassium carbonate (250mg.) was added and the mixture was stirred 20h. Analysis by t.l.c. showed only 46.

Using similar conditions, but with the addition of tetra-n-butyl-ammonium bromide as a phase transfer catalyst, the reaction mixture was found to contain α-ionone, 39, as the only volatile product.

c) Using p-toluenesulphonic acid and 46.[48]

A sample of slightly impure 46 (200mg.) was dissolved in a sample (6ml.) of a solution of p-toluenesulphonic acid (600mg.) in redistilled chlorobenzene (60ml.). After refluxing for 27h., t.l.c. analysis showed the removal of 46 and a plethora of products. The solvent was evaporated and the residue was dissolved in diethyl ether (30ml.) and washed with saturated aqueous sodium carbonate (2x20ml.), dried and filtered. Bulb to bulb distillation provided material with aroma properties not dried fruit like, and with more than ten components by t.l.c. None of these components reacted with a 2,4-dinitrophenylhydrazine visualisation reagent.

Attempted preparation of methyl (4E)-3-methylene-2-oxo-5-(2,6,6-trimethylcyclohex-2-enyl)pent-4-enoate, 49.

a) Using manganese dioxide and 46.

Active manganese dioxide was prepared as follows.[181] Potassium permanganate (9.6g.) was dissolved in water (60ml.). Manganese sulphate monohydrate (11.1g.) in water (15ml.) and sodium hydroxide (4.7g.) in water (12ml.) were added simultaneously with stirring at 60°C, dropwise over 30m. After stirring a further 1h. at 60°C, the reaction mixture was allowed to reach ambient temperature. Centrifuging, decanting and

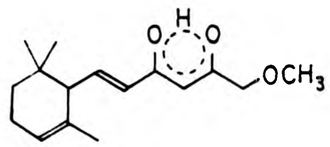
washing with water provided the active manganese dioxide, which was dried in an oven and pulverised to a powder. A sample of 46 (30mg.) was treated with a portion of the active manganese dioxide (50mg.) in tetrachloromethane for 3d. Filtration followed by evaporation of the solvent gave no product. Boiling the filtered manganese dioxide in chloroform failed to extract any product.

b) Using silver (1) oxide and 46.[24]

A sample of 46 (10mg.) was dissolved in diethyl ether (1ml.). Silver (1) oxide (20mg.) was added and stirred overnight. T.l.c. analysis showed numerous products, none of which gave a positive reaction with a 2,4-dinitrophenylhydrazine visualisation reagent.[174] Filtration and evaporation of solvent gave material (10mg.), which did not smell dried fruit-like.

c) Using barium permanganate and 46.

Barium permanganate was prepared by the following procedure.[182] Powdered potassium hydroxide (11.2g.) and manganese dioxide (8.7g.) were fused in a muffle furnace at 350°C for 3h. The resulting dark green solid was powdered and added in water (150ml.) to a solution of barium hydroxide (7.0g.) in water (100ml.), adjusted to pH = 7 by the addition of 2N aqueous hydrolchloric acid. The resulting dark purple mixture was stirred for 1h. Vacuum filtration provided the blue powdery crystals of barium permanganate, which were washed with water (100ml.) and dried in an oven. A portion of these crystals (2.0g.) was added to a sample of the hydroxyester 46 (200mg.) in redistilled, dry benzene (5ml.). The resultant mixture was refluxed for 30m. T.l.c. analysis showed no residual 46 and five components. Filtration through silica and evaporation of the solvent provided an oil (70mg.). The aroma of this oil was not dried fruit-like and none of the components reacted with a 2,4-dinitrophenylhydrazine visualisation reagent.[174]



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Attempted preparation of methyl 2-methoxy-3-methyl-5-(2,5,5-trimethylcyclohex-2-enyl)-pentadienoate.[49]

50% Sodium hydride in mineral oil (960mg., 20mmol.) in diethyl ether (10ml.) and ethanol (0.1ml.) was stirred at 0°C. Methyl methoxyacetate (3.12g., 30mmol.) was added dropwise over 30m. and α-ionone (39) (1.92g., 10mmol.) was added dropwise over 30m. After stirring overnight, water (20ml.) was added dropwise over 30m. at -78°C. After allowing to reach ambient temperature, the reaction mixture was extracted with diethyl ether (3x50ml.). Evaporation of the solvent, followed by bulb to bulb distillation and radial chromatography of a portion of the crude product provided a sample of pure 50 (90mg., 15%). This material gave a positive iron (3) chloride test for β-diketones[50] as indicated by a dark red colour.

R_F (E.A. 0.1, P.E. 0.9) = 0.40.

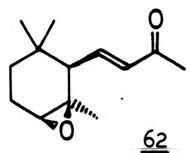
i.r. (film): 2960, 2930, 2870, 1645, 1585, 1450, 1200, 1120 cm^{-1}.

m.s.:- m/e = 264 (71%), 163 (100), 135 (62), 115 (46), 97 (51), 91 (54), 55 (44), 45 (62).

(M^+ (264) = $C_{16}H_{24}O_3$, M^+ (163) = $C_{10}H_{11}O_2$)

^1H n.m.r.: 6.75 (1H, ABq, 15Hz., 9Hz.), 5.87 (1H, d, 15Hz.), 5.72 (1H, s), 5.6-5.4 (1H, m), 4.00 (2H, s), 3.44 (3H, s), 2.31 (1H, d, 9Hz.), 2.2-1.1 (4H, m), 1.58 (3H, s), 0.93 (3H, s), 0.86 (3H, s) .

The compound decomposed during a ^{13}C n.m.r. experiment.



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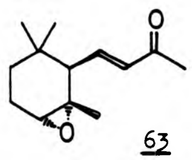

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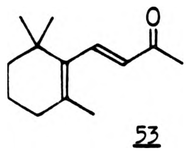

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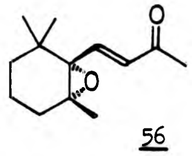

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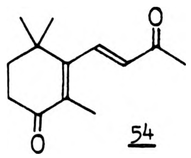

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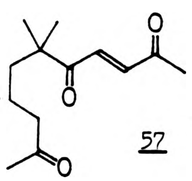

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Preparation of the cis and trans isomers [36] of (E)-4-(1,3,3-trimethyl-7-oxabicyclo[4,1]hept-2-yl)buten-2-one (4,5-epoxy megastigma-7(E)-9-one), 62 and 63.

α-Ionone 39 (190mg., 0.99mmol.), 80% pure m-chloroperoxybenzoic acid (220mg., 1.02mmol.) and dichloromethane (10ml.) were stirred together at ambient temperature for 48h. After washing with saturated aqueous sodium carbonate (3x5ml.), water (2x5ml.) and saturated aqueous sodium chloride (2x5ml.), the solution was dried and filtered. Evaporation of the solvent gave the impure products as a mixture (160mg., 78%). Repeated radial chromatography separated the isomeric products. Thus, a sample of the pure cis isomer 62 (60mg., 29%) was obtained.

R_F (E.A. 0.1, P.E. 0.9) = 0.39.

R_T (OV1, Et_2O, 150°C) = 3.7m.

m.s.:- m/e = 179 (13%), 111 (20), 109 (44), 95 (26), 55 (16), 43 (100), 41 (29), 39 (19). M^+(208) = $C_{13}H_{20}O_2$. M^+(109) = C_7H_9O.

^1H n.m.r.:- 6.74 (1H, ABq, 10Hz., 16Hz.), 6.09 (1H, d, 16Hz.), 3.10 (1H, t, 2Hz.), 2.29 (3H, s), 2.2-1.0 (5H, m), 1.26 (3H, s), 0.95 (3H, s), 0.77 (3H, s)δ.

^{13}C n.m.r.:- 198.6 (s), 146.3 (d), 134.0 (d), 59.4 (d), 58.8 (s), 52.5 (d), 31.2 (s), 28.5 (t), 27.9 (q), 27.3 (q), 26.4 (q), 24.0 (q), 21.7 (t) p.p.m.

Likewise, a sample of a mixture of 62 and 63 (20mg., 10%) was obtained, which was enriched in 63. The ratio 62:63 was about 40:60 by n.m.r. Identical retention times made g.c. isomeric ratio determination impossible. T.l.c. and n.m.r. spectral data are given for the trans isomer only. The m.s. data refer to the mixture.

R_F (E.A. 0.1, P.E. 0.9) = 0.36.

R_T (OV1, Et_2O, 150°C) = 3.7m.

m.s.:- m/e = 111 (26%), 109 (51), 95 (33), 69 (18), 55 (21), 43 (100), 41 (40), 28 (44). $M^{+\cdot}$(208) = $C_{13}H_{20}O_2$. M^{+}(109) = C_7H_9O.

^1H n.m.r.:- 6.69 (1H, Abq, 11Hz., 16Hz.), 6.15 (1H, d, 16Hz.), 3.01 (1H, t, 2Hz.), 2.30 (3H, s), 2.2-1.0 (5H, m), 1.20 (3H, s), 0.87 (3H, s), 0.81 (3H, s)δ.

^{13}C n.m.r.:- 197.6 (s), 145.1 (d), 134.2 (d), 59.8 (d), 58.8 (s), 54.1 (d), 32.6 (t), 31.8 (s), 29.5 (q), 27.7 (q), 23.6 (q), 21.5 (q), 21.5 (t) p.p.m.

M.s. metastable ions for the transition m/e = x → 109 implied values for x as m/e = 208, 192, 167 and 153. The former two derived values corresponded to metastable ions of very weak intensity. ^{13}C n.m.r. data was obtained by additive transfer of the data for pure 62 to the data for the mixture.

Purification of megastigma-5,7(E)-dien-9-one (β-ionone), 53.

Spinning band distillation of 98% pure commercial β-ionone 53 gave material of ~99.5% purity. Radial chromatography of this material gave 53 of >99.8%.

R_F (E.A. 0.1, P.E. 0.9) = 0.45.

R_T (OV1, Et_2O, 150°C) = 4.7m.

i.r. (film):- 2960, 2930, 2870, 1695, 1675, 1605, 1360, 1255 cm^{-1}.

^1H n.m.r.:- 7.27 (1H, d, 16Hz.), 6.10 (1H, d, 16Hz.), 2.29 (3H, s), 2.2-1.3 (6H, m), 1.77 (3H, s), 1.08 (6H, s)δ.

^{13}C n.m.r.:- 198.3 (s), 143.0 (d), 136.0 (s), 135.8 (s), 132.4 (d), 39.8 (t), 34.1 (s), 33.6 (t), 28.8 (q), 27.2 (q), 21.7 (q), 18.9 (t) p.p.m.

Impurities isolated impure were the epoxide 56 and α-ionone 39. Data for 39 is presented elsewhere. The epoxide 56 was obtained in purity >99.8% by radial chromatography.

m.p.:- 43-5°C (lit.[183] 46-8°C).

R_F (MeOH 0.01, E.A. 0.10, P.E. 0.89) = 0.29.

R_T (OV1, Et_2O, 150°C) = 4.3m.

i.r.[67] (film):- 2960, 2930, 2870, 1675, 1625, 1360, 1255, 985 cm^{-1}.

m.s.:- m/e = 135 (9%), 124 (9), 123 (100), 95 (5), 55 (7), 43 (52), 41 (14), 39 (9). $M^{+\cdot}$(193) = $C_{12}H_{17}O_2$.

^1H n.m.r.[67]:- 7.01 (1H, d, 16Hz.), 6.26 (1H, d, 16Hz.), 2.27 (3H, s), 2.2-1.3 (6H, m), 1.15 (6H, s), 0.93 (3H, s)δ.

^{13}C n.m.r.[72]:- 196.7 (s), 142.6 (d), 132.5 (d), 70.6 (s), 65.9 (s), 35.5 (t), 33.6 (s), 29.8 (t), 28.3 (q), 25.9 (q), 20.8 (q), 16.9 (t) p.p.m.

Preparation of megastigma-5,7(E)-dien-4,9-dione, 54.

a) Using t-butyl chromate.[59]

t-Butanol was distilled from calcium oxide and a sample (3.0g., 43mmol.) was dissolved in hexane (100ml.). Chromium (6) trioxide (2.0g., 20mmol.) was added in portions with stirring. The orange solution was stirred vigorously for 1h. The solution was decanted. The decanted solution was dried over granular calcium chloride. The solution was decanted and cooled to -78°C. A yellow solid appeared and the liquid was decanted from this solid. The solid was recrystallised at -78°C twice from hexane (100ml.). All of the above operations were achieved in a nitrogen atmosphere. The yellow solid t-butyl chromate so obtained was dissolved in hexane (100ml.) and β-ionone 53 (4.0g., 21mmol.) in hexane (50ml.) was added at 5°C. The brown solution was stirred 40h. at ambient temperature. T.l.c. analysis implied only partial reaction, so another batch of t-butyl chromate was prepared as above and added to the reaction mixture. After stirring 5d., the reaction mixture was washed with saturated aqueous sodium carbonate (2x100ml.) and filtered through silica. The solution was

washed with water (3x100ml.) and saturated aqueous sodium chloride (2x100ml). After drying and filtering, the solvent was evaporated to give a dark red oil (2.01g.), which turned brown on standing. Radial chromatography of a sample (1.00g.) separated the various components. β-Ionone 53 (580mg.) was eluted first followed by the epoxide 56 (60mg., 14%) isolated impure as a yellow oil. The identity of these compounds was deduced by comparison of chromatographic and spectral data with genuine samples. A sample of 56 has already been described as an impurity in commercial β-ionone 53 (vide supra). Next to be eluted was 54 isolated as a yellow solid (150mg., 33%) of purity ~95%.

m.p. = 38-44°C (lit.[184] 51-52°C).

R_F (MeOH 0.01, E.A. 0.1, P.E. 0.89) = 0.21.

R_T (OV1, Et_2O, 150°C) = 7.6 (95%)m.

i.r.[56] (Nujol mull):- 2960, 2930, 2870, 1700, 1680, 1455, 1380, 1370 cm^{-1}.

m.s.[56,58]:- m/e= 206 (49%), 164 (23), 163 (78), 135 (23), 122 (28), 121 (37), 43 (100), 41 (27). M^+(206) = $C_{13}H_{18}O_2$. (m/e = 191 (10%)).

^1H n.m.r.[56]:- 7.26 (1H, d, 17Hz.), 6.19 (1H, d, 17Hz.), 2.55 (2H, t, 7Hz.), 2.36 (3H, s), 1.90 (2H, t, 7Hz.), 1.81 (3H, s), 1.21 (6H, s)δ.

^{13}C n.m.r.:- 198.3 (s), 157.9 (s), 140.4 (d), 133.5 (d), 37.6 (t), 35.7 (s), 34.2 (t), 27.3 (q), 13.5 (q) p.p.m.

The last compound to be isolated was 57 (30mg., 7%). Further purification by radial chromatography gave a sample of 57 (10mg.) of ~80% purity as an oil.

R_F (MeOH 0.01, E.A. 0.10, P.E. 0.89) = 0.13.

R_T (OV1, Et_2O, 150°C) = 7.8m.

i.r. (film)[61]:- 2960, 2930, 2870, 1715, 1680, 1360, 1250, 1075 cm^{-1}.

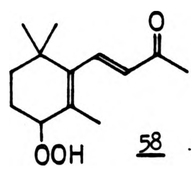
m.s.:- m/e = 123 (40%), 109 (57), 98 (91), 69 (64), 45 (39), 43 (95), 41 (43), 28 (100). $M^{+\cdot}$(224) = $C_{13}H_{20}O_3$.

^1H n.m.r.[61]:- 7.35 (1H, d, 15Hz.), 6.97 (1H, d, 15Hz.), 2.5-2.2 (5H, m), 2.13 (3H, s), 1.7-1.4 (2H, m), 1.18 (6H, s)δ.

b) Using acidic sodium chlorate and sodium iodide in biphasic conditions.[62]

Sodium chlorate (13.2g., 124mmol.) and sodium iodide (0.56g., 3.7mmol.) was dissolved in water (50ml.). This was added to 99.5% pure β-ionone 53 (4.8g., 25mmol.) in chloroform (125ml.) with stirring. Concentrated sulphuric acid (55μl.) was added and the reaction mixture was stirred for 24h. at 45°C. The aqueous layer was at pH = 6 and was adjusted to pH=2 by the addition of concentrated sulphuric acid (30μl.). After stirring for 3d., analysis by t.l.c. showed unreacted ionone. The chloroform layer was separated and a fresh batch of the aqueous acidic sodium chlorate and sodium iodide reagent was added. After stirring for 10d., the organic layer was separated and the peach coloured solution was washed with saturated aqueous sodium bicarbonate (100ml.) and water (100ml.). The solution had become lime green in colour. It was dried, filtered and then the solvent was evaporated to give an oil (5.02g.). Extensive radial chromatography separated the compounds which are presented in order of elution. The first compound was not characterised or identified before it decomposed to mostly β-ionone, 53, on storage at 0°C. It was an impure pale yellow oil (10mg.), which gave a positive Beilstein test[50] for halogen.

R_F (MeOH 0.01, E.A. 0.10, P.E. 0.89) = 0.54.



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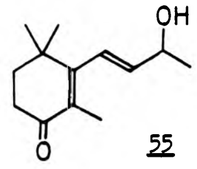

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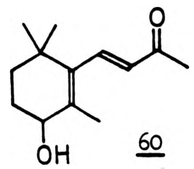

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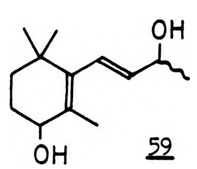

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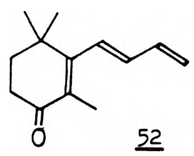

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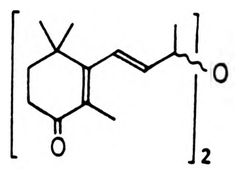

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The next compound to be eluted was β-ionone 53 (3.2g.). Following this, the epoxide 56 (310mg., 36%) was isolated. Both these compounds were identified by comparison of chromatographic and spectral data with genuine samples. The epoxide 56 was characterised as an impurity in commercial β-ionone (vide supra). The next compound to be isolated was of unknown structure. It was an oil (10mg.) and gave a positive o-dianisidine aldehyde test,[50] the colour obtained being reddish-brown.

R_F (MeOH 0.02, E.A. 0.20, P.E. 0.78) = 0.37.

m.s.:- m/e = 123 (100%), 109 (58), 95 (38), 81 (29), 55 (29), 43 (91), 41 (51), 39 (33). $M^{+\cdot}$(192) = $C_{13}H_{20}O$.

^1H n.m.r.:- 10.34 (1H, d, 8Hz.), 6.00 (1H, d, 8Hz.), 5.45 (1H, m), 3.2-3.1 (1H, m), 2.5-1.2 (5H, m), 1.71 (3H, s), 1.16 (6H, s)δ.

^{13}C n.m.r.:- 193.0 (d), 127.2 (d), 59.0 (d), 32.6 (t), 26.8 (q), 23.0 (q), 21.1 (q), 20.1 (t) p.p.m.

Note that any quaternary carbon resonances were not observed due to the low signal to noise ratio.

The next compound to be eluted was identified tentatively as the hydroperoxide 58 and was isolated slightly impure as an oil (10mg., 1%). The sample decomposed before full characterisation.

R_F (MeOH 0.02, E.A. 0.20, P.E. 0.78) = 0.31.

m.s.:- m/e = 146 (13%), 109 (17), 95 (14), 69 (22), 55 (16), 43 (100), 41 (27), 29 (13). $M^{+\cdot}$(224) = $C_{13}H_{20}O_3$. (m/e = 191 (4%).

^1H n.m.r.:- 7.21 (1H, d, 17Hz.), 6.14 (1H, d, 17Hz.), 2.32 (3H, s), 2.3-1.1 (5H, m), 1.87 (3H, s), 1.09 (6H, s)δ.

The last compound, isolated impure, was the dione 54 (270mg., 31%), identified by comparison of chromatographic and spectral data with a genuine sample obtained previously (vide supra).

Preparation of 9-hydroxymegastigma-5,7(E)-dien-4-one, 55.

The dione 54 (360mg., 1.7mmol.) was dissolved in methanol (2.4ml.) with rapid stirring at 0°C. Sodium borohydride (20.0mg., 0.6mmol.) was added and the reaction was stirred for 15m. After allowing to ambient temperature, water (10ml.) was added and the reaction mixture was stirred for 5m. This solution was extracted with diethyl ether (3x10ml.). The solvent was evaporated, from the combined extracts, to give an oil (310mg.). Radial chromatography separated the three components. Unreacted dione 54 (60mg.) was identified from its chromatographic and spectral data. Next to be eluted was the desired product 55 as an oil (230mg., 77%).

R_F (MeOH 0.02, E.A. 0.20, P.E. 0.78) = 0.15.

^1H n.m.r.[55]:- 6.27 (1H, d, 16Hz.), 5.73 (1H, ABq, 6Hz., 16Hz.), 4.48 (1H, quintet, 6Hz.), 2.51 (2H, t, 7Hz.), 1.86 (2H, t, 7Hz.), 1.81 (3H, s), 1.37 (3H, d, 6Hz.), 1.18 (6H, s)δ.

This compound decomposed on storage to a mixture of 55 and its isomer 60, identified by comparison of the ^1H n.m.r. spectral data with literature values.[185]

Also isolated slightly impure were the diols 59 (10mg., 3%).

R_F (MeOH 0.02, E.A. 0.20, P.E. 0.78) = 0.07.

i.r.[63] (film):- 3420 (br.), 2960, 2930, 2860, 1640, 1445, 1020, 960 cm^{-1}.

^1H n.m.r.[63]:- 6.08 (1H, d, 16Hz.), 5.55 (1H, ABq, 6Hz., 16Hz.), 4.6-3.9 (2H, m), 2.0-1.1 (4H, m), 1.82 (3H, s), 1.34 (3H, d, 6Hz.), 1.04 (3H, s), 1.00 (3H, s)δ.

Preparation of megastigma-5,7(E),9-trien-4-one, 52.

A sample of 55 (210mg., 1.0mmol.) was dissolved in toluene (0.9ml.). p-Toluenesulphonic acid (4.5mg., 0.03mmol.) was added and the mixture was refluxed for 2h., then stirred overnight. Diethyl ether

(30ml.) was added and the solution was washed with saturated aqueous sodium bicarbonate (3x10ml.), water (10ml.) and saturated aqueous sodium chloride (10ml.). After drying and filtering, the solvent was evaporated to give an oil (160mg.). Repeated radial chromatography separated the major products. The first to be eluted was the trienone 52 (70mg., 36%) as a pale yellow oil of purity >99.8%.

R_F (MeOH 0.02, E.A. 0.20, P.E. 0.78) = 0.60.

R_T (OV1, Et_2O, 150°C) = 4.4m.

^1H n.m.r.[55]:- 6.7-6.1 (3H, m), 5.4-5.1 (2H, m), 2.52 (2H, t, 7Hz.), 1.88 (2H, t, 7Hz.), 1.88 (3H, s), 1.19 (6H, s)δ.

^{13}C n.m.r.:- 198.9 (s), 160.3 (s), 137.1 (d), 136.9 (d), 129.5 (d), 119.0 (t), 37.4 (t), 35.6 (s), 34.3 (t), 27.5 (q), 13.6 (q) p.p.m.

The second component was the novel ether 61 (70mg., 34%) identified from its spectral characteristics.

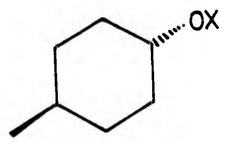
R_F (MeOH 0.02, E.A. 0.20, P.E. 0.78) = 0.35.

i.r. (film):- 2970, 2930, 1670, 1365, 1355, 1335, 1190, 1170 cm^{-1}. (1600 cm^{-1}.)

m.s.:- m/e = 149 (42%), 83 (51), 69 (68), 57 (57), 56 (44), 55 (84), 43 (98), 41 (100). M^{+}(398) = $C_{26}H_{38}O_3$. (m/e = 191 (36%), 163 (37)).

^1H n.m.r.:- 6.20 (2H, d, 16Hz.), 5.61 (ABq, 7Hz., 16Hz.) and 5.57 (ABq, 7Hz., 16Hz.) (2H), 4.10 (2H, quintet, 6Hz.), 2.7-2.3 (4H, m), 2.0-1.7 (4H, m), 1.85 (s) and 1.81 (s) (6H), 1.33 (6H, d, 6Hz.), 1.19 (s) and 1.15 (s) (12H)δ.

^{13}C n.m.r.:- 199.3 (s), 160.1 (s), 139.1 (d) and 138.9 (d), 127.1 (d) and 126.1 (d), 73.8 (d) and 73.4 (d), 37.3 (t), 35.4 (s), 34.3 (t), 27.4 (q), 22.3 (q) and 21.3 (q), 13.4 (q) p.p.m.



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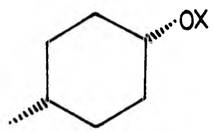

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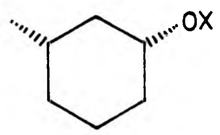

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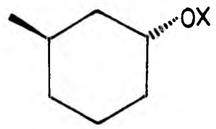

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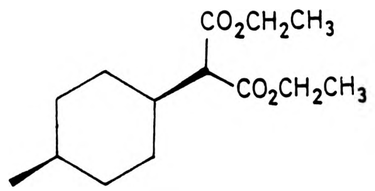

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Preparation of trans-4-methylcyclohexyl-p-toluenesulphonate, 92.

p-Toluenesulphonylchloride (2.10g., 11mmol.) was dissolved in distilled and dried pyridine (8ml.). Trans-4-methylcyclohexanol 91 (1.14g., 10mmol.) was added dropwise over 5m. After stirring at ambient temperature for 13h., the reaction mixture was poured into 2N aqueous hydrochloric acid (55ml.). This mixture was then extracted with diethyl ether (3x30ml.) and the combined extracts were washed with water (50ml.), dried and filtered. Evaporation of the solvent provided the crude product (2.56g., 90%) which was purified by radial chromatography to give the pure product 92 (1.92g., 72%) as white crystals.

m.p. = 70-72°C (lit.[93] 70.8 - 71.8°C).

R_F (MeOH 0.01, E.A. 0.10, P.E. 0.89) = 0.41.

^1H n.m.r.:- 7.78 (2H, d, 8Hz.), 7.31 (2H, d, 8Hz.), 4.6-4.2 (1H, m), 2.42 (3H, s), 2.1-1.0 (9H, m), 0.84 (3H, d, 6Hz.)δ.

Preparation of cis-4-methylcyclohexyl-p-toluenesulphonate, 93.

Commercial cis-4-methylcyclohexanol 94 (2.0g.) was purified by radial chromatography to give the pure material (1.56g.). A sample of the pure material was reacted in similar conditions as in the preparation of the trans- isomer 92. The product was purified by recrystallisation from ethyl acetate and hexane to give white needle crystals of pure 93 (2.41g., 63%).

m.p. = 73.0-73.5°C (lit.[93] 72.0-72.8°C).

R_F (MeOH 0.01, E.A. 0.10, P.E. 0.89) = 0.44.

^1H n.m.r.:- 7.81 (2H, d, 8Hz.), 7.33 (2H, d, 8Hz.), 4.8-4.6 (1H, m), 2.45 (3H, s), 2.1-1.1 (9H, m), 0.90 (3H, d, 5Hz.)δ.

Preparation of a mixture of cis-and trans- isomers of 3-methylcyclohexyl-p-toluenesulphonate, 97 and 98.

Using similar conditions as those employed in the preparation of 92, commercial 3-methylcyclohexanol (11.4g., 100mmol.), of isomeric mixture

70:30, 95:96, provided the crude product (2.0g.) which was purified by radial chromatography to give a pure sample of a 70:30 mixture of 97 and 98 as an oil (1.51g., 65%).

R_F (MeOH 0.01, E.A. 0.10, P.E. 0.87) = 0.41.

^1H n.m.r.:- 7.78 (2H, d, 8Hz.), 7.32 (2H, d, 8Hz.), 4.9-4.7 (m) and 4.6-4.2 (m) (1H), 2.42 (3H, s), 2.1-1.0 (9H, m), 1.0-0.7 (3H, m)δ.

No separation of 97 and 98 was effected by radial chromatography, vacuum distillation or low temperature recrystallisation.

Preparation of diethyl (cis-4-methylcyclohexyl)malonate, 99.

a) Using sodium ethoxide in ethanol.[93]

Sodium (310mg.) was dissolved in ethanol (6.8ml.). Diethyl malonate (2.05ml.) was added, followed by the tosylate 92 (1.50g., 5.4mmol.) in ethanol (10ml.). After heating for 24h. at 60°C, t.l.c. analysis showed that some 92 remained unreacted. After adding 2N aqueous hydrochloric acid (25ml.), the reaction mixture was extracted with diethyl ether (3x50ml.). The combined ethereal extracts were washed with water (100ml.) and saturated aqueous sodium carbonate (100ml.), dried and filtered. Evaporation of the solvent provided the crude product, which was purified by repeated radial chromatgoraphy. Some 92 (640mg.) was obtained along with the product 99 (300mg., 42%) as an oil.

R_F (MeOH 0.01, E.A. 0.10, P.E. 0.89) = 0.38.

i.r. (film):- 2930, 1760, 1735, 1295, 1240, 1180, 1140, 1040 cm^{-1}.

m.s.:- m/e = 160 (41%), 81 (26), 67 (26), 55 (42), 53 (24), 41 (48), 39 (27), 29 (100). M^+(256) - not observed.

^1H n.m.r.:- 4.18 (2H, q, 7Hz.), 3.39 (1H, d, 10Hz.), 2.0-1.2 (10H, m), 1.25 (3H, t, 7Hz.), 1.0-0.7 (3H, m)δ.

^{13}C n.m.r.:- 168.9 (s), 61.1 (t), 55.7 (d), 35.8 (d), 30.6 (t), 29.6 (d), 26.5 (t), 19.9 (q), 14.1 (q) p.p.m.

Note that this compound showed impurity in some of the spectral data and one spot by t.l.c. analysis.

b) Using n-butyllithium in hexane and diethyl ether.

1.6M n-Butyllithium in hexane (300μl.) was added dropwise to diethyl malonate (60mg.) in diethyl ether (4ml.). Addition of 92 (100mg., 0.36mmol.) in diethyl ether (4ml.) gave a solution which did not contain 99 by t.l.c. analysis, after stirring for 10d. at ambient temperature. Addition of 12-crown-4 (100mg.) still failed to provide 99 after a further 10d.

Attempted preparation of dimethyl (cis-4-methylcyclohexyl)malonate, 100.

T.l.c. scale experiments were undertaken in which 92 was exposed to an excess of the dimethyl malonate anion prepared by addition of either of the following reagents: 1.6M n-butyllithium in hexane and tetrahydrofuran or diethyl ether, or sodium hydride in tetrahydrofuran, with or without 18-crown-6. None of these reagents provided any adducts with t.l.c. characteristics consistent with the desired product 100.

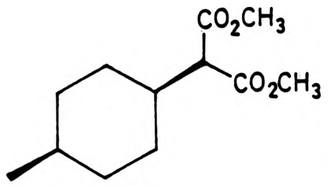
Attempted preparation of (cis-4-methylcyclohexyl)acetic acid, 85.

a) By decarboxylation of (cis-4-methylcyclohexyl)malonic acid.[93]

A sample of the diethyl malonate 99 (300mg., 1.2mmol.) was dissolved in ethanol (2.5ml.) and potassium hydroxide (800mg.) in water (1.1ml.) was added. After refluxing for 90m., the reaction mixture was one phase and was allowed to reach ambient temperature. 6N Aqueous hydrochloric acid was added until the solution was pH=2. Extraction with diethyl ether (4x50ml.) provided a combined ethereal phase, which was dried and filtered. Evaporation of the solvent provided a white solid, which was recrystallised from diethyl ether and hexane to give a sample of (cis-4-methylcyclohexyl)malonic acid (150mg., 63%).

m.p. = 212°C.

R_F (MeOH 0.02, E.A. 0.20, P.E. 0.78) = 0.11.



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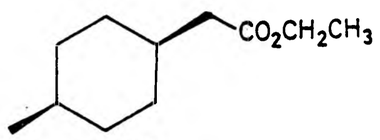

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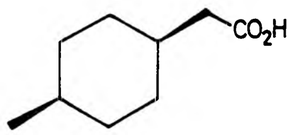

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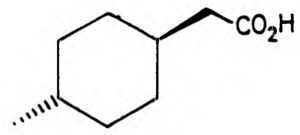


86

^{1}H n.m.r.:- 3.32 (1H, d, 10Hz.), 2.4-1.0 (10H, m), 1.0-0.7 (3H, m)δ.

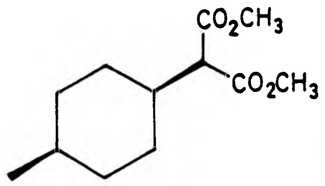
The n.m.r. spectral data showed evidence of an impurity in this material. The multiplet at 1.0-0.7δ was essentially a doublet at 0.92 (6Hz.)δ, with low intensity peaks superimposed. Also there was a low intensity doublet at 3.48 (7Hz.)δ.

A sample (20mg.) of the malonic acid was heated at 180°C for 1h. The product was a charred, black material. Extraction with chloroform provided a low yield (5mg.) of an uncharacterised material. Heating another sample at 135°C for 3h. caused no change in the material. Refluxing another sample in 2.5M aqueous hydrochloric acid[95] (8ml.) for 6h. caused no production of the acetic acid 85. Flash vacuum pyrolysis of a sample (30mg.) involved flaming the sample at low pressure (0.5mmHg.) in bulb to bulb apparatus into a bulb cooled by a cardice/acetone bath. The product was a yellow oil (10mg.) which contained the acids 85 and 86, in the ratio 83:17, of purity ~85%. The identification was made by comparison with genuine samples of these acids (vide infra) using g.c. and t.l.c. analysis.

b) By decarboethoxylation of the malonate ester, 99.

Using the method of Krapcho and Lovey,[96] a sample of 99 (150mg.) in dimethyl sulphoxide (5ml.) was treated with sodium chloride (35mg.) in water (1ml.) at reflux for 10h. No reaction was evident and the ester 99 was returned by addition of water (20ml.), extraction with dichloromethane (2x50ml.) and radial chromatography.

Using the method of Ho,[97] a sample of 99 (230mg.) was treated with boric acid (30mg.) at 170-190°C for 7h. The reaction mixture was bulb to bulb distilled to give the crude product (130mg.) as a mixture of 99 and 105. Separation of these by radial chromatography and saponification the impure 105, so obtained, gave a sample (40mg.) of the impure acids 85 and 86 in the ratio 84:16, as determined by g.c. and



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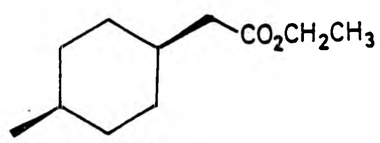

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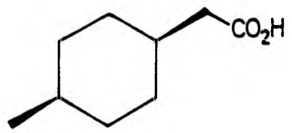

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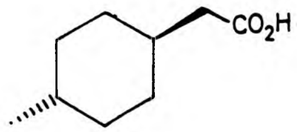

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^{1}H n.m.r.:- 3.32 (1H, d, 10Hz.), 2.4-1.0 (10H, m), 1.0-0.7 (3H, m)δ.

The n.m.r. spectral data showed evidence of an impurity in this material. The multiplet at 1.0-0.7δ was essentially a doublet at 0.92 (6Hz.)δ, with low intensity peaks superimposed. Also there was a low intensity doublet at 3.48 (7Hz.)δ.

A sample (20mg.) of the malonic acid was heated at 180°C for 1h. The product was a charred, black material. Extraction with chloroform provided a low yield (5mg.) of an uncharacterised material. Heating another sample at 135°C for 3h. caused no change in the material. Refluxing another sample in 2.5M aqueous hydrochloric acid[95] (8ml.) for 6h. caused no production of the acetic acid 85. Flash vacuum pyrolysis of a sample (30mg.) involved flaming the sample at low pressure (0.5mmHg.) in bulb to bulb apparatus into a bulb cooled by a cardice/acetone bath. The product was a yellow oil (10mg.) which contained the acids 85 and 86, in the ratio 83:17, of purity ~85%. The identification was made by comparison with genuine samples of these acids (vide infra) using g.c. and t.l.c. analysis.

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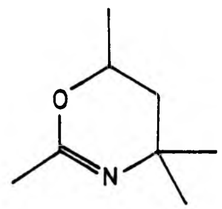

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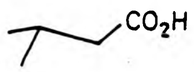

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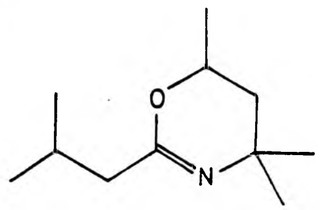


108

t.l.c. analysis and by comparison with genuine samples (vide infra).

Isolation of cis-3-methylcyclohexanol.

Commercial 3-methyl cyclohexanol (100g.) was distilled using the spinning band technique. From a 70:30 mixture of cis and trans isomers, enriched fractions were obtained, of various isomeric make-up. Thus, some material (1.5g.), of isomeric purity >99% and chemical purity >99.8%, was obtained and was identified as the cis isomer, 95.

R_T (OV1, CH_2Cl_2, 100°C) = 3.63.

^1H n.m.r.:- 3.8-3.3 (1H, m), 2.2-1.0 (9H, m), 0.92 (3H, d, 6Hz.)δ.

^{13}C n.m.r.:- 70.9 (d), 44.9 (t), 35.6 (t), 34.3 (t), 31.6 (d), 24.3 (t), 22.3 (q) p.p.m.

Silylation of the commercial 3-methylcyclohexanol was achieved in the following manner.[94] A sample of the 70:30 95:96 mixture (37.4g., 330mmol.) was dissolved in dichloromethane (100ml.) and triethylamine (33.1g., 330mmol.) in an ice bath. Trimethylsilylchloride (35.8g., 330mmol.) was added dropwise such that the reaction mixture temperature did not exceed 30°C. After 30m., water (500ml.) and dichloromethane (250ml.) was added. The separated organic phase was washed with water (250ml.), 0.5N aqueous hydrochloric acid (250ml.), water (250ml.), saturated aqueous copper (2) sulphate (2x250ml.) and saturated aqueous sodium chloride (2x250ml.). After drying, filtering and evaporation of the solvent, the crude material was spinning band distilled. Analysis by g.c. showed enrichment but not separation of the component silyl ethers. The alcohols could be returned by treatment of the silyl ethers with 10% potassium fluoride in methanol for 6h.

Preparation of ammonium acetate from 2,4,4,6-tetramethyl-dihydro-1,3-oxazine, 107.

a) By saponification of a quaternary salt of 107.[112,113]

A sample of 107 (200mg., 1.4mmol.) was dissolved in diethyl ether

(1ml.) and a large excess of iodomethane (3ml.) was added. After stirring for 60h., the solvent was evaporated to give pale yellow crystals (380mg., 96%) of the adduct. Saponification was achieved with saturated aqueous ammonium hydroxide (4ml.) at 50°C overnight. Evaporation of the reaction mixture provided ammonium acetate (100mg., 94%) identified by comparison with a genuine sample.

b) By epoxidation of 107[114] followed by saponification.

A sample of 107 (140mg., 1.0mmol.) was treated with m-chloroperoxybenzoic acid (190mg., 1.2mmol.) in dichloromethane (2ml.). The intensely blue solution was stirred for 15m. and then the solvent was evaporated. 1N Aqueous sodium hydroxide (3ml.) was added and the reaction mixture was stirred overnight at 50°C. The solution was acidified to pH=2 with 2N aqueous hydrochloric acid and extracted with diethyl ether (2x20ml.). The solvent was evaporated from the combined extracts and concentrated aqueous ammonium hydroxide (6ml.) was added. Evaporation of the reaction mixture provided the crude product that was recrystallised to give a sample of ammonium acetate (30mg., 40%) identified by comparison with a genuine sample.

Preparation of 2-(2-methylpropyl)-4,4,6-trimethyl-dihydro-1,3-oxazine, 108.

The distilled oxazine 107 (220mg., 1.6mmol.) of purity ~99.5% was dissolved in tetrahydrofuran (5ml.) at -78°C. 1.6M n-Butyllithium in hexane (1.0ml., 1.6mmol.) was added dropwise and the reaction mixture was stirred for 2h. 2-Bromopropane (150µl.) was added dropwise over 30m. and the reaction mixture was allowed to reach ambient temperature. After stirring for 12h., ice cold water (20ml.) was added and resulting solution was extracted with diethyl ether (30ml.). The separated ethereal layer was dried and filtered. Evaporation of the solvent provided the crude product which was purified by radial chromatography to

give slightly impure 108 (120mg., 40%) as an oil.

Repeating the experiment, but with one equivalent of 12-crown-4 in the reaction mixture, increased the yield of 108 to 85% of pure material. The 12-crown-4 was separated from the product, when the mixture was bulb to bulb distilled from lithium chloride.

R_F (MeOH 0.01, E.A. 0.10, P.E. 0.89) = 0.45.

^1H n.m.r.:- 4.3-3.9 (1H, m), 2.2-1.9 (2H, m), 1.9-1.1 (3H, m), 1.26 (3H, d, 6Hz.), 1.18 (6H, s), 0.92 (3H, d, 6Hz.)δ.

Repetition of this experiment provided additional material.

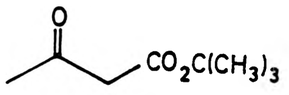
Preparation of 3-methylbutanoic acid (isovaleric acid), 80.

a) By acid catalysed hydrolysis[109] of 108.

A sample of 108 (120mg.) was treated with 2N aqueous hydrochloric acid (3ml.) at reflux for 2h. The product was isolated by extraction with diethyl ether (2x10ml.). Evaporation of the solvent from the combined extracts provided material containing many products, none of which was 80, as analysed by t.l.c. and n.m.r. spectroscopy.

b) By treatment with iodomethane followed by saponification.

A sample of 108 (500mg., 2.2mmol.) was dissolved in diethyl ether (3ml.). A large excess of iodomethane (4ml.) was added and the reaction mixture was stirred overnight. Evaporation of the solvent provided a solid, which was reacted with 1N aqueous sodium hydroxide (5ml.) overnight. The solution was washed with diethyl ether (20ml.), acidified to pH=2 with 2N aqueous hydrochloric acid and extracted with diethyl ether (3x20ml.). The combined extracts were dried and filtered. Evaporation of the solvent provided the crude product which was purified by bulb to bulb distillation. The pure product (120mg., 54%) had identical t.l.c. and n.m.r. spectral characteristics to a genuine sample of 80.



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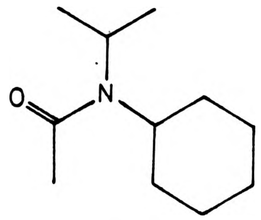

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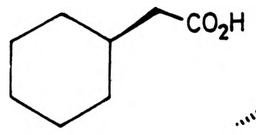

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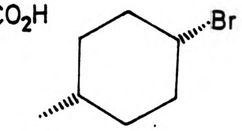

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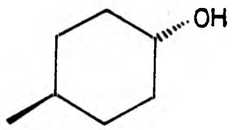

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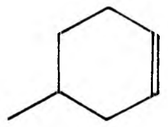


111

c) From t-butyl acetate and lithium di(2-propyl)amine.[119]

Di(2-propyl)amine (1.01g., 10mmol.) was dissolved in tetrahydrofuran (4ml.) at -78°C. 1.6M n-Butyllithium in hexane (6.25ml., 10mmol.) was added dropwise over 15m. After stirring for 1h., t-butyl acetate (1.16g., 10mmol.) was added dropwise over 15m. After stirring for 1h., 2-bromopropane (1.42g., 1.15mmol.) in hexamethylphosphoric acid triamide (2.69g.) was added dropwise over 15m. The reaction mixture was stirred for 1h. at -35°C and was then poured onto ice cold water (20ml.). After acidification to pH=2 with 4N aqueous hydrochloric acid, the mixture was extracted with diethyl ether (3x30ml.). The extracts were combined, dried and filtered. The product (400mg.) gave a positive ferric chloride test[50] for β-dicarbonyl compounds and was identified as impure 121 from its ^{1}H n.m.r. spectrum.

^{1}H n.m.r.:- 3.35 (2H, s), 2.25 (3H, s), 1.47 (9H, s)δ.

Treatment of this material with trifluoroacetic acid (5ml.) for 30m., followed by rotary evaporation, provided a trace amount of 80 by g.c. analysis.

d) From t-butyl acetate and lithium cyclohexyl(2-propyl)amine.[120]

Cyclohexyl(2-propyl)amine (2.26g., 16mmol.) was dissolved in tetrahydrofuran (16ml.) was added at -78°C to 1.6M n-butyllithium in hexane (10ml., 16mmol.) dropwise over 15m. After 10m., t-butyl acetate (1.35ml., 16mmol.) was added to the yellow solution dropwise over 10m. After 10m., this solution was added dropwise over 15m. to 2-bromopropane (2.95g., 24mmol.) in dimethylsulphoxide (7ml.) at ambient temperature. Analysis by t.l.c. after 1h. showed a single product. Diethyl ether (100ml.) was added and the resulting solution was washed with water (50ml.), dried and filtered. The crude product, contaminated with starting materials, was isolated by rotary evaporation to give impure 122, tentatively identified from its ^{1}H n.m.r. spectrum.

R_F (E.A. 0.1, P.E. 0.9) = 0.45.

^1H n.m.r.:- 3.8-3.6 (2H, m), 2.2-1.1 (11H, m), 1.82 (3H, s), 1.01 (6H, d, 7Hz.)δ.

Treatment with trifluoroacetic acid as in method c), failed to provide any detected amount of 80.

e) From t-butyl acetate and n-butyllithium with 12-crown-4.[121]

1.6M n-Butyllithium in hexane (6.9ml.) was added dropwise over 15m. to a mixture of t-butyl acetate (1.16g.) and 12-crown-4 (1.93g.) in tetrahydrofuran (20ml.) at -78°C. After 1h., 2-bromopropane (1.35g.) was added dropwise over 15m. The reaction mixture was stirred for 1h. at -78°C and then allowed to ambient temperature. Analysis of the mixture showed only starting materials by g.c. after 2h. The crude material was recovered by rotary evaporation. Distillation from lithium chloride effected separation of the 12-crown-4 and the distilled material was then treated with trifluoroacetic acid (1ml.) for 15m. Evaporation of the trifluoroacetic acid provided material that did not contain 80 as assessed by g.c. analysis.

Preparation of cyclohexylacetic acid, 76.

a) From bromocyclohexane.

Using the conditions used for the method b) preparation of 3-methylbutanoic acid 80, bromocyclohexane (134μl., 1.1mmol.) was reacted with 107 (192μl., 1.2mmol.). The crude product was distilled from lithium chloride. The distilled product was reacted with an excess of iodomethane (3ml.), followed by saponification of the salt so prepared. Work up provided a sample of 76 (40mg., 26%), which was identified by t.l.c. and mixed m.p. analysis with a genuine sample of 76.

b) From cyclohexanol.

N-Bromosuccinimide (350mg., 2.0mmol.) was dissolved in diethyl ether (5ml.). Triphenylphoshine (520g., 2.0mmol.) in diethyl ether (4ml.) was

added dropwise, followed after 30m. by cyclohexanol (200mg., 2.0mmol.) in diethyl ether (1ml.). After refluxing for 24h., the solvent was evaporated. The crude product was bulb to bulb distilled to provide a sample of the crude bromide (90mg.), which was reacted as in method a) with 107 (0.85mmol.). The product (20mg., 7%) was identical to a genuine sample by t.l.c. and melting point analysis.

Preparation of cis-4-methyl-bromocyclohexane, 110.

a) Using N-bromosuccinimide and triphenylphosphine in tetrahydrofuran.[116]

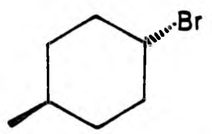
N-Bromosuccinimide (1.78g., 10mmol.) in tetrahydrofuran (40ml.) was stirred at ambient temperature. Triphenylphosphine (2.62g., 10mmol.) in tetrahydrofuran (10ml.) was added dropwise and the reaction mixture was stirred for 30m. Trans-4-methylcyclohexanol, 91, (1.00g., 8.5mmol.) in tetrahydrofuran (5ml.) was added dropwise and the reaction mixture was stirred for 2h. The solvent was carefully evaporated and the material so obtained was taken up in diethyl ether (100ml.) and water (50ml.). The separated ethereal layer was distilled to give a yellow oil (980mg.), which gave a positive Beilstein test.[50] A further distillation and radial chromatography of a portion (100mg.) provided a sample of 1,4-dibromobutane, which had identical spectral characteristics to a genuine sample.

b) Using N-bromosuccinimide and triphenylphosphine in diethyl ether.

The experiment described in method a) was repeated, using diethyl ether as the solvent, and the reaction mixture was refluxed for 50h. The crude product (730mg.) contained 91, 110 and 111 from g.c. and n.m.r. analysis. A portion of this was separated by radial chromatography. A sample of the 99% pure bromide (100mg., 34%) was so obtained.

R_T (SP1000, Et_2O, 100°C) = 6.3m.

^1H n.m.r.[115c]:- 4.8-4.5 (1H, m), 2.3-1.2)9H, m), 1.1-0.8 (3H, m)δ.



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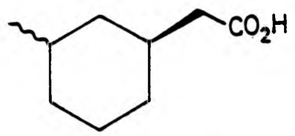
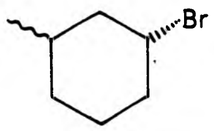


cis 157
trans 158



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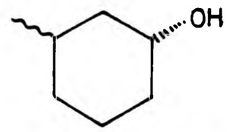
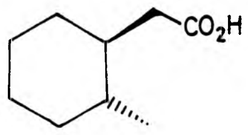
trans 115



cis 116
trans 117



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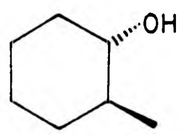

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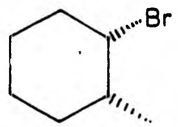

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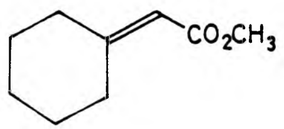

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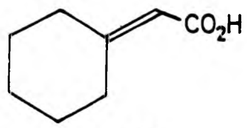


131

Attempted preparation of trans-(4-methylcyclohexyl)acetic acid, 86.

Using the procedure described for the preparation of cyclohexylacetic acid 76 via method b), trans-4-methylcyclohexanol, 91, (3.15g., 27.6mmol.) was reacted with N-bromosuccinimide and triphenylphosphine to give the impure bromide 110. This material was reacted with the anion of 107 in the manner previously described, but the acid 85 could not be detected in the product mixture. The elimination product 111 was identified from its ^{1}H n.m.r. spectrum by comparison with a genuine sample.

Attempted preparation of trans-4-methyl bromocyclohexane, 118.

a) Using N-bromosuccinimide and triphenylphosphine.

Cis-4-methylcyclohexanol 93 (115mg., 1.0mmol.), which had been purified by radial chromatography, was subjected to conditions similar to those described for the preparation of cyclohexylacetic acid 76 via method b). The products were separated by radial chromatography, but 118 was not isolated. The major product was the elimination product 111, identified by comparison of n.m.r. and g.c. data with a genuine sample.

b) Using diethyl azodicarboxylate, triphenylphosphine and zinc bromide.[117]

Cis-4-methylcyclohexanol (115mg., 1.0mmol.) and triphenylphosphine (790mg., 3.0mmol.) and zinc bromide (225mg., 1.0mmol.) were stirred at ambient temperature in tetrahydrofuran (20ml.). Diethyl azodicarboxylate (520mg., 3.ommol.) in tetrahydrofuran (2ml.) was added dropwise and the reaction mixture was then stirred for 60h. Silica chromatography and bulb to bulb distillation gave fractions which did not contain the bromide 118. The alkene 111 was identified by g.c. and n.m.r. spectroscopy by comparison with the genuine material.

Attempted preparation of the cis- and trans- isomers of (3-methylcyclohexyl)acetic acid, 157 and 158 via the cis- and trans-3-methylbromocyclohexane intermediates, 114 and 115.

A mixture of 72:28 cis:trans isomers of 3-methylcyclohexanol 116 and 117 (3.50g., 31mmol.) was reacted with N-bromosuccinimide and triphenylphosphine in a fashion similar to the conditions used to prepare cyclohexylacetic acid, 76. Purification procedures provided an impure sample (800mg., 16%) of the bromides 115 and 114 in the ratio 89:11, as analysed by g.c. and ^{1}H n.m.r. spectroscopy.[115c] Likewise, a sample (1.75g., 15mmol.) of 116 and 117 of ratio 92:8 provided the trans-bromide 115 nearly exclusively. The 89:11 mixture of 115 and 114 was subjected to the anion of 107, quaternisation with iodomethane and saponification sequence. Analysis of the product showed a trace of the cis- acid 157, identified by comparison with a genuine sample (vide infra).

Attempted preparation of trans-(2-methylcyclohexyl)acetic acid, 90.

Using conditions similar to those described for the preparation of cyclohexylacetic acid 76 via method b), trans-2-methylcyclohexanol 112 (2.0g., 1.7mmol.) provided a sample of the cis-bromide 113 (190mg., 7%), after radial chromatography, as an oil of 94% purity.

R_T (SP1000, Et_2O, 100°C) = 4.7m.

^{1}H n.m.r.[115c]:- 4.7-4.4 (1H, m), 2.3-1.1 (9H, m), 1.1-0.8 (3H, m)δ.

Reaction of this bromide with the anion of 107, quaternisation with iodomethane and saponification failed to provide any isolable amounts of 90. However, g.c. analysis of the products implied the presence of a small amount of 90, by comparison with a genuine sample (vide infra).

Preparation of methyl cyclohexylideneacetate, 132, and cyclohexylideneacetic acid, 131.

To a solution of trimethylphosphonoacetate (2.0g., 11mmol.) in toluene (35ml.), which had been freshly distilled from calcium hydride,

was added in portions over a period of 10m. sodium hydride as a 50% dispersion in mineral oil (520mg., 11mmol.). After stirring for 30m., distilled cyclohexanone 130 (980mg., 10mmol.) was added dropwise with stirring over 10m., then stirred overnight. T.l.c. analysis showed no cyclohexanone. The reaction mixture was filtered through silica gel in a sintered glass funnel. The solvent was evaporated and traces of residual toluene removed by azeotroping with dry methanol. Radial chromatography gave a sample of methyl cyclohexylideneacetate 132 (720mg., 47%) of purity 99.8%, as a colourless oil.

R_F (E.A. 0.1, P.E. 0.9) = 0.63.

R_T (SP1000, P.E., 100°C) = 17.5m.

i.r. (film):- 2930, 1720, 1650, 1440, 1240, 1210, 1160, 1130 cm^{-1}.

m.s.:- m/e = 154 (100%), 123 (48), 95 (54), 94 (43), 79 (49), 67 (52), 41 (44), 39 (63). $M^{\cdot+}$(154) = $C_9H_{14}O_2$.

^1H n.m.r. 5.65 (1H, s), 3.70 (3H, s), 3.1-2.7 (1H, m), 2.4-2.0 (2H, m), 1.9-1.4 (6H, m)δ.

^{13}C n.m.r.:- 167.1 (s), 163.5 (s), 112.8 (d), 50.6 (q), 38.1 (t), 30.0 (t), 28.8 (t), 27.9 (t), 26.4 (t) p.p.m.

To a sample of the methyl ester 132 (310mg., 2.0mmol.), so prepared, was added 2N potassium hydroxide in 50:50 aqueous methanol (3ml.). After refluxing overnight, water (15ml.) was added, followed by cold concentrated hydrochloric acid until the reaction mixture was at pH=2. Extraction with diethyl ether (3x20ml.) gave a combined organic phase which was washed with water (10ml.), dried and filtered. After solvent evaporation, a white crystalline solid (250mg., 90%) was obtained. Recrystallisation from formic acid provided white needle crystals of >99.8% pure 131 (160mg., 57%).

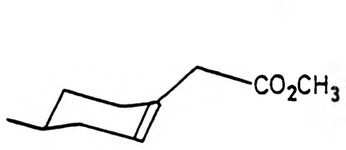
m.p. = 90-1°C, (lit.[129] = 88°C).

R_F (MeOH 0.02, E.A. 0.20, P.E. 0.78) = 0.44.

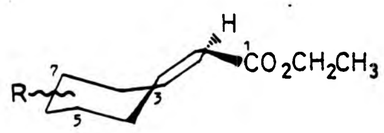
R=H 130
R=4-CH_3 133
R=3-CH_3 139
R=2-CH_3 142

R=H 132
R=6-CH_3 134
R=5-CH_3 137
R=7-CH_3 138
R=4-CH_3 143
R=8-CH_3 144

R=H 131
R=6-CH_3 136
R=5-CH_3 140
R=7-CH_3 141
R=4-CH_3 148
R=8-CH_3 147



135



R=4-CH_3 145
R=8-CH_3 146

R_T (SP1000, Et_2O, 150°C) = 34.6m.

i.r. (CCl_4):- 2920, 2840, 1690, 1640, 1270, 1240, 1220, 1180 cm^{-1}. (3500–2500 cm^{-1}).

m.s.:- m/e = 140 (100%), 97 (42), 95 (45), 81 (31), 80 (74), 67 (34), 41 (38), 39 (42). $M^{\cdot+}$(140) = $C_8H_{12}O_2$.

^1H n.m.r.:- 5.65 (1H, s), 3.0–2.7 (2H, m), 2.4–2.0 (2H, m), 1.85–1.40 (6H, m)δ.

^{13}C n.m.r.:- 172.6 (s), 166.3 (s), 112.8 (d), 38.2 (t), 30.2 (t), 28.7 (t), 27.9 (t), 26.3 (t) p.p.m.

Preparation of methyl (4-methylcyclohexylidene)acetate, 134 and (4-methylcyclohexylidene)acetic acid, 136.

To a solution of trimethylphosphonoacetate (18.2g., 100mmol.) in toluene (120ml.), which had been freshly distilled from calcium hydride, was added in portions, over a period of 10m., sodium hydride as a 50% despersion in mineral oil (4.8g., 100mmol.), with dry nitrogen flowing through the apparatus. After stirring for 30m., 4-methylcyclohexanone 133 (11.2g., 100mmol.) was added dropwise with stirring over 15m. In order to retain mobility, extra toluene (30ml.) was added. After stirring for 1h., the reaction mixture was heated to 65°C for 15m., then allowed to reach ambient temperature with constant stirring. T.l.c. implied that there was no unreacted 133. The reaction mixture was washed with water (3x100ml.), dried over calcium chloride, filtered and the solvent evaporated. A sample (3.0g.) of the crude product (21.4g.) so obtained, was purified by repeated radial chromatography to give ~99.8% pure 134 (930mg., 40%).

R_F (E.A. 0.1, P.E. 0.9) = 0.65.

R_T (SP1000, Et_2O, 100°C) = 19.8m.

i.r. (film):- 2920, 2845, 1720, 1650, 1435, 1195, 1150, 1130 cm^{-1}.

m.s.:- m/e = 168 (100%), 137 (30), 126 (67), 111 (42), 94 (50), 67 (32), 41 (35), 39 (30). M^{+}(168) = $C_{10}H_{16}O_2$.

^{1}H n.m.r.:- 5.60 (1H, s), 3.8-3.5 (1H, m), 3.64 (3H, s), 2.4-1.0 (8H, m), 0.90 (3H, d, 7Hz.)δ.

^{13}C n.m.r.:- 167.2 (s), 163.4 (s), 112.7 (d), 50.7 (q), 37.3 (t), 36.5 (t), 35.8 (t), 32.3 (d), 29.0 (t), 21.6 (q) p.p.m.

Material of 95% purity could be obtained by bulb to bulb distillation of the crude product. Thus, a sample (2.0g.) of crude 134 gave 95% pure 134 (1.45g., 93%), contaminated with another product, which could be isolated impure by repeated radial chromatography. Its identity was confirmed as 135, by comparison of chromatographic and spectral data with a pure sample prepared by an alternative procedure (vide infra).

A sample of the 95% pure ester 134 (840mg., 5.0mmol.) was refluxed overnight with 2N potassium hydroxide in 50:50 aqueous methanol (3ml.). Water (30ml.) was added and the aqueous layer extracted with diethyl ether (30ml.), then acidified to pH=2 with cold concentrated hydrochloric acid. The aqueous layer was extracted with diethyl ether (5x30ml.) and these extracts were combined, dried and filtered. The solvent was evaporated to give impure 136 (720mg., 94%) as a yellow oil, which crystallised on standing. Radial chromatography followed by repeated recrystallisation from hexane gave white crystals of 136 (240mg., 31%), which were >99.8% pure.

m.p. 65-6°C (lit.[136] 60-2°C).

R_F (MeOH 0.02, E.A. 0.20, P.E. 0.78) = 0.21.

R_T (SP1000, Et_2O, 150°C) = 43.0m.

i.r. (CCl_4):- 2950, 2935, 2870, 2860, 1695, 1650, 1275, 1220 cm^{-1}. (3400-2500 cm^{-1}).

m.s.:- m/e = 154 (100%), 139 (38), 112 (84), 111 (44), 97 (35), 94 (53), 69 (28), 41 (34). $M^{+\cdot}(154) = C_9H_{14}O_2$.

^1H n.m.r.:- 5.64 (1H, s), 3.9-3.6 (1H, m), 2.5-1.1 (8H, m), 0.92 (3H, d, 6Hz.)δ.

^{13}C n.m.r.:- 172.6 (s), 166.2 (s), 112.7 (d), 37.6 (t), 36.5 (t), 35.8 (t), 32.1 (d), 29.3 (t), 21.5 (q) p.p.m.

Preparation of enriched (Z)-and (E)-isomeric mixtures of methyl (3-methylcyclohexylidene)acetate, 137 and 138, and (3-methylcyclohexylidene)acetic acid, 140 and 141.

To a solution of trimethylphosphonoacetate (2.0g., 11mmol.) in toluene (35ml.), which had been freshly distilled from calcium hydride, was added sodium hydride as a 50% dispersion in mineral oil (520mg., 11mmol.) in portions over a period of 10m. After stirring 30m., 3-methylcyclohexanone 139 (1.12g., 10mmol.) was added dropwise over 10m. The ketone 139 was shown to be 97.8% pure by g.c., with two impurities which possessed identical retention times to the isomeric 3-methyl cyclohexanols. After stirring the reaction mixture for 48h., t.l.c. implied that it contained no residual 139. Filtration through a sintered glass funnel containing silica gel, followed by evaporation of the solvent, gave the crude product. This was further purified by bulb to bulb distillation and radial chromatography to give a mixture of 137 and 138 (860mg., 51%). The ratio of the products 137:138 was 54:46. Further radial chromatographic purification gave a sample of purity >99.8% and isomeric ratio 50:50.

R_F (E.A. 0.1, P.E. 0.9) = 0.44.

R_T (SP1000, Et_2O, 150°C) = 7.4 (50%), 6.9 (50%)m.

i.r. (film):- 2940, 2920, 2850, 1720, 1645, 1210, 1195, 1155 cm^{-1}.

Further extensive radial chromatography, in which enriched fractions were further enriched in a stepwise fashion, provided a sample of

enriched 137 (70mg.) of chemical purity >99.8% and containing 85% of the (Z) isomer 137. Likewise, a sample of enriched 138 (40mg.) of purity >99.8%, containing 80% of the (E) isomer 138, was obtained.

(137)

R_T (SP1000, Et_2O, 130°C) = 7.4m.

m.s.:- m/e = 168 (100%), 153 (89), 121 (56), 94 (59), 93 (54), 67. (49), 41 (53), 39 (49). $M^{+\cdot}$(168) = $C_{10}H_{16}O_2$.

^1H n.m.r.:- 5.60 (1H, s), 3.8-3.5 (1H, m), 3.66 (3H, s), 2.4-1.1 (8H, m), 0.96 (3H, d, 6Hz.)δ.

^{13}C n.m.r.:- 167.1 (s), 162.9 (s), 112.8 (d), 50.6 (q), 37.9 (t), 37.6 (9t), 34.7 (t), 34.2 (d), 27.4 (t), 22.2 (q) p.p.m.

(138)

R_T (SP1000, Et_2O, 130°C) = 6.9m.

m.s.:- m/e = 168 (100%), 153 (90), 121 (57), 94 (52), 93 (55), 67 (49), 41 (54), 39 (56). $M^{+\cdot}$(168) = $C_{10}H_{16}O_2$.

^1H n.m.r.:- 5.60 (1H, s), 3.8-3.5 (1H, m), 3.66 (3H, s), 2.4-1.1 (8H, m), 0.94 (3H, d, 6Hz.)δ.

^{13}C n.m.r.:- 167.2 (s), 163.0 (s), 112.8 (d), 50.7 (q), 46.1 (t), 34.9 (d), 34.6 (t), 29.3 (t), 26.6 (t), 22.1 (q) p.p.m.

The enriched samples, so obtained, were saponified in a similar fashion. 2N Potassium hydroxide in 50:50 aqueous methanol (1.5ml.) was added to the sample and then heated at 50°C with stirring for 20h. Allowing the reaction mixture to reach ambient temperature, water (6ml.) and hexane (6ml.) was added. The aqueous layer was separated and acidified to pH=2 with cold concentrated hydrochloric acid. Extraction with hexane (3x10ml.) gave a combined organic phase, which was washed with water (6ml.) and then the solvent was evaporated. Residual water was azeotroped with dry ethanol. A solid was thus obtained. Using the

enriched 137 sample (40mg.) as the starting material, enriched 140 (30mg., 82%) was obtained as a white solid. Sublimation provided a >99.8% pure mixture of 140 and 141 (20mg., 55%) in the ratio 81:19 as white crystals.

R_F (MeOH 0.02, E.A. 0.20, P.E. 0.78) = 0.48.

R_T (SP1000, $HCCl_3$, 180°C) = 13.6 (81%), 13.0 (19%)m.

i.r. (CCl_4):- 2930, 1695, 1650, 1420, 1275, 1260, 1230, 1185 cm^{-1}. (3400-2500 cm^{-1}).

m.s.:- m/e = 154 (99%), 139 (100), 111 (63), 94 (100), 69 (70), 55 (71), 41 (88), 39 (83). $M^{+\cdot}$(154) = $C_9H_{14}O_2$.

^1H n.m.r.:- 5.62 (1H, s), 3.9-3.6 (1H, m), 2.5-1.1 (8H, m), 0.97 (3H, d, 6Hz.)δ.

^{13}C n.m.r.:- 171.8 (s), 165.8 (s), 112.6 (d), 38.0 (t), 37.8 (t), 34.5 (t), 34.2 (d), 27.3 (t), 22.2 (q) p.p.m.

Using the enriched sample of 138 (70mg.), as the starting material, enriched 141 (30mg., 47%) was obtained as a white solid. Sublimation of this product provided a >99.8% pure sample of the mixture of 140 and 141 in the ratio 13:87, as white crystals.

R_F (MeOH 0.02, E.A. 0.20, P.E. 0.78) = 0.46.

R_T (SP1000, $HCCl_3$, 180°C) = 14.1 (13%), 13.4 (87%)m.

i.r. (CCl_4)[136]:- 2940, 2920, 2860, 1685, 1640, 1250, 1220, 1210 cm^{-1}. (3400-2400 cm^{-1}).

m.s.:- m/e = 154 (100%), 139 (71), 111 (43), 94 (92), 69 (52), 67 (43), 41 (50), 39 (44). $M^{+\cdot}$(154) = $C_9H_{14}O_2$.

^1H n.m.r.[136]:- 5.62 (1H, s), 3.8-3.5 (1H, m), 2.5-1.1 (8H, m), 0.96 (3H, d, 6Hz.)δ.

^{13}C n.m.r.[136]:- 172.3 (s), 166.0 (s), 112.8 (d), 46.4 (t), 35.0 (d), 34.5 (t), 29.6 (t), 26.7 (t), 22.1 (q) p.p.m.

A sample of the enriched 140 mixed with a sample of the enriched 141

gave two peaks by g.c. Together with spectral evidence, the equivalence of the major peak of one sample with the minor peak of the other was demonstrated.

Preparation of enriched methyl (E)-(2-methylcyclohexylidene)acetate, 144, and enriched (E)-(2-methylcyclohexylidene)acetic acid, 147.

To a solution of methyl diethylphosphonoacetate (23.12g., 110mmol.) in toluene (350ml.), which had been freshly distilled from calcium hydride, was added sodium hydride (2.6g., 110mmol.) in portions over 10m. After stirring for a further 30m., 2-methylcyclohexanone 142 (11.2g., 100mmol.) was added dropwise over 10m. After stirring for a further 24h., the reaction mixture was filtered through silica gel in a sintered glass funnel. The solvent was then evaporated and traces of toluene removed by azeotroping with methanol. Distillation gave a mixture of 143, 144, 145 and 146, by g.c., in ~80% yield. The mixture was treated with 2N potassium hydroxide in 50:50 aqueous methanol (90ml.) at 50°C overnight. Addition of water (50ml.), extraction with ether (3x50ml.), acidification of the aqueous phase with cold concentrated hydrochloric acid and extraction of this phase with diethyl ether (3x70ml.) gave, after evaporation, the acids 147 and 148 (8.3g., 75%) of 97% chemical purity. The use of recrystallisation, radial chromatography or sublimation failed to satisfactorily separate the isomeric acids. A sample of the acid mixture of 147 and 148 (6.5g.) was dissolved in dried methanol (70ml.) and a catalytic amount of p-toluenesulphonic acid (50mg.) was added. Heating at 50°C for 22h. gave an impure mixture of the corresponding methyl esters 143 and 144, which were isolated by adding saturated aqueous sodium carbonate (40ml.), extracting with an ethyl acetate/hexane mixture of ratio 25:75 (5x50ml.) and evaporation of the solvent from the combined organic phase. The ratio of 143:144 was 25:75 by g.c. Repeated radial chromatography

enabled a sample (600mg.) of 143:144, 4:96 to be isolated, of >99.8% purity.

R_T (SP1000, P.E., 100°C) = 26.2 (96%), 20.7 (4%)m.

^1H n.m.r.:- 5.61 (1H, s), 3.70 (3H, s), 3.7-3.4 (1H, m), 2.4-1.2 (8H, m), 1.07 (3H, d, 7Hz.)δ.

^{13}C n.m.r.:- (143) 167.2 (s), 112.6 (d), 50.6 (q), 33.2 (t), 33.0 (t), 30.9 (d), 28.3 (t), 20.4 (t), 18.4 (q) p.p.m.

(144) 167.2 (s), 110.5 (d), 50.6 (q), 39.9 (d), 37.1 (t), 29.4 (t), 28.4 (t), 25.2 (t), 18.4 (q) p.p.m.

Note that the ^{13}C n.m.r. spectral values given were derived from a 36:64 ratio mixture of 143 and 144, obtained during the enrichment process.

A sample (200mg.) of the enriched ester so prepared was reacted with 2N potassium hydroxide in 50:50 aqueous methanol (6ml.) at 50°C overnight. Addition of water (5ml.), extraction with diethyl ether (3x5ml.) and acidification of the aqueous phase with cold concentrated hydrochloric acid, gave a cloudy solution. This solution was extracted with diethyl ether (3x10ml.) and the combined extracts gave, after evaporation, the respective acids. Sublimation gave white crystals of 147 and 148 (150mg., 82%) in a 96:4 ratio and >99.8% purity.

R_F (MeOH 0.02, E.A. 0.20, P.E. 0.78) = 0.55.

R_T (SP1000, EtOH, 180°C) = 13.6 (96%), 11.9 (4%)m.

i.r. (CCl_4):- 2970, 2930, 2860, 1690, 1640, 1270, 1250, 1210 cm^{-1}. (3400-2500 cm^{-1}).

m.s.:- m/e = 154 (47%), 95 (29), 94 (100), 81 (25), 79 (36), 67 (40), 41 (33), 28 (73). $M^{\cdot+}$(154) = $C_9H_{14}O_2$.

^1H n.m.r.:- 5.60 (1H, s), 3.7-3.3 (1H, m), 2.5-1.2 (8H, m), 1.08 (3H, d, 7Hz.)δ.

^{13}C n.m.r.:- 171.8 (s), 169.3 (s), 110.6 (d), 40.2 (d), 37.1 (t), 29.6 (t), 28.4 (t), 25.1 (t), 18.4 (q) p.p.m.

Preparation of enriched ethyl (Z)-(2-methylcyclohexylidene)acetate, 145, and (Z)-(2-methylcyclohexylidene)acetic acid, 148.

1.2M n-Butyllithium[186] in hexane(6ml., 7.2mmol.) was added dropwise to cyclohexyl(2-propyl)amine (1.05g., 7.4mmol.) with rapid stirring at 0°C over a period of 10m. After stirring for a further 10m., freshly purified and dried tetrahydrofuran (16ml.) was added. The temperature was lowered to -78°C and the reaction mixture was stirred a further 10m. Ethyl trimethylsilylacetate (1.15g., 7.2mmol.) was then added dropwise over 10m., and the reaction stirred a further 30m. Keeping the temperature at -78°C, dried 2-methylcyclohexanone (810mg., 7.2mmol.) was added dropwise over 15m. The reaction mixture was allowed to reach ambient temperature and was stirred overnight. Water (3ml.) was added and the reaction mixture acidified to pH=2 with cold 2N aqueous hydrochloric acid. Extraction with hexane (2x50ml.) gave an organic solution which was separated from the aqueous phase and washed with more of the acid (30ml.) and water (30ml.), then evaporated. A t.l.c. showed a complex mixture of products. The ratio of 145:146 was 80:20 by g.c. The most mobile, by t.l.c., ultraviolet active component was separated by radial chromatography into fractions which totalled 820mg. in mass. One of these fractions was enriched in 145 and was further purified by bulb to bulb distillation, to yield the ethyl esters 145 and 146 (210mg., 20%) in the ratio 90:10 and of purity ~98.5%.

R_T (SP1000, $HCCl_3$, 130°C) = 11.2 (10%), 9.4 (89%)m.

^1H n.m.r.[139]:- 5.55 (1H, s), 4.2-3.9 (1H, m), 4.13 (2H, q, 7Hz.), 2.6-1.2 (8H, m), 1.28 (3H, t, 7Hz.), 1.15 (3H, d, 7Hz.)δ.

^{13}C n.m.r.:- 167.1 (s), 166.4 (s), 113.1 (d), 59.4 (t), 33.2 (t), 33.0 (t), 30.9 (d), 28.3 (t), 20.4 (t), 18.4 (q), 14.4 (q) p.p.m.

The remaining fractions of the impure product were combined and g.c. analysis showed an isomeric ratio of 82:18 145:146 and a purity of ~95%.

To a 98.5% pure sample of 145:146 (200mg., 1.1mmol.) of ratio 90:10 (vide supra) was added 2N potassium hydroxide in 50:50 aqueous methanol (6ml.). The reaction mixture was stirred at ~50°C for 17h., and then water (20ml.) and hexane (20ml.) was added. Separation and acidification of the aqueous layer to pH=2 was followed by extraction with dichloromethane (3x40ml.). The organic layers were separated and combined, then washed with water (20ml.). Solvent evaporation, using ethanol to azeotrope residual water, yielded a yellow solid which was sublimed at reduced pressure to give the acids 147 and 148 (150mg., 91%) in the ratio of 9:91 and purity >99.8%.

R_F (MeOH 0.02, E.A. 0.20, P.E. 0.78) = 0.47.

R_T (SP1000, $HCCl_3$, 180°C) = 14.9 (9%), 13.1 (91%)m.

i.r. (CCl_4):- 2970, 2940, 2860, 1690, 1640, 1270, 1250, 1195 cm^{-1}. (3400-2500 cm^{-1}).

m.s.:- m/e = 154 (62%), 97 (45), 94 (100), 79 (51), 67 (55), 55 (51), 41 (62), 39 (62). $M^{\ddagger}$(154) = $C_9H_{14}O_2$.

^1H n.m.r.:- 5.58 (1H, s), 4.2-3.8 (1H, m), 2.8-1.3 (8H, m), 1.16 (3H, d, 7Hz.)δ.

^{13}C n.m.r.:- 172.4 (s), 170.5 (s), 112.6 (d), 33.4 (t), 33.0 (t), 30.9 (d), 28.2 (t), 20.2 (t), 18.3 (q) p.p.m.

The two g.c. peaks were found to possess identical retention times to the previously prepared (E) enriched acids, by analysis of a mixture of the two products.

Preparation of methyl (4-methylcyclohex-1-enyl)acetate, 135.

Cyclohexyl(2-propyl)amine (560mg., 2.0mmol.) was dissolved in tetrahydrofuran (6ml.), which had been freshly distilled from calcium hydride. After cooling the mixture to -78°C with stirring, 1.3M

149

132

150

156

76

n-butyllithium in hexane(3.1ml., 2.0mmol.) was added dropwise over 10m. After stirring for 30m., a sample of the previously prepared 95% pure 134 (330mg., 2.0mmol.) in tetrahydrofuran (2ml.) was added dropwise over 10m. The reaction mixture was stirred for 3h. at -78°C, and then allowed to reach ambient temperature. After stirring for a further 34h., dry methanol (4ml.) was added dropwise over 10m. The orange coloured solution became yellow on acidification with cold 2N aqueous hydrochloric acid. Extraction with diethyl ether (3x10ml.) gave a combined organic phase of yellow colour. After storage overnight at 4°C over anhydrous potassium carbonate, the solution was filtered and the solvent evaporated to give the impure product. This impure material was subjected to bulb to bulb distillation, radial chromatography and further bulb to bulb distillation. This treatment gave the product 135 (30mg., 9%) as a colourless oil of purity ~99.8%.

R_F (E.A. 0.1, P.E. 0.9) = 0.47.

R_T (SP1000, Et_2O, 100°C) = 16.2m.

i.r. (film):- 2940, 2905, 2860, 1740, 1430, 1255, 1165, 1125 cm^{-1}.

m.s.:- m/e = 108 (19%), 95 (19), 94 (100), 93 (24), 85 (19), 79 (32), 67 (23), 28 (38). $M^{+\cdot}$(168) = $C_{10}H_{16}O_2$.

^1H n.m.r.:- 5.55 (1H, s), 3.68 (3H, s), 2.96 (2H, s), 2.3-1.1 (7H, m), 0.94 (3H, d, 7Hz.)δ.

^{13}C n.m.r.:- 172.3 (s), 130.6 (s), 125.2 (d), 51.6 (q), 43.1 (t), 33.9 (t), 30.9 (t), 28.4 (t), 28.0 (d), 21.6 (q) p.p.m.

Preparation of cyclohex-1-enylacetic acid, 149.

Trimethylphosphonoacetate (2.0g., 11mmol.) was dissolved in freshly distilled toluene (35ml.). 50% Sodium hydride in mineral oil (520mg., 11mmol.) was added in portions over 10m. and the mixture stirred for 30m. Cyclohexanone 130 (980mg., 10mmol.) was added dropwise over 10m. and the mixture stirred overnight. The mixture was filtered through

silica in a sintered glass tunnel and then the solvent was evaporated, azeotroping the residual toluene with methanol. Bulb to bulb distillation gave the ester 132 (1.21g.) impure with the ketone 130. Treatment of a sample (1.0g.) of this impure ester, with 2N potassium hydroxide in 50:50 aqueous methanol at reflux temperature for 6h., gave a single phase. Water (45ml.) was added and the reaction mixture was acidified to pH=2 with concentrated hydrochloric acid. Extraction with diethyl ether (3x60ml.) gave a combined organic phase, which was washed with water (30ml.) and saturated aqueous sodium chloride solution (30ml.), dried and filtered. Evaporation of the solvent gave the conjugated acid 132 (790mg.) as white crystals, which were purified by recrystallisation from aqueous formic acid to give crystals (660mg.) of m.p. = 88-90°C. A solution of cyclohexyl(2-propyl)amine (3.3ml., 20mmol.) in tetrahydrofuran (30ml.) was cooled to -78°C and 1.6M n-butyllithium in hexane (12.5ml., 20mmol.) was added dropwise over 10m. After stirring for 30m., this solution was added to the crystals dropwise over 10m. The reaction mixture was stirred for 3h. at -78°C, then allowed to reach ambient temperature and stirred overnight. Methanol (20ml.) was added dropwise over 10m. and the reaction mixture was stirred overnight. Evaporation of the solvent to a quarter of the original volume was followed by the addition of water (10ml.). Acidification with 2N aqueous hydrochloric acid gave a solution which was extracted with diethyl ether (3x50ml.). The combined extracts were washed with water (10ml.), dried and filtered. Evaporation of the solvent gave the impure product (690mg.) as a brown oil. Radial chromatography, recrystallisation from aqueous formic acid and sublimation at high vacuum gave the >99.8% product 149 (160mg., 14% overall) as white crystals.

m.p. = 33-34°C (lit.[141] = 34°C).

silica in a sintered glass tunnel and then the solvent was evaporated, azeotroping the residual toluene with methanol. Bulb to bulb distillation gave the ester 132 (1.21g.) impure with the ketone 130. Treatment of a sample (1.0g.) of this impure ester, with 2N potassium hydroxide in 50:50 aqueous methanol at reflux temperature for 6h., gave a single phase. Water (45ml.) was added and the reaction mixture was acidified to pH=2 with concentrated hydrochloric acid. Extraction with diethyl ether (3x60ml.) gave a combined organic phase, which was washed with water (30ml.) and saturated aqueous sodium chloride solution (30ml.), dried and filtered. Evaporation of the solvent gave the conjugated acid 132 (790mg.) as white crystals, which were purified by recrystallisation from aqueous formic acid to give crystals (660mg.) of m.p. = 88-90°C. A solution of cyclohexyl(2-propyl)amine (3.3ml., 20mmol.) in tetrahydrofuran (30ml.) was cooled to -78°C and 1.6M n-butyllithium in hexane (12.5ml., 20mmol.) was added dropwise over 10m. After stirring for 30m., this solution was added to the crystals dropwise over 10m. The reaction mixture was stirred for 3h. at -78°C, then allowed to reach ambient temperature and stirred overnight. Methanol (20ml.) was added dropwise over 10m. and the reaction mixture was stirred overnight. Evaporation of the solvent to a quarter of the original volume was followed by the addition of water (10ml.). Acidification with 2N aqueous hydrochloric acid gave a solution which was extracted with diethyl ether (3x50ml.). The combined extracts were washed with water (10ml.), dried and filtered. Evaporation of the solvent gave the impure product (690mg.) as a brown oil. Radial chromatography, recrystallisation from aqueous formic acid and sublimation at high vacuum gave the >99.8% product 149 (160mg., 14% overall) as white crystals.

m.p. = 33-34°C (lit.[141] = 34°C).

R_F (MeOH 0.02, E.A. 0.20, P.E. 0.78) = 0.33.

R_T (SP1000, Et_2O, 150°C) = 34.5m.

m.s.:- m/e = 140 (23%), 95 (12), 81 (31), 80 (100), 79 (27), 67 (16), 41 (20), 39 (18). $M^{+\cdot}$(140) = $C_8H_{12}O_2$.

^1H n.m.r.:- 5.63 (1H, s), 3.00 (2H, s), 2.3-1.8 (4H, m), 1.8-1.4 (4H, m)δ.

^{13}C n.m.r.:- 178.3 (s), 130.5 (s), 126.2 (d), 43.4 (t), 28.4 (t), 25.4 (t), 22.8 (t), 22.0 (t) p.p.m.

Purification of cyclohex-2-enylacetic acid, 150.

A sample of 93% pure 150 (1.00g.) was purified by use of radial chromatography and sublimation onto a cardice cold-finger. The product (300mg.) was a colourless oil of >99.8% purity.

R_F (MeOH 0.02, E.A. 0.20, P.E. 0.78) = 0.41.

R_T (SP1000, P.E., 150°C) = 33.6m.

i.r. (film)[141]:- 3020, 2920, 2860, 2840, 1710, 1435, 1410, 1290 cm^{-1}. (3500-2500 cm^{-1}).

m.s.:- m/e = 81 (40%), 80 (100), 79 (45), 77 (13), 67 (20), 53 (15), 41 (28), 39 (28). $M^{+\cdot}$(140) = $C_8H_{12}O_2$.

^1H n.m.r.[141]:- 6.1-5.4 (2H, m), 3.1-0.9 (9H, m)δ.

^{13}C n.m.r.:- 179.4 (s), 129.8 (d), 128.2 (d), 40.6 (t), 32.0 (d), 28.8 (t), 25.0 (t), 21.0 (t) p.p.m.

Purification of methyl cyclohexylacetate, 156.

Commercial methyl cyclohexylacetate 156 (300mg.) of purity ~99% was purified by radial chromatography to give >99.8% pure 156 (220mg.).

R_T (SP1000, Et_2O, 100°C) = 10.0m.

R_T (SP1000, Et_2O, 130°C) = 4.6m.

Purification of cyclohexylacetic acid, 76.

Commercial cyclohexylacetic acid 76 (300mg.) was recrystallised from hexane to give 76 (220mg.) of purity >99.8%.

R_T (SP1000, Et_2O, 150°C) = 23.7m.

134

cis 152
trans 153

cis 85
trans 86

cis 159
trans 160

cis 154
trans 87

137

138

Preparation of a mixture of cis and trans isomers of methyl (4-methylcyclohexyl)acetate, 152 and 153.

a) Using magnesium in methanol.[131]

Methyl (4-methylcyclohexylidene)acetate 134 (1.00g., 5.9mmol.) of 95% purity was dissolved in methanol (75ml.). Magnesium turnings (5.7g., 237mmol.) were added and the reaction stirred until initiated. When reacting vigorously, the reaction mixture was kept below 30°C by cooling in an ice-bath. After stirring for 28h., ice cold 6N aqueous hydrochloric acid (120ml.) was added dropwise over 30m. The aqueous phase was extracted with dichloromethane (3x100ml.). The combined organic extracts were washed with water (50ml.) and the solvent was evaporated, azeotroping the residual water with ethanol. The ratio of the resulting product mixture was 59:41 of 152:153. Radial chromatography provided a sample (680mg., 67%) of >99.8% pure 152/153 in a similar ratio, as a colourless oil. Only at high loadings, which caused unacceptable loss of resolution, could the compound mixture be observed on t.l.c. plates using conventional visualisation agents. This property was general for the saturated methyl esters prepared here.

R_T (SP1000, P.E., 130°C) = 5.61 (41%), 6.17 (59%)m.

i.r. (film):- 2945, 2920, 2850, 1745, 1440, 1290, 1170, 1125 cm^{-1}.

m.s.:- m/e = 97 (25%), 96 (27), 81 (22), 75 (62), 74 (100), 55 (46), 43 (41), 41 (38). M^{+}(170) = $C_{10}H_{18}O_2$.

^1H n.m.r.:- (152) 3.65 (3H, s), 2.5-1.1 (12H, m), 0.91 (3H, d, 7Hz.)δ.

(153) 3.65 (3H, s), 2.5-1.1 (12H, m), 0.87 (3H, d, 6Hz.)δ.

^{13}C n.m.r.:- (152) 173.6 (s), 51.2 (q), 39.0 (t), 32.4 (d), 30.5 (d), 30.5 (t), 28.6 (t), 20.2 (q) p.p.m.

(153) 173.6 (s), 51.2 (q), 41.9 (t), 34.9 (t), 34.7 (d), 33.0 (t), 32.4 (d), 22.5 (q) p.p.m.

The n.m.r. assignments of the individual isomers were made by comparison with their respective acids (vide infra) and from literature values.[88,89]

b) Using calcium in methanol.

The reaction was carried out as for the magnesium in methanol reduction except that the reaction was done on a tenth scale, and calcium (1.00g., 25mmol.) was added instead of the magnesium. The reaction also required extra methanol (15ml.) in order to retain mobility. The product was not purified, but contained the unsaturated ester 134 (31%), the products 152 (30%) and 153 (31%), and an impurity (6%), by g.c. If the reaction was cooled down to below ambient temperature, the reaction was effectively stopped, as indicated by the absence of hydrogen evolution. Attempted separation of the products from the starting material by radial chromatography was abandoned as impractical.

c) Using magnesium and palladium on carbon in methanol[148] and methyl (4-methylcyclohexylidene)acetate, 134.

Methyl (4-methylcyclohexylidene)acetate, 134 (340mg., 2.0mmol.), of 95% purity was dissolved in methanol (6ml.) and 10% palladium on carbon (10mg.) and magnesium turnings (240mg., 10mmol.) were added. After heating to initiate the reaction, the reaction mixture was kept at below 30°C. When all the magnesium had dissolved, the reaction mixture was added to ice cold 3N aqueous hydrochloric acid (6ml.). Extraction with diethyl ether (3x20ml.) gave a combined ethereal solution which was washed with water (10ml.), dried and filtered. Evaporation of the solvent gave the impure products (310mg., 91%), which were identified by g.c. as the impure esters 152 and 153 in the ratio 58:42.

d) Using magnesium and palladium on carbon in methanol[148] and methyl (4-methylcyclohex-1-enyl)acetate, 135.

Methyl (4-methylcyclohex-1-enyl)acetate 135 (90mg., 0.54mmol.) was

dissolved in methanol (1.5ml.) and 10% palladium on carbon (5mg.) was added. Addition of magnesium turnings (60mg., 2.5mmol.) caused a spontaneously initiated reaction, which was stirred for 48h. The reaction mixture was then poured onto ice cold 3N aqueous hydrochloric acid (1.5ml.) and extracted with diethyl ether (3x5ml.). The combined extracts were washed with water (5ml.), filtered and then the solvent was evaporated. The product (80mg.) was mostly unreacted 135 (84%) with a large number of other components, by g.c., two of which were the esters 152 (3%) and 153 (2%). An ^{1}H n.m.r. spectrum confirmed that the product was impure 135.

Preparation of a mixture of cis and trans isomers of (4-methylcyclohexyl)acetic acid, 85 and 86.

a) By saponification of a mixture of the methyl esters, 152 and 153.

A sample (500mg., 2.9mmol.) of the esters, prepared by magnesium in methanol reduction of 134, was saponified by heating at 50°C overnight with 2N potassium hydroxide in 50:50 aqueous methanol. The reaction mixture was allowed to reach ambient temperature. Water (20ml.) was added and the resultant solution was washed with hexane (20ml.). The separated aqueous layer was acidified to pH=2 with cold 6N aqueous hydrochloric acid and was then extracted with dichloromethane (3x20ml.). The combined extracts were washed with water (20ml.) and the solvent was evaporated, traces of water being azeotroped with ethanol. Sublimation of the yellow oil so obtained onto a cold finger yielded the acids 85 and 86 (430mg., 94%) in the ratio 58:42 as white crystals. These crystals melted at ambient temperature, to give a colourless oil of >99.8% purity.

R_F (MeOH 0.02, E.A. 0.20, P.E. 0.78) = 0.45.

R_T (SP1000, $HCCl_3$, 180°C) = 12.1 (58%), 10.8 (42%)m.

i.r. (film):- 2940, 2910, 2840, 1710, 1445, 1410, 1295, 1240 cm^{-1}.

m.s.:- m/e = 97 (100%), 96 (25), 81 (39), 61 (21), 60 (41), 55 (61), 41 (34), 39 (23). $M^{+\cdot}$(156) = $C_9H_{16}O_2$.

^1H n.m.r.:- (85) 2.33 (2H, d, 7Hz.), 2.1-1.0 (13H, m), 0.92 (3H, d, 6Hz.)δ.

(86) 2.21 (2H, d, 6Hz.), 2.1-1.0 (13H, m), 0.88 (3H, d, 5Hz.)δ.

^{13}C n.m.r.:- (85) 180.1 (s), 39.1 (t), 32.1 (d), 30.5 (t), 29.9 (d), 20.2 (q) p.p.m.

(86) 178.9 (s), 41.9 (t), 34.9 (t), 34.4 (d), 33.0 (t), 32.4 (d), 22.5 (q) p.p.m.

Assignments were facilitated by the comparison of the data obtained here with that obtained from the products of copper (1) hydride reduction of 134 (section b) and with literature values.[88,90,100]

b) Using copper (1) hydride from sodium bis(2-methoxyethoxy)aluminium hydride.[151]

Copper (1) bromide (1.80g., 9.75mmol.) in tetrahydrofuran (60ml.) was stirred at 0°C. 3.4M Sodium bis (2-methoxyethoxy)aluminium-hydride in toluene (5.6ml., 19.5mmol.) was added dropwise over 10m. After stirring for a further 30m., the reaction mixture was cooled to -78°C and 2-butanol (1.71ml., 19.5mmol.) was added dropwise over 10m. Methyl (4-methylcyclohexylidene)acetate 134 (550mg., 3.27mmol.) in tetrahydrofuran (200ml.) was added dropwise over 10m. and the reaction mixture was stirred for 3h. at -78°C. After heating rapidly in a water bath to 30°C, the reaction mixture was stirred overnight. Saturated aqueous ammonium chloride solution (6ml.) and dichloromethane (60ml.) were added. The resulting mixture was filtered through silica, the silica being washed with dichloromethane (2x40ml.). The combined organic phase was washed with water (40ml.) and the solvent was evaporated. Traces of water were removed by azeotroping with ethanol.

The product (600mg.) was a colourless oil. No trace of 134 was detected by g.c. Ethanol (9.6ml.) was added to the product and silver (1) nitrate (720mg.) in water (1ml.) was added to the solution. After adding 1.12M aqueous potassium hydroxide (9.6ml.), the reaction mixture was stirred overnight. The precipitate was separated by filtration and it was washed with water (10ml.). Acidification of the aqueous solution with cold 6N aqueous hydrochloric acid provided a cloudy solution, which was extracted with dichloromethane (3x20ml.). The combined extracts were evaporated to give an oil, which was sublimed onto a cold finger. The >99.8% pure product (100mg., 20%) was a mixture of 85 and 86, in the ratio 79:21 and was a colourless oil.

R_F (MeOH 0.02, E.A. 0.20, P.E. 0.78) = 0.45.

R_T (SP1000, $HCCl_3$, 180°C) = 12.1 (79%), 10.8 (21%)m.

The spectral data were virtually identical to the 85/86 mixture prepared by method a) except that certain n.m.r. peaks were stronger enabling the assignments of individual isomers to be confirmed.

The experiment was attempted as above with less copper (1) hydride, but not all the starting material 134 was reacted in these conditions. Hence, when 6.5mmol. of the copper(1) hydride reagent is employed, the g.c. analysis implied the presence of 134 (29%) in the reaction mixture. The same conditions of 6.5mmol. of the copper (1) hydride reagent, but allowing the reaction to slowly reach room temperature rather than rapidly heating it, gave a small amount of the aldehydes 159 and 160 and the presumed corresponding alcohols, from g.c. analysis. The aldehydes 159 and 160 (30mg.) were isolated impure by radial chromatographic separation, in the ratio 85:15.

R_F (MeOH 0.01, E.A. 0.10, P.E. 0.89) = 0.35.

R_T (SP1000, $HCCl_3$, 130°C) = 4.6 (60%), 4.1 (10%)m.

^1H n.m.r.:- (159) 9.85-9.79 (1H, m), 2.5-1.0 (12H, m), 0.92 (3H, d, 7Hz.)δ.

^{13}C n.m.r.:- (159) 39.0 (t), 32.1 (d), 30.5 (t), 29.9 (d), 28.6 (t), 20.2 (q) p.p.m.

The ^{13}C n.m.r. spectrum of 159 did not give a signal for the carbonyl carbon. This resonance may be greater than 200 p.p.m. The resonances of the other isomer 160 were observed for the methyl and methylene carbons.

^{13}C n.m.r.:- (160) 41.8 (t), 34.9 (t), 32.9 (t), 22.5 (q) p.p.m.

The corresponding acids were prepared by silver (1) oxide oxidation of the aldehydes 159 and 160 in conditions similar to that described above. The acids had chromatographic and spectral properties identical to those of the acids prepared previously.

The conditions of the reaction were varied with respect to mole equivalents of copper (1) hydride, reaction time and mole equivalents of 2-butanol used to prepare the copper (1) hydride. It was found that the optimal conditions were as initially described, using 1.5-2.5 mole equivalents of the copper (1) hydride reagent. In these conditions, a mixture of the saturated esters, aldehydes and compounds of higher retention times were observed by g.c. analysis. The latter peaks were assumed to be due to the saturated alcohols. The unsaturated ester was not observed.

It should be noted that the reaction was sensitive to traces of water, necessitating rigorous drying of solvents, reagents and apparatus prior to use.

c) Using copper (1) hydride from lithium tri-sec-butylborohydride.[123]

Copper (1) bromide (240mg., 1.63mmol.) in tetrahydrofuran (10ml.) at $0^{o}C$ was stirred while 0.5M lithium tri-sec-butylborohydride in tetrahydrofuran (6.5ml.) was added dropwise over 10m. The resulting brown-black suspension was stirred for 30m. at $0^{o}C$, then cooled to $-78^{o}C$. 2-Butanol (285μl., 3.25mmol.) was added dropwise over 10m.,

followed by 134 (220mg., 1.3mmol.) in tetrahydrofuran (5ml.). After stirring for 3h., the reaction mixture was rapidly heated to 30°C, then stirred at ambient temperature overnight. The precipitate, which appeared to contain metallic copper, was removed by filtration and the solvent was evaporated from the separated solution. G.c. analysis of the crude product so obtained gave no peaks corresponding to the aldehydes 159 and 160 or the esters 134, 152 and 153. Many other peaks were observed, but no attempt was made to separate and characterise these components.

d) Using copper (1) hydride from lithium tris(t-butoxy)aluminium hydride.[158]

Copper (1) bromide (930mg., 6.5mmol.) was dissolved in tetrahydrofuran (5ml.), was stirring at 0°C. Lithium tris(t-butoxy)aluminium hydride (3.31g., 13mmol.) was added as a slurry in tetrahydrofuran (25ml.). After stirring at 0°C for 30m., the yellow mixture was cooled to -78°C. 2-Butanol (1.15ml., 13mmol.) was added dropwise over 5m., followed by methyl (4-methyl cyclohexylidene)-acetate 134 (110mg., 0.6mmol.) in tetrahydrofuran (5ml.) over 5m. After stirring for 2h., the reaction was allowed to reach ambient temperature. T.l.c. and g.c. analyses implied that 134 was the major component in the reaction mixture, even after stirring at room temperature overnight.

Preparation of a mixture of cis and trans isomers of methyl (3-methylcyclohexyl)acetate, 154 and 87.

a) Using magnesium in methanol.[131]

A mixture of (Z)- and (E)- isomers of methyl (3-methylcyclohexylidene)acetate, 137 and 138 (530mg., 3.1mmol.), in the ratio of 50:50, was dissolved in methanol (40ml.). About forty equivalents of magnesium turnings (2.7g.) was added. After stirring for 2d., the system was

acidified with cold 6N aqueous hydrochloric acid to pH=2 and was extracted with hexane (3x100ml.). The solvent of the combined organic extracts was evaporated and the crude ester mixture was purified by radial chromatography. The product was isolated as an oil (200mg., 37%) of purity >99.8% and proved to be 87 and 154 in the ratio 61:39.

R_T (SP1000, MeOH, 130°C) = 6.01 (61%), 5.5 (39%)m.

i.r. (film)[91]:- 2965, 2915, 2840, 1740, 1440, 1320, 1250, 1170 cm^{-1}.

m.s.:- m/e = 97 (13%), 96 (19), 95 (13), 75 (36), 74 (100), 55 (25), 43 (23), 41 (20). $M^{+\cdot}$(170) = $C_{10}H_{18}O_2$.

^1H n.m.r.:- (154) 3.68 (3H, s), 2.19 (2H, d, 7Hz.), 2.0-1.0 (13H, m), 0.87 (3H, d, 6Hz.)δ.

(87) 3.68 (3H, s), 2.3-2.2 (2H, m), 2.0-1.0 (13H, m), 0.92 (3H, d, 7Hz.)δ.

^{13}C n.m.r.:- (154) 174.3 (s), 51.3 (q), 42.1 (t), 41.9 (t), 35.0 (t), 35.0 (d), 32.7 (t), 32.5 (d), 26.0 (t), 22.8 (q) p.p.m.

(87) 173.6 (s), 51.3 (q), 39.5 (t), 38.9 (t), 33.5 (t), 31.4 (t), 29.9 (d), 27.2 (d), 20.7 (q), 20.7 (t) p.p.m.

The n.m.r. assignments were made by comparison with the assignments for the respective acids (vide infra) and from literature values.[91]

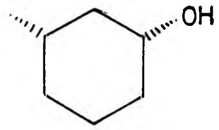
b) Using calcium in methanol.

A mixture of (Z) and (E) isomers of methyl (3-methylcyclohexylidene)acetate, 137 and 138 (200mg., 1.2mmol.), was added to calcium (16g.) in methanol (120ml.). Stirring with a powerful mechanical stirrer was only possible by periodically adding methanol, as required. After 2d., the reaction was acidified to pH=2 by addition of ice cold 6N aqueous hydrochloric acid and was then extracted with hexane (3x100ml.). Filtration through silica and evaporation of the solvent,

cis 157
trans 158

161

162



95

from the combined organic extracts, provided a crude product, which was a mixture of the saturated esters, 87 and 154, and the unsaturated esters, 137 and 138, by g.c. analysis. Chromatography on silica did not cleanly separate the saturated esters from the unsaturated esters. The ratio of 87:154 was 51:49 and the ratio of saturated:unsaturated esters was 39:61, by g.c.

Preparation of a mixture of cis and trans isomers of (3-methylcyclohexyl)acetic acid, 157 and 158.

a) By saponification of a mixture of the methyl esters, 87 and 154.

A sample of the 61:39 mixture of 87 and 154 (150mg., 0.88mmol.) was treated with 2N potassium hydroxide in 50:50 aqueous methanol at 50°C overnight. Water (10ml.) and hexane (10ml.) were added. The separated aqueous phase was acidified to pH=2 by addition of ice cold 6N aqueous hydrochloric acid and was then extracted with dichloromethane (3x15ml.). The combined extracts were washed with water (10ml.) and the solvent was evaporated. Traces of water were removed by azeotroping with ethanol. The crude product (120mg., 88%) was purified to >99.8% by sublimation onto a cold finger. The oil (100mg., 73%) so obtained was 157 and 158 in the ratio 36:64.

R_F (MeOH 0.02, E.A. 0.20, P.E. 0.78) = 0.43.

R_T (SP1000, $HCCl_3$, 180°C) = 11.4 (64%), 10.4 (36%)m.

i.r. (film):- 2945, 2920, 2855, 2840, 1710, 1410, 1305, 1290 cm^{-1}. (3600-2400 cm^{-1}).

m.s.:- m/e = 97 (100%), 96 (30), 81 (41), 67 (17), 60 (34), 55 (52), 41 (31), 39 (19). $M^{\cdot+}$(156) = $C_9H_{16}O_2$.

^1H n.m.r.:- (157) 2.23 (2H, d, 7Hz.), 2.0-1.0 (10H, m), 0.88 (3H, d, 6Hz.)δ.

(158) 2.35-2.25 (2H, m), 2.0-1.0 (10H, m), 0.92 (3H, d, 7Hz.)δ.

^{13}C n.m.r.:- (157) 179.6 (s), 42.0 (t), 41.8 (t), 34.8 (t), 34.8 (d), 32.5 (t), 32.5 (d), 26.0 (t), 22.7 (q) p.p.m.

(158) 179.9 (s), 39.4 (t), 38.8 (t), 33.4 (t), 31.3 (t), 29.7 (d), 27.2 (d), 20.6 (q), 20.6 (t) p.p.m.

Assignments of n.m.r. data were facilitated by comparison with the data obtained from the products of copper (1) hydride reduction of 137 and 138 (section b)) and with literature values.[90]

b) Using copper (1) hydride.

Copper (1) bromide (1.20g., 6.5mmol.) in tetrahydrofuran (40ml.) was stirred at 0°C. 3.4M Sodium bis(2-methoxyethoxy)aluminium hydride in toluene (3.7ml.) 13mmol.) was added dropwise over 10m. to give a black slurry. After stirring for 30m. at 0°C, the reaction mixture was cooled to -78°C. 2-Butanol (1.14ml.) was added dropwise over 10m., followed by a 50:50 mixture of 137 and 138 (550mg., 3.3mmol.) in tetrahydrofuran (20ml.) dropwise over 10m. The reaction mixture was stirred for 3h., then rapidly brought to 30°C by placing the reaction vessel in a warm water bath. After stirring at ambient temperature overnight, saturated aqueous ammonium chloride (4ml.) and dichloromethane (40ml.) were added. The mixture was filtered through silica and the silica was then washed with dichloromethane (3x20ml.). The combined dichloromethane filtrates were washed with water (40ml.) and the solvent was evaporated. The resulting oil was dissolved in ethanol (9.6ml.) and silver (1) nitrate (720mg.) in water (1ml.) was added. Addition of 1.12M aqueous potassium hydroxide (9.6ml.) caused a black precipitate to appear. The mixture was stirred for 18h. and then filtered, the precipitate being washed with water (10ml.). The filtrate was washed with hexane (3x10ml.) and these washings were retained. The aqueous phase was acidified to pH=2 by adding ice cold 6N aqueous hydrochloric acid and was then extracted with dichloromethane (6x20ml.). The

combined dichloromethane extracts were washed with water (20ml.). Evaporation of the solvent provided the impure products (200mg., 39%). Purification by sublimation onto a cold finger provided a sample (160mg., 31%) of the >99.8% pure products 157 and 158 in the ratio 20:80 as a colourless oil.

R_F (MeOH 0.02, E.A. 0.20, P.E. 0.78) = 0.43.

R_T (SP1000, $HCCl_3$, 180°C) = 11.4 (80%), 10.4 (20%)m.

The spectral data were virtually identical to the 157/158 mixture prepared by method a) except that certain n.m.r. peaks were stronger enabling the assignment of individual isomers to be confirmed.

The hexane washings, which had been retained, were combined and the solvent was evaporated to give an oil (160mg.). This material was purified by radial chromatography to give a sample (40mg.) of a >99.8% pure mixture of 161 and 162 as an oil.

R_T (SP1000, P.E., 130°C) = 10.2 (77%), 9.1 (23%)m.

i.r. (film):- 3330, 2910, 2860, 2840, 1455, 1375, 1050, 1010 cm^{-1}. (3550-3000 cm^{-1}).

m.s.:- m/e = 124 (22%), 96 (28), 95 (100), 81 (44), 68 (32), 67 (40), 55 (60), 41 (44). M^+(142) - not observed.

^1H n.m.r.:- (161) 3.65 (2H, t, 7Hz.), 2.0-1.0 (12H, m), 0.87 (3H, d, 6Hz.)δ.

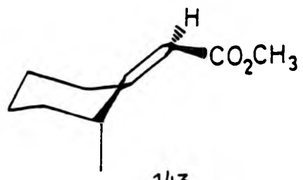
(162) 3.65 (2H, t, 7Hz.), 2.0-1.0 (12H, m), 0.91 (3H, d, 7Hz.)δ.

^{13}C n.m.r.:- (161) 60.8 (t), 42.3 (t), 40.5 (t), 35.2 (t), 34.4 (d), 33.0 (t), 32.7 (d), 26.3 (t), 22.9 (q) p.p.m.

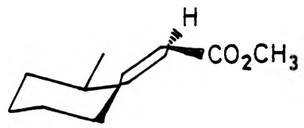
(162) 61.3 (t), 39.3 (t), 37.5 (t), 33.8 (t), 31.6 (t), 29.0 (d), 27.2 (d), 20.8 (q), 20.8 (t) p.p.m.

The n.m.r. spectral data were assigned by comparison with the data obtained for the esters 87 and 154, the acids 157 and 158 and

cis 155
trans 88

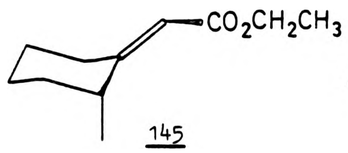


143



144

cis 89
trans 90



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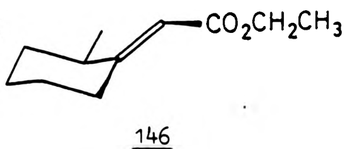

146

cis-3-methylcyclohexanol 95.

A sample of the alcohols 161 and 162 (30mg.) was dissolved in acetone (0.5ml.). Jones' reagent[24] (0.5ml.) was added and the reaction was stirred for 10m. Extraction with hexane (3x2ml.) provided an organic phase containing the product acids 157 and 158. Evaporation of the solvent gave a slightly impure oil (20mg.) with t.l.c. and g.c. properties identical to the acids prepared previously. The ratio 157:158 was 21:79.

Preparation of a mixture of cis and trans isomers of methyl (2-methylcyclohexyl)acetate, 155 and 88.

A mixture of the (Z)- and (E)- isomers of methyl (2-methylcyclohexylidene)acetate, 143 and 144 (710mg., 4.2mmol.), in the ratio 143:144 of 24:76 was stirred in methanol (55ml.). Magnesium turnings[131] (4.03g.) were added and the reaction mixture was stirred for 25h. at ambient temperature, cooling when necessary to prevent the temperature exceeding 40°C. Ice cold 6N aqueous hydrochloric acid (50ml.) was added dropwise over 30m. and the resulting acidic solution was extracted with dichloromethane (3x50ml.). The combined extracts were washed with water (40ml.) and the solvent was evaporated. G.c. analysis of the products showed no starting material. Bulb to bulb distillation followed by radial chromatography provided a sample of the products (480mg., 67%) of >99.8% purity. The ratio 88:155 was 38:62.

R_T (SP1000, $HCCl_3$, 130°C) = 6.8 (62%), 5.7 (38%)m.

i.r. (film):- 2940, 2920, 2850, 1745, 1435, 1290, 1240, 1165 cm^{-1}.

m.s.:- m/e = 97 (41%), 96 (32), 81 (21), 75 (39), 74 (100), 55 (37), 43 (25), 41 (25). $M^{+\cdot}$(170) = $C_{10}H_{18}O_2$.

^1H n.m.r.:- (155) 3.68 (3H, s), 2.35-2.00 (2H, m), 2.0-1.0 (13H, m), 0.86 (3H, d, 7Hz.)δ.

(88) 3.68 (3H, s), 2.35-2.00 (2H, m), 2.0-1.0 (13H, m), 0.86 (3H, d, 7Hz.)δ.

^{13}C n.m.r.:- (155) 173.7 (s), 51.3 (q), 37.1 (d), 36.6 (t), 32.7 (d), 32.1 (t), 28.2 (t), 24.4 (t), 22.4 (t), 14.7 (q) p.p.m.

(88) 173.7 (s), 51.3 (q), 41.5 (d), 39.3 (t), 37.2 (d), 35.7 (t), 32.7 (t), 26.5 (t), 20.2 (q) p.p.m.

The n.m.r. assignments were made by comparison with the assignments for the respective acids (vide infra).

Preparation of a mixture of cis- and trans- isomers of (2-methylcyclohexyl) acetic acid, 89 and 90.

a) By saponification of a mixture of the methyl esters, 88 and 155.

A sample of the esters, 88 and 155 (400mg., 2.4mmol.) was treated with 2N potassium hydroxide in 50:50 aqueous methanol (8ml.) at 50°C overnight. After the addition of water (30ml.), the solution was washed with hexane (2x30ml.). After acidification to pH=2 with cold 6N aqueous hydrochloric acid, the solution was extracted with dichloromethane (3x30ml.). The combined dichloromethane extracts were washed with water (30ml.) and the solvent was evaporated to give the crude product (380mg., 100%). This product was purified by high vacuum sublimation on to a cold finger to give a sample of 89 and 90, in the ratio 62:38, of purity 99.8% and as a colourless oil (250mg., 68%).

R_F (MeOH 0.02, E.A. 0.20, P.E. 0.78) = 0.49.

R_T (SP1000, $HCCl_3$, 180°C) = 13.3 (62%), 11.4 (38%)m.

i.r. (film):- 2955, 2925, 2860, 1710, 1450, 1415, 1300, 1260 cm^{-1}. (3500-2500 cm^{-1}).

m.s.:- m/e = 97 (100%), 96 (62), 81 (62), 67 (22), 60 (28), 55 (59), 41 (42), 39 (24). $M^{\cdot+}$(156) = $C_9H_{16}O_2$.

^1H n.m.r.:- (89) 2.4-2.0 (2H, m), 2.0-1.0 (13H, m), 0.88 (3H, d, 7Hz.)δ.

(90) 2.4-2.0 (2H, m), 2.0-1.0 (13H, m), 0.88 (3H, d, 7Hz.)δ.

^{13}C n.m.r.:- (89) 180.5 (s), 36.9 (d), 36.7 (t), 32.5 (d), 32.0 (t), 28.0 (t), 24.3 (t), 22.2 (t), 14.7 (q) p.p.m.

(90) 180.5 (s), 41.2 (d), 39.4 (t), 37.1 (d), 35.6 (t), 32.6 (t), 26.4 (t), 20.2 (q) p.p.m.

Assignments of n.m.r. data were facilitated by comparison with the data obtained from the products of copper (1) hydride reduction of a mixture of 143 and 144 (section c)) or 145 and 146 (section b)) and with literature values.[90]

b) Using copper (1) hydride and (Z)-enriched ethyl (2-methylcyclohexylidene)acetate.

Copper (1) bromide (900mg., 4.9mmol.) in tetrahydrofuran (30ml.) was stirred at 0°C. 3.4M Sodium bis (2-methoxyethoxy)aluminium hydride in toluene (2.8ml.) 9.8mmol.) was added dropwise over 10m. giving a black slurry. After stirring for 30m., the reaction mixture was cooled to -78°C. 2-Butanol (850 1.) was added dropwise over 10m. causing effervescence. A sample of 145 and 146 of isomeric ratio 82:18 (560mg., 3.0mmol.) in tetrahydrofuran (15ml.) was added dropwise over 10m. After stirring at -78°C for 3h., the reaction mixture was rapidly brought to 30°C, by placing in a warm water bath, and was then stirred overnight at ambient temperature. Saturated aqueous ammonium chloride (3ml.) and dichloromethane (30ml.) were added and the resultant slurry was filtered through silica. The silica was washed with dichloromethane (3x15ml.) and the dichloromethane filtrates were combined. The solution so obtained was washed with water (30ml.) and the solvent was evaporated. Analysis by g.c. showed that none of the unsaturated ester starting material remained. The impure mixture of compounds was dissolved in ethanol (7.5ml.) and silver (1) nitrate (540mg.) in water (0.75ml.) was added. After adding 1.12M aqueous potassium hydroxide (7.5ml.), the reaction mixture was stirred for 19h. Filtration and washing of the

^{13}C n.m.r.:- (89) 180.5 (s), 36.9 (d), 36.7 (t), 32.5 (d), 32.0 (t), 28.0 (t), 24.3 (t), 22.2 (t), 14.7 (q) p.p.m.

(90) 180.5 (s), 41.2 (d), 39.4 (t), 37.1 (d), 35.6 (t), 32.6 (t), 26.4 (t), 20.2 (q) p.p.m.

Assignments of n.m.r. data were facilitated by comparison with the data obtained from the products of copper (1) hydride reduction of a mixture of 143 and 144 (section c)) or 145 and 146 (section b)) and with literature values.[90]

b) Using copper (1) hydride and (Z)-enriched ethyl (2-methylcyclohexylidene)acetate.

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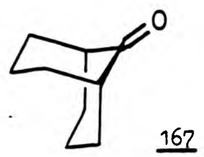
^{13}C n.m.r.:- (89) 180.5 (s), 36.9 (d), 36.7 (t), 32.5 (d), 32.0 (t), 28.0 (t), 24.3 (t), 22.2 (t), 14.7 (q) p.p.m.

(90) 180.5 (s), 41.2 (d), 39.4 (t), 37.1 (d), 35.6 (t), 32.6 (t), 26.4 (t), 20.2 (q) p.p.m.

Assignments of n.m.r. data were facilitated by comparison with the data obtained from the products of copper (1) hydride reduction of a mixture of 143 and 144 (section c)) or 145 and 146 (section b)) and with literature values.[90]

b) Using copper (1) hydride and (Z)-enriched ethyl (2-methylcyclohexylidene)acetate.

Copper (1) bromide (900mg., 4.9mmol.) in tetrahydrofuran (30ml.) was stirred at 0°C. 3.4M Sodium bis (2-methoxyethoxy)aluminium hydride in toluene (2.8ml.) 9.8mmol.) was added dropwise over 10m. giving a black slurry. After stirring for 30m., the reaction mixture was cooled to -78°C. 2-Butanol (850 l.) was added dropwise over 10m. causing effervescence. A sample of 145 and 146 of isomeric ratio 82:18 (560mg., 3.0mmol.) in tetrahydrofuran (15ml.) was added dropwise over 10m. After stirring at -78°C for 3h., the reaction mixture was rapidly brought to 30°C, by placing in a warm water bath, and was then stirred overnight at ambient temperature. Saturated aqueous ammonium chloride (3ml.) and dichloromethane (30ml.) were added and the resultant slurry was filtered through silica. The silica was washed with dichloromethane (3x15ml.) and the dichloromethane filtrates were combined. The solution so obtained was washed with water (30ml.) and the solvent was evaporated. Analysis by g.c. showed that none of the unsaturated ester starting material remained. The impure mixture of compounds was dissolved in ethanol (7.5ml.) and silver (1) nitrate (540mg.) in water (0.75ml.) was added. After adding 1.12M aqueous potassium hydroxide (7.5ml.), the reaction mixture was stirred for 19h. Filtration and washing of the



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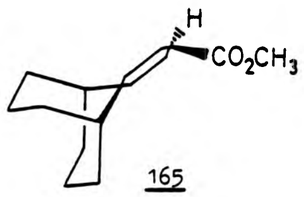

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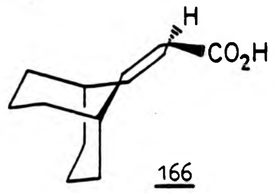

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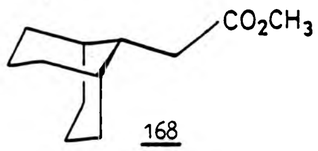

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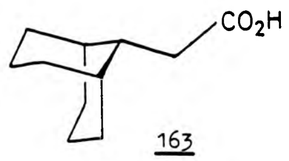

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solid with water (10ml.) provided an aqueous solution, which was washed with hexane (3x10ml.). The solution was then acidified to pH=2, by addition of ice cold 6N aqueous hydrochloric acid, and was then extracted with dichloromethane (3x20ml.). The combined dichloromethane extracts were dried and the solvent was evaporated to give a yellow oil (480mg., 92%) of the impure product. Bulb to bulb distillation and repeated radial chromatography provided a small sample (40mg., 9%) of ~99.5% pure mixture of 89 and 90 in the ratio 87:13.

R_F (MeOH 0.02, E.A. 0.20, P.E. 0.78) = 0.49..

R_T (SP1000, $HCCl_3$, 180°C) = 13.3 (86%), 11.4 (13%)m.

The spectral data were virtually identical to the 89/90 mixture prepared by method a) except that certain n.m.r. peaks were stronger enabling the assignment of individual isomers to be confirmed.

c) Using copper (1) hydride and (E)-enriched methyl (2-methylcyclohexylidene)acetate.

This experiment was performed in identical conditions to that described as method b) except that the starting material was 143 and 144 of isomeric ratio 4:96. The impure product was bulb to bulb distilled to give a slightly impure mixture of 89 and 90 (240mg., 51%) of isomeric ratio 87:13. This product possessed identical chromatographic and spectral characteristics to the 89/90 product mixture previously prepared by method b).

Preparation of methyl (9-bicyclo[3,3,1]nonylidene)acetate, 165.

Trimethylphosphonoacetate (2.0g., 11mmol.) was dissolved in toluene (45ml.) and 50% sodium hydride in mineral oil (520mg., 11mmol.) was added in portions over 10m.[19] After stirring a further 30m., 9-bicyclo[3,3,1]nonanone 167 (200mg., 1.45mmol.) in toluene (10ml.) was added dropwise over 10m. After stirring for 50h., the reaction mixture was filtered through silica and the silica was washed with toluene

(100ml.). The solvent was evaporated from the combined filtrates using methanol to azeotrope residual traces of toluene. The impure product (570mg.) was purified by radial chromatography to give a sample (70mg., 26%) of ~99.8% pure 165.

R_T (SP1000, $HCCl_3$, 150°C) = 13.1m.

i.r. (film):- 2910, 2840, 1705, 1650, 1435, 1390, 1260, 1160 cm^{-1} (1490 cm^{-1}).

m.s.:- m/e = 194 (100%), 134 (55), 120 (60), 91 (100), 79 (65), 77 (56), 41 (59), 39 (53). M^{+}(194) = $C_{12}H_{18}O_2$.

^1H n.m.r.:- 5.68 (1H, s), 4.2-3.9 (1H, m), 3.70 (3H, s), 2.5-2.2 (1H, m), 2.2-1.3 (12H, m)δ.

^{13}C n.m.r.:- 174.0 (s), 171.9 (s), 109.4 (d) 50.6 (q), 41.5 (d), 34.5 (t), 33.6 (t), 32.8 (d), 21.3 (t) p.p.m.

Other fractions provided material of ~99% purity (140 mg., 51%), in addition to the pure sample.

This reaction was repeated in order to obtain more material.

Preparation of (9-bicyclo[3,3,1]nonylidene)acetic acid, 166.

A sample of ~99% pure 165 (120mg., 0.6mmol.) was reacted with 2N potassium hydroxide in 50:50 aqueous methanol (2ml.) at 50°C overnight. Water (10ml.) and diethyl ether (10ml.) were added and the separated aqueous phase was acidified to pH=2 with ice cold concentrated hydrochloric acid. This acidic solution was extracted with diethyl ether (3x15ml.) and the combined extracts were washed with water (10ml.) and saturated aqueous sodium chloride. Evaporation of the solvent gave a solid of sharp melting point (166°C). Recrystallisation from ethanol provided material of similar melting point. The white prisms of 166 (100mg., 98%) were of >99.8% purity.

m.p. = 166°C.

R_T (SP1000, EtOH, 200°C) = 19.1m

i.r. (CCl_4):- 2910, 2840, 1685, 1640, 1270, 1205, 1190, 1120 cm^{-1}. (3400-2500 and 1490 cm^{-1}).

m.s.:- m/e = 180 (100%), 138 (55), 120 (70), 95 (66), 93 (58), 91 (55), 79 (57), 67 (63). $M^{+\cdot}(180) = C_{11}H_{16}O_2$.

^1H n.m.r.:- 5.68 (1H, s), 4.2-3.9 (1H, m), 2.6-2.3 (1H, m), 2.2-1.4 (12H, m)δ.

^{13}C n.m.r.:- 175.6 (s), 172.7 (s), 109.2 (d), 41.9 (d), 34.7 (t), 33.8 (t), 33.2 (d), 21.4 (t) p.p.m.

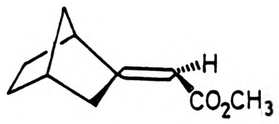
The methyl ester 165 was returned by treatment with acidic methanol. Thus, the acid 166 (70mg., 0.42mmol.) was refluxed in methanol (5ml.) and p-toluenesulphonic acid (10mg.) for 50h. The solvent was evaporated and the crude product was purified by radial chromatography. A sample of the pure methyl ester (70mg., 92%) so obtained was identical by g.c. and ^1H n.m.r. analysis to a genuine sample.

Preparation of methyl (9-bicyclo[3,3,1]nonyl)acetate, 168.

A sample of ~99% 165 (170mg., 0.94mmol.) was dissolved in methanol (12ml.). Forty equivalents of magnesium turnings (910mg.)[131] were added and the reaction mixture was stirred for 80h., with cooling in a water bath applied when the reaction temperature exceeded 40°C. Ice cold 6N aqueous hydrochloric acid (20ml.) was added dropwise over 30m. The resultant solution was extracted with diethyl ether (3x30ml.). The solvent was evaporated from the combined extracts to give the impure product. Radial chromatography provided a >99.8% pure sample of 168 (140mg., 76%).

R_T (SP1000, Et_2O, 150°C) = 12.9m.

i.r. (film):- 2900, 2870, 1740, 1435, 1315, 1270, 1190, 1155 cm^{-1}. (1490 cm^{-1}).



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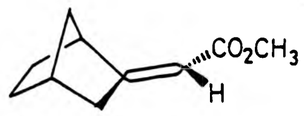

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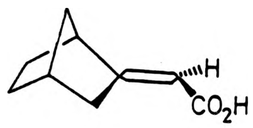

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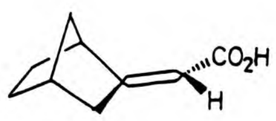

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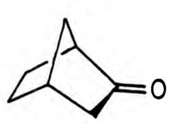

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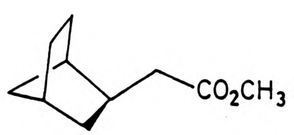

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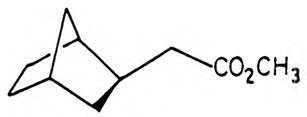

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m.s.:- m/e = 196 (54%), 164 (61), 136 (89), 135 (59), 107 (60), 81 (72), 75 (64), 74 (100). $M^{+\cdot}$(196) = $C_{12}H_{20}O_2$.

^{1}H n.m.r.:- 3.68 (3H, s), 2.49 (2H, d, 7Hz.), 2.2-1.4 (15H, m)δ.

^{13}C n.m.r.:- 174.2 (s), 51.4 (q), 38.8 (d), 37.8 (t), 33.8 (t), 31.9 (d), 25.3 (t), 22.5 (t), 21.6 (t) p.p.m.

This reaction was repeated in order to provide additional material.

Preparation of (9-bicyclo[3.3.1]nonyl)acetic acid, 163.

A sample of the saturated ester 168 (170mg., 0.87mmol.) was reacted with 2N potassium hydroxide in 50:50 aqueous methanol (1.5ml.) at 50°C overnight. Water (8ml.) and diethyl ether (10ml.) were added. The separated aqueous phase was acidified to pH=2 using ice cold concentrated hydrochloric acid and was then extracted with diethyl ether (3x10ml.). The combined ethereal extracts were filtered through silica and then the solvent was evaporated. A portion of the resultant white solid, with a broad melting point range (118-126°C) was purified to >99.8% by sublimation. White crystals (110mg., 70%) of 163 were obtained.

m.p. = 160-161°C.

R_F (MeOH 0.02, E.A. 0.20, P.E. 0.78) = 0.47.

R_T (SP1000, Et_2O, 180°C) = 39.4m.

i.r. (CCl_4):- 2980, 2900, 2870, 1710, 1455, 1410, 1315, 1280 cm^{-1}. (3400-2500 and 1490 cm^{-1}).

m.s.:- m/e = 182 (72%), 164 (65), 136 (100), 94 (59), 81 (88), 79 (48), 67 (60), 41 (49). $M^{+\cdot}$(182) = $C_{11}H_{18}O_2$.

^{1}H n.m.r.:- 2.50 (2H, d, 7Hz.), 2.2-1.3 (15H, m)δ.

^{13}C n.m.r.:- 179.9 (s), 38.6 (d), 37.6 (t), 33.6 (t), 31.7 (d), 25.1 (t), 22.2 (t), 21.5 (t) p.p.m.

Preparation of enriched (Z) and enriched (E) isomers of methyl (2-bicyclo[2.2.1]heptylidene)acetate, 172 and 171, and (2-bicyclo[2,2,1]heptylidene)acetic acid, 175 and 174.[165]

Trimethylphosphonoacetate (8.0g., 22mmol.) in toluene (180ml.) was

treated with sodium hydride (1.14g., 22mmol.) in portions over 10m.[19] After rigorous stirring for 1h., 2-bicyclo[2,2,1]heptanone (norcamphor) 173 (2.2g., 20mmol.) in toluene (20ml.) was added dropwise over 10m. After stirring for 65h., the reaction mixture was filtered through silica. The silica was washed with hexane (200ml.). Evaporation of the solvent from the combined filtrates provided the crude product. Radial chromatography separated the unreacted ketone 173 (1.8g.) from the products 171 and 172 (380mg., 42%). The above procedure was repeated in order that more product was available. The combined material was subjected to extensive careful radial chromatography. Enriched fractions were combined and re-chromatographed to provide more enriched fractions, and so on. After several enrichment steps, samples of the esters enriched to >90% isomeric purity were obtained. Hence, 172 was an oil (30mg.).

R_T (SP1000, Et_2O, 130°C) = 10.8 (9%), 10.1 (91%)m.

i.r. (film):- 2950, 1720, 1660, 1295, 1220, 1160, 1140 cm^{-1}.

m.s.:- m/e = 166 (28%), 138 (100), 106 (22), 91 (24), 79 (42), 77 (22), 39 (27), 28 (49). M^{+}(166) = $C_{10}H_{14}O_2$.

^1H n.m.r.:- (172) 5.55 (1H, s), 4.05-3.90 (1H, m), 3.67 (3H, s), 2.5-1.0 (9H, m)δ.

^{13}C n.m.r.:- 170.2 (s), 167.3 (s), 109.4 (d), 50.7 (q), 42.9 (d), 41.0 (t), 39.4 (t), 35.5 (d), 28.5 (t), 27.7 (t) p.p.m.

Likewise, a sample of enriched 171 was an oil (50mg.).

R_T (SP1000, Et_2O, 130°C) = 10.8 (95%), 10.1 (5%)m.

i.r. (film):- 2950, 1720, 1665, 1440, 1360, 1205, 1150, 730.

m.s.:- m/e = 166 (29%), 138 (100), 135 (22), 106 (22), 91 (21), 79 (44), 77 (20), 39 (26). M^{+}(166) = $C_{10}H_{14}O_2$.

^1H n.m.r.:- (171) 5.75, (1H, s), 3.67 (3H, s), 2.95-2.75 (1H, m), 2.6-2.4 (2H, m), 2.0-1.1 (7H, m)δ.

^{13}C n.m.r.:- (171) 171.3 (s), 167.6 (s), 108.4 (d), 50.6 (q), 47.1 (d), 39.9 (t), 39.0 (t), 36.4 (d), 28.7 (t), 27.8 (t) p.p.m.

The enriched ester isomers were saponified similarly. Thus, the sample (30mg., 0.18mmol.) of enriched 172 was treated with 2N potassium hydroxide in 50:50 aqueous ethanol at 50°C for 22h. Water (5ml.) and diethyl ether (5ml.) were added. The separated aqueous phase was acidified to pH=2 with ice cold concentrated hydrochloric acid and was then extracted with diethyl ether (3 x 8ml.). The combined extracts were washed with water (5ml.) and saturated aqueous sodium chloride (5ml.). Evaporation of the solvent provided the crude product, which was purified by sublimation to give the >99.8% pure acid mixture, 174/175, as needle crystals (20mg., 73%). The g.c. peaks were not discrete in a variety of conditions. The product ratios were assumed to be akin to the ester starting material and this hypothesis was supported by the spectral data.

R_F (MeOH 0.02, E.A. 0.20, P.E. 0.78) = 0.34.

R_T (SP1000, $HCCl_3$, 180°C) = 15.2, shoulder at 15.7m.

i.r. (CCl_4):- 2960, 2870, 1685, 1650, 1415, 1295, 1260, 1240 cm^{-1}.

m.s.:- m/e = 152 (34%), 124 (100), 91 (21), 79 (46), 77 (19), 67 (26), 41 (24), 39 (32). $M^{\cdot+}$(152) = $C_9H_{12}O_2$.

^1H n.m.r.:- (175) 5.57 (1H, s), 4.10-3.90 (1H, s), 2.6-1.1 (9H, m)δ.

^{13}C n.m.r.:- (175) 172.2 (s), 171.1 (s), 109.5 (d), 43.1 (d), 41.3 (t), 39.4 (t), 35.5 (d), 28.5 (t), 27.6 (t) p.p.m.

The enriched ester 171 (50mg., 0.30mmol.) was likewise saponified except on a proportionally larger scale. The >99.8% pure product was obtained as white crystals (40mg., 87%). The g.c. peaks were better resolved, but still not discrete. The product ratio was probably 95:5,

174:175.

R_F (MeOH 0.02, E.A. 0.20, P.E. 0.78) = 0.34.

R_T (SP1000, Et_2O, 180°C) = 15.7 (96%), 15.2 (4%)m.

i.r. (CCl_4):- 2950, 2870, 1685, 1650, 1420, 1295, 1285, 1240 cm^{-1}.

m.s.:- m/e = 152 (31%), 124 (100), 91 (24), 79 (61), 77 (27), 67 (26), 41 (24), 39 (32). $M^{+\cdot}$(152) = $C_9H_{12}O_2$.

^1H n.m.r.[165]:- (174) 5.75 (1H, s), 2.95-2.80 (1H, m), 2.60-2.40 (2H, m), 2.0-1.1 (7H, m)δ.

^{13}C n.m.r.:- (174) 174.3 (s), 172.9 (s), 108.6 (d), 47.5 (d), 40.2 (t), 39.0 (t), 36.3 (d), 28.6 (t), 27.7 (t) p.p.m.

Preparation of the enriched endo- isomers of methyl (2-bicyclo[2,2,1]-heptyl)acetate, 176, and (2-bicyclo[2,2,1]heptyl)acetic acid, 164.[161]

A sample of the unsaturated esters 171 and 172 (300mg., 1.81mmol.) of isomeric ratio 62:38 was dissolved in methanol (20ml.). The addition of forty equivalents of magnesium turnings (1.5g.)[131] caused effervescence in an exothermic reaction. The reaction mixture was kept below 40°C using a cold water bath. After stirring for 100h., 6N aqueous hydrochloric acid (35ml.) was added dropwise over 30m. and then the solution was extracted with diethyl ether (3x70ml.). The combined extracts were washed with water (70ml.) and filtered through silica. Evaporation of the solvent gave the crude material (300mg.), which was purified by bulb to bulb distillation to give enriched 176 as an oil (270mg., 89%).

R_T (SP1000, Et_2O, 130°C) = 6.5m.

i.r. (film):- 2940, 2860, 1740, 1435, 1290, 1270, 1205, 1165 cm^{-1}.

m.s.:- m/e = 99 (73%), 95 (50), 94 (67), 79 (52), 74 (46). 67 (100), 41 (57), 39 (47). $M^{+\cdot}$(168) = $C_{10}H_{18}O_2$.

^1H n.m.r.:- (176) 3.65 (3H, s), 2.4-1.0 (13H, m)δ.

^{13}C n.m.r.:- (176) 173.8 (s), 51.4 (q), 40.3 (d), 39.9 (t), 37.4 (t), 37.2 (d), 36.7 (d), 36.5 (t), 30.1 (t), 22.6 (t) p.p.m.

The g.c. analysis did not resolve the endo- and exo- isomers. Spectroscopic analysis of the enriched 176 and analysis of the corresponding acid mixture implied a product ratio of about 91:9, 176:178.

The corresponding acid was prepared by saponification. A sample of the enriched endo- ester 164 was treated with 2N potassium hydroxide in 50:50 aqueous methanol (6ml.) at 50°C for 24h. Water (30ml.) and diethyl ether (30ml.) were added. The separated aqueous phase was acidified to pH=2 with ice cold concentrated hydrochloric acid and was then extracted with diethyl ether (3x50ml.). The combined extracts were washed with water (30ml.) and then the solvent was evaporated. The crude product so obtained was purified by sublimation onto a cold finger. The product acids (110mg., 48%) were obtained as an oil. Again, g.c. analysis failed to separate the isomers into completely discrete peaks, some overlap being evident in a variety of conditions. However, spectral data supported a 83:164 ratio of 9:91.

R_F (MeOH 0.02, E.A. 0.20, P.E. 0.78) = 0.40.

R_T (SP1000, EtOH, 180°C) = 13.0 (91%), 12.7 (9%)m. (Peaks not completely resolved).

i.r. (film):- 2940, 2860, 1705, 1450, 1410, 1295, 1275, 1220 cm^{-1}.

m.s.:- m/e = 95 (34%), 94 (98), 85 (77), 79 (38), 68 (38), 67 (100), 41 (35), 39 (29). M^{+}(154) = $C_9H_{14}O_2$. (m/e = 136 (37%)).

^{1}H n.m.r.:- (164) 2.5-1.0 (m)δ.

^{13}C n.m.r.:- (164) 180.2 (s), 40.1 (d), 39.8 (t), 37.3 (t), 37.1 (d), 36.7 (d), 36.2 (t), 30.0 (t), 22.6 (t) p.p.m.

Preparation of the exo- isomer of methyl (2-bicyclo[2,2,1]heptyl)acetate, 178.[186]

A sample of exo- (2-bicyclo[2,2,1]heptyl)acetic acid (300mg., 1.95mmol.) in methanol (20ml.) and p-toluenesulphonic acid (20mg.) was heated at 60°C for 24h. The solvent was evaporated and the crude product was purified by bulb to bulb distillation. A sample of >99.8% pure ester 178 (300mg., 92%) was obtained as a colourless oil.

R_T (SP1000, Et_2O, 130°C) = 6.6m.

i.r. (film):- 2940, 2860, 1740, 1435, 1300, 1260, 1215, 1175 cm^{-1}.

m.s.:- m/e = 99 (96%), 95 (100), 94 (67), 79 (41), 74 (73), 67 (83), 41 (50), 39 (35). M^{+}(168) = $C_{10}H_{16}O_2$.

^1H n.m.r.:- 3.65 (3H, s), 2.4-1.0 (13H, m)δ.

^{13}C n.m.r.:- 173.4 (s), 51.2 (q), 41.2 (t), 41.2 (d), 38.5 (d), 37.9 (t), 36.9 (d), 35.2 (t), 29.9 (t), 28.6 (t) p.p.m.

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175. Cigarettes were kindly donated by Gallaher Ltd., Belfast.

176. This spectrum was run on a Perkin-Elmer R-24 60MHz. spectrometer.

177. The distillation was achieved in the following manner. A glass tube was washed with methanolic potassium hydroxide and water, then dried. The sample was placed in the closed end of the tube and the open end was attached directly to an oil pump. The closed half of the tube was then placed in a preheated glass oven. The fore-run was flamed away, then the middle fraction was collected at the top

end of the tube. The fraction was collected by breaking the tube, after vacuum release. The complete distillation procedure took less than one minute.

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