STUDIES IN NATURAL PRODUCTS

Sesquiterpenoids of *Brachylaena hutchinsii*

A thesis submitted to the University of Glasgow

for the degree of Ph.D.

by

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To my Mother

Studies in Natural Products

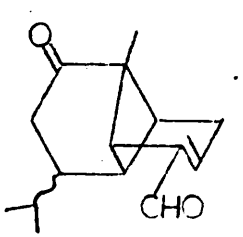
Sesquiterpenoids of Brachylaena hutchinsii

A.G.Y. MacKintosh

Summary

The chemical constituents of Brachylaena hutchinsii, a hardwood indigenous to East Africa, have been examined, and sesquiterpenoids of the heartwood extract and of the steam-volatile oil (Essential Oil Muhuhu) have been investigated.

The first part of the thesis is concerned with the chemical composition of the heartwood and, in particular, with the structures of the Brachylaenalones, principal sesquiterpenoid constituents of an extract of the heartwood. In the present work, structures (A), proposed earlier (C.J.W. Brooks and M.M. Campbell, Chem.Comm., 1969, 630) for these compounds, are re-examined and revised to structures (B) in the light of further evidence. In



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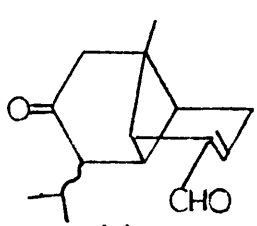


(B)

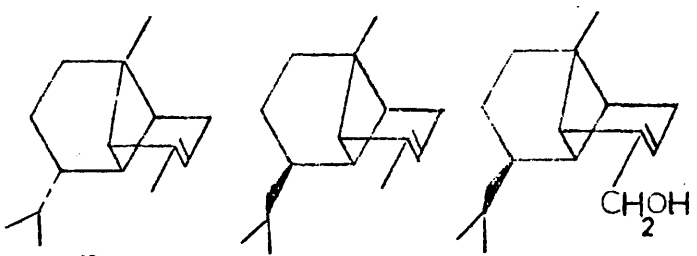
addition, the products of hydride reduction of the Brachylaenalones,

previously reported to be three diastereomeric diols, are re-investigated and, in consequence of a refinement in analytical procedure, the occurrence of the fourth, expected diol is established. This work has necessitated the use of a wide range of analytical techniques, and physico-chemical data of the Brachylaenalones, the diols and the corresponding ketols have been recorded and analysed.

Chemical correlation of the Brachylaenalones with the expected parent hydrocarbons, copaene and ylangene, has been attempted by several routes, but the desired transformations have been accompanied by side reactions, leading to complex mixtures of products in each case. Reduction of the dithioketal derivative of one ketoaldehyde over Raney nickel catalyst yielded a mixture of products comprising neither copaene nor ylangene: reduction of each ketoaldehyde according to the Wolff-Kishner method, however, afforded the parent hydrocarbons as minor products.

In the second part of the thesis, a survey of the chemical constituents of the steam-volatile oil from the heartwood is described, and comparisons are drawn with the composition of the heartwood extract. Separations of the oil into fractions by gas-liquid chromatography (analytical and preparative), together with combined gas chromatographic-mass spectrometric analyses of individual fractions, supplemented in some instances by infra-red spectrometry, has allowed the documentation of molecular weights and functional types

present in the oil, and the tentative identifications of copaene (C), ylangene (D) and ylangenol (E). The Brachylaenalones were not present in the oil, which comprised mainly compounds of molecular weights in the range 200-222.



(C) (D) (E)

ACKNOWLEDGEMENTS

I wish to express my gratitude to Dr. C.J.W. Brooks, D.Sc., for his guidance and interest at all times, and to Professor R.A. Raphael, F.R.S., for providing the opportunity to carry out this research.

Thanks are also due to the technical staff of the Department for excellent service.

The work was carried out during the tenure of a Demonstratorship.

Department of Chemistry, University of Glasgow, August, 1971.

CONTENTS

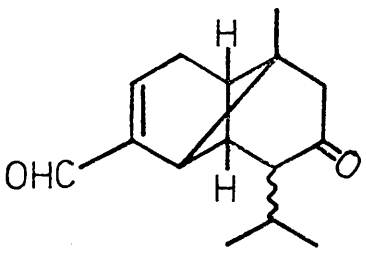
PART 2: CHEMICAL CONSTITUENTS OF THE STEAM-VOLATILE OIL FROM BRACHYLAENA HUTCHINSII (ESSENTIAL OIL MUHUHU)

2.1 DISCUSSION OF RESULTS

Conventions and Nomenclature

In the text that follows, numbers appearing as a superscript thus [12] denote references, while those written in line with the text indicate drawings of chemical formulae, and may be referred to as 'structure 4' or 'the Brachylaenalones (1,2)'.

In drawings of chemical structure, stereochemistry is not implied unless specifically indicated: a thickened or broken bond denotes a substituent located respectively above or below the plane of the paper; a wavy bond indicates an epimeric mixture.



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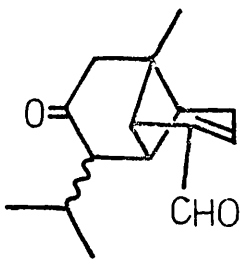

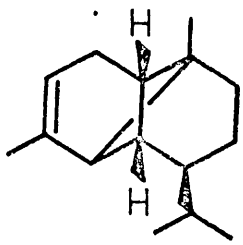

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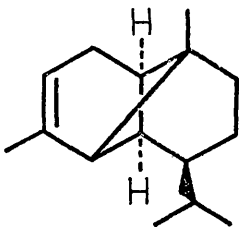

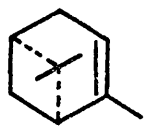

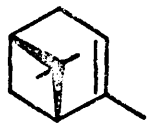

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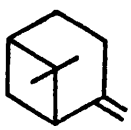

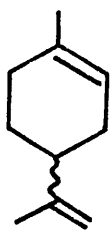

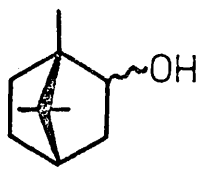

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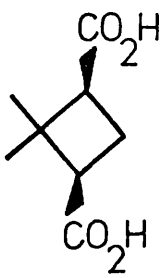

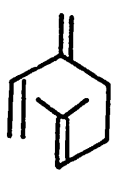

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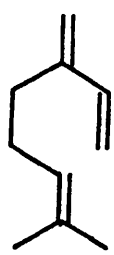

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INTRODUCTION

The sesquiterpenoids comprise a large group of C-15 compounds found in nature, primarily in plants, but also within the animal kingdom and as mould metabolites. Since the first recognition in 1833 of the natural occurrence of sesquiterpenoids,[1] the wide diversity of acyclic, monocyclic and fused-ring structures occurring within this field has provided many interesting and challenging problems for the organic chemist. In the area of structural elucidation, recent years have seen a great increase in the use of established spectrometric methods, notably infra-red (IR) and nuclear magnetic resonance (NMR) spectrometry, while the advent of combined gas-chromatography - mass spectrometry (GC - MS) has afforded to the chemist a most powerful technique for the direct analysis of complex mixtures separable by gas - liquid chromatography (GLC). In addition, great progress has been made in the solution of stereochemical problems, and the availability of X-ray and optical techniques has been of increasing importance in this area.

The major part of this thesis is concerned with the structural elucidation of two sesquiterpenoid ketoaldehydes (78,79), isolated from the heartwood of Brachylaena hutchinsii Hutch. (Compositae), from East Africa, and possessing structures based on the copaene (3) and ylangene (4) skeletons.

Since the number of naturally occurring compounds containing a cyclobutane ring is comparatively small, it is interesting to review briefly the occurrence of these more generally in the field of mono-and sesquiterpenoids.

Monoterpenoids

α-Pinene is one of the most important of terpene hydrocarbons, giving rise to all the known cyclobutanoid monoterpenoids. It is widely distributed in nature in both (+)- (5) and (-)- (6) forms, and often in conjunction with the β-isomer (7) which is, however, generally present to a lesser extent. In particular, it is found in the majority of oils from the Coniferae and is the principal constituent of oil of turpentine, known from early times. α- and β-Pinene may be considered as the parent hydrocarbons of many terpenes, since monocyclic terpenes [e.g. dipentene (8)] are produced by ring fission and the camphane skeleton [e.g. borneol (9)] from molecular re-arrangement.

The presence of a cyclobutane ring in pinene was established firmly in 1895 in a series of experiments by von Baeyer,[2] but not until 1929 was confirmation of this formula achieved in a synthesis by Kerr[3] of cis-norpinic acid (10), obtained by von Baeyer as an oxidation product of α-pinene. This was the first structural elucidation of a natural product containing a cyclobutane ring.

Much of the present interest in pinene has centred on

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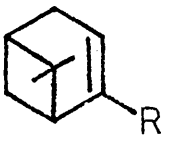
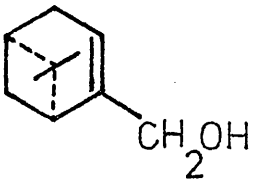
13, R = CHO
14, R = CO_2H





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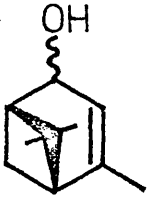

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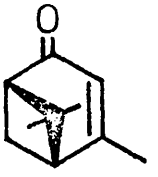

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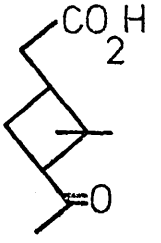

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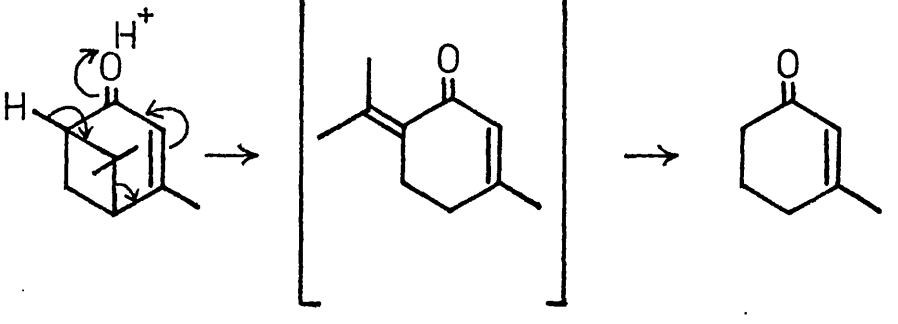

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its products of re-arrangement. In the field of its oxygenated derivatives, for example, Suga *et al.*[4] have described a pinacol-type conversion of 1β-hydroxy - 2β-tosyloxy-pinane to the strained bicyclohexane system, providing information on the conformation and reactivity of these structural types. Decyclisation of β-pinene by pyrolysis yields myrcene (11),[5] the starting point in the synthesis of many commercial perfumery chemicals.

The reduction of pinene represents another area of activity and has been the subject of an investigation by Cocker *et al.*[6] who found that the catalytic reduction of α-pinene at elevated temperatures yields more (+)-*trans*- than (+)-*cis*-pinane, in agreement with the results of earlier workers.[7] Brown *et al.*[8] studied the reduction of α-pinene to *cis*-pinane with diborane, where the intermediate alkyl borane proved to be of value in the stereospecific conversion of *cis*-olefins to optically active alcohols. It is of interest to note that in these reductions, cyclobutane ring cleavage apparently did not take place.

Other naturally occurring monoterpenoids of structural type similar to pinene include myrtenol (12), myrtenal (13) and the corresponding acid, myrtenic acid (14). The relationship of these compounds to their parent hydrocarbon is apparent in the oxidation of α-pinene with selenium dioxide, resulting in the formation of myrtenal and, when a deficiency of the oxidising agent is used, the corresponding alcohol.[9] (+)-

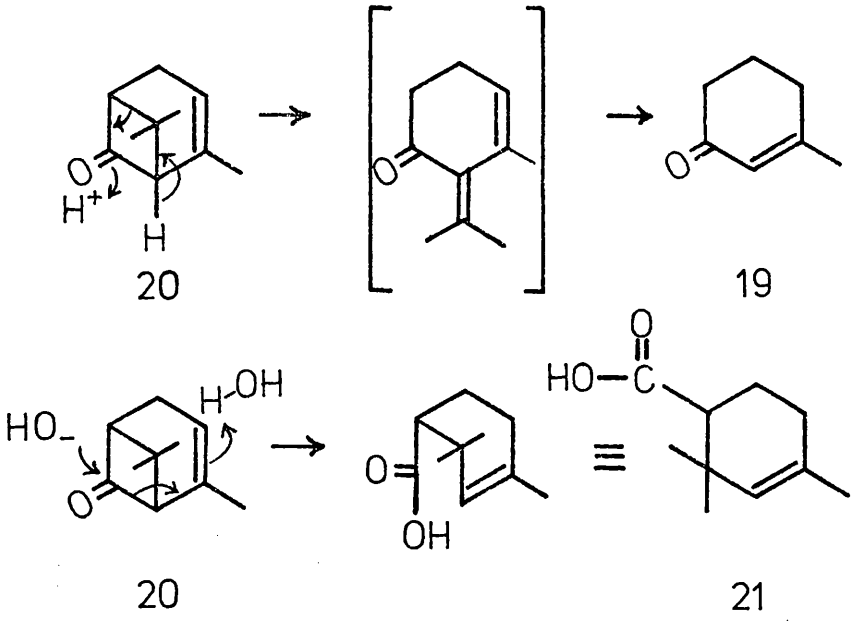
Myrtenol (15) was first obtained from the leaves and flowers of Myrtus communis L. in 1905,[10] and its enantiomer fifty years later from Valeriana officinalis.[11] The (+)-aldehyde was isolated from oil of false camphor-wood in 1911[12] and the corresponding acid first obtained more recently from Chaemaecyparis species.[13]

Autoxidation of α-pinene results in formation of the monoterpenoids, verbenol (16) and verbenone (17).[14] Verbenone was first isolated in 1900 from the essential oil of Verbena triphylla, and its structure elucidated by oxidation to pinonic acid (18).[15] Verbenol was first shown to occur in the essential oil from the oleoresin of Boswellia carterii in 1913,[16] and its structure was determined later in the same year[15] by comparison of its oxidation product with an authentic sample of verbenone. The stereochemistry of each of the epimeric verbenols was defined in 1940,[17] in an examination of the products of reduction of verbenone. Similarly, stereochemical relationships between the verbanols and verbanones, which have not been obtained from any natural source, were unambiguously defined in 1969.[18] The cyclobutane ring of verbenone is unstable to mineral acid, on account of its position α to the enone system. Accordingly, treatment with dilute sulphuric acid results in the formation of 1-methylcyclohex-1-en-3-one (19).[15]

Photolysis of verbenone results in the formation of chrysanthenone (20),[19] the structure of which was characterised

Figure 1

Reactions of Chrysanthenone (20) in Acid and Base



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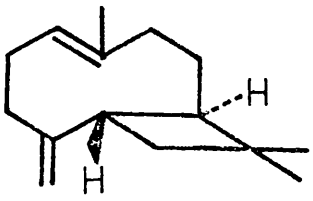
after its first isolation in 1957 from the leaves of Chrysanthemum sinense Sabin.[20] The rearrangements of chrysanthenone in acid and base have been studied. The reaction of chrysanthenone on digestion with acid parallels the reaction of verbenone under the same conditions, with production of 1-methylcyclohex-1-en-3-one (19). In the presence of base, however, a different pattern of cleavage is observed,[21] with formation of 2,2,4-trimethylcyclohex-3-ene-1-carboxylic acid [(21), Fig. 1]

The selenium dioxide oxidation of α-pinene was discussed above. In a parallel oxidation of β-pinene,[22] a small yield of the monoterpenoid, pinocarvone was produced. (-)-Pinocarvone (22) has been found to occur in the essential oil of Eucalyptus globulus,[23] in admixture with the corresponding alcohol, (-)-pincarveol.[24] The trans-configuration (23) has been assigned to the naturally occurring alcohol.[23]

A recent review of gem-dimethyl-cyclobutanoids related to pinonic acid (18) has been presented by Subramanian et al.,[25] with particular reference to stereochemical information resulting from NMR, optical rotatory dispersion (ORD) and circular dichroism (CD) studies. A more general review of the stereochemistry of compounds in the pinane series has been written by Teisseire et al.[26]

Sesquiterpenoids

In the sesquiterpenoid field, cyclobutane rings occur in a considerably greater variety of molecular structures. Among the earliest known examples of cyclobutanoid



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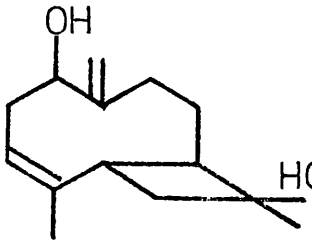

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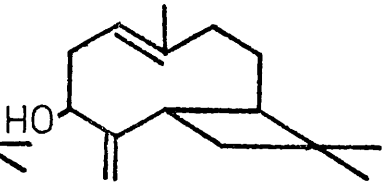

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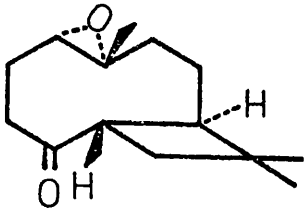

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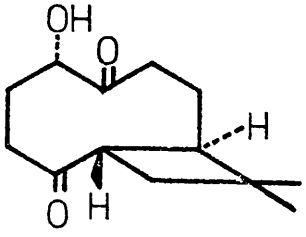

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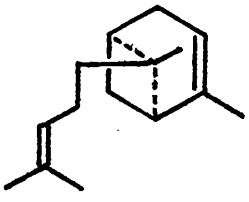

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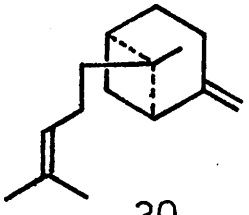

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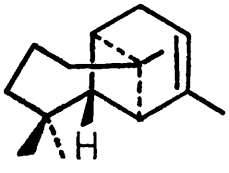

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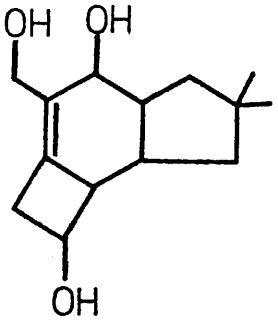

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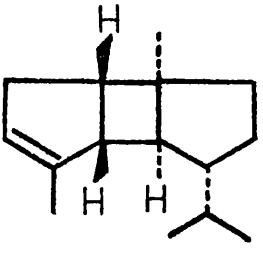

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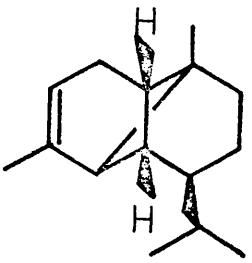
sesquiterpenoids are those within the caryophyllane group. Caryophyllene (24) was discovered in 1892[27] in oil of cloves (Eugenis caryophyllata), and its structure was finally established in the early 1950's. The structures of α-betulenol (25) and β-betutenol (26)[28] are based on the same skeleton. More recently, two new nor-sesquiterpenoids of the same group, kobusone (27) and iso-kobusone (28) have been discovered[29] in the essential oil of nutgrass (Cyperus rotundus Linn.) of Japanese origin.

Other bicyclic sesquiterpenoids containing the cyclobutane ring include α-bergamotene (29) and β-bergamotene (30), of the bisabolane group. α-Bergamotene was originally isolated from bergamot oil (Oleum bergamottae verum) in 1950 by Herout et al.,[30] but was not characterised until 1963 by Kovats.[31] The structure of β-bergamotene, isolated in 1963 from the root oil of Valeriana Wallichi,[32] was established earlier in the same year. β-Bergamotene may be regarded as bicyclic sesquiterpenoid analogue of β-pinene. A tricyclic analogue of α-pinene is represented by α-longipinene (31), first shown to occur as a minor sesquiterpenoid in the wood of Pinus silvestris L.[33] It was later isolated from Swedish sulphate turpentine[34] and its structure established by Lindstrom and Westfelt.[35]

A tricyclic sesquiterpenoid of a different structural type is illudol (32), first isolated in 1967 as a metabolite of Clitocybe illudens,[36] and at present the subject of an attempted total synthesis.[37] Synthesis is a comparatively recent area of development in the field of terpenoids; its



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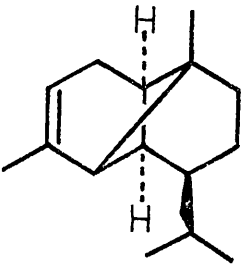

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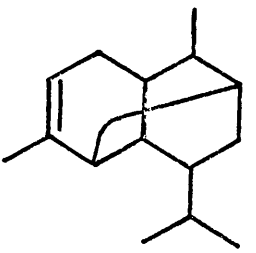

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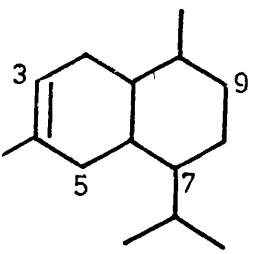

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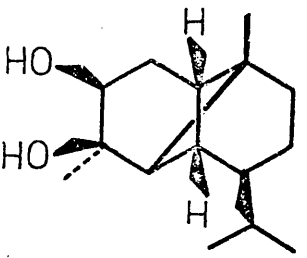

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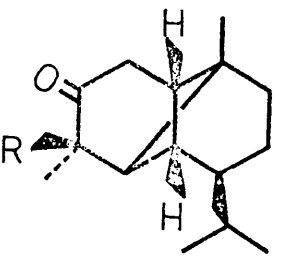
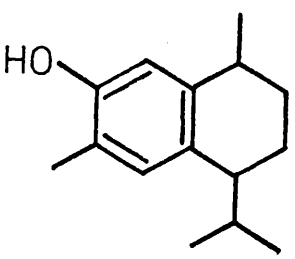


37, R = OH
38, R = H



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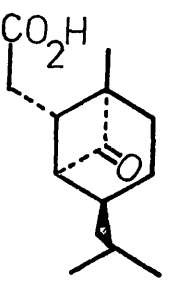

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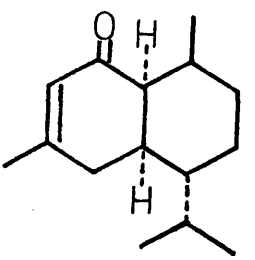

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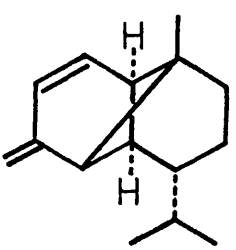

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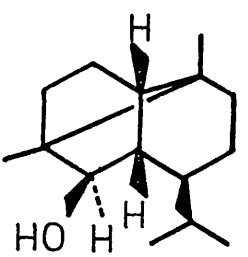

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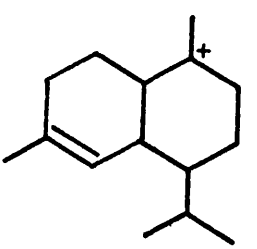


44

importance lies in the fact that the structure of a compound is seldom unambiguously proved until its total synthesis has been achieved. Thus the structure of α-bourbonene (33), isolated with its β-isomer from the essential oil of Geranium Bourbon in 1966,[38] was confirmed two years later by its total synthesis.[39]

Copaene, Ylangene and Related Compounds

Of particular relevance to the present work are the structures of the tricyclic sesquiterpenoids, α-copaene (3), its stereoisomer α-ylangene (4) and the corresponding β-isomers with double bonds exocyclic, according to the accepted nomenclature. These compounds have been the subject of a great deal of research since the first isolation of α-copaene from African oil of copaiba (from Oxystigma manii Harms.) in 1914.[40] The literature regarding the copaenes and ylangenes, however, has been sometimes confused, and only recently have unambiguous structural elucidations of these compounds been achieved.

As early as 1914, α-copaene was known to be a tricyclic sesquiterpenoid with one double bond,[41] and at that time it was believed to contain a cyclopropane ring, a postulate which gained support from the findings of later workers. e.g. [42] In 1950, Birch[43] pointed out that on the basis of available data, a structure such as 34 was not precluded, but Vonasek et al.[44] proposed the presence of a cyclopropane

methylene group in copaene, primarily from a study of its infra-red spectrum. This spectral evidence was rejected by Büchi *et al.*[45] who, by means of a series of transformations of copaene, proved its cyclobutanoid structure. A keto-acid was obtained by ozonolytic cleavage of the double bond of copaene, and this was found to isomerise to a homogeneous substance on treatment with mild alkali. Since inversion adjacent to the carboxylate anion was considered improbable under the conditions employed, inversion adjacent to the ketone was inferred, requiring an asymmetric centre at C-5 and thus implicating C-5 as a terminus of the missing bond in 35 . An analysis of the NMR spectrum of copaene revealed a quaternary carbon at C-10, indicating that the remaining terminus was at this centre. Treatment of copaene with osmium tetroxide gave 36 , which was oxidised to a cyclohexanone, the infra-red spectrum of which was consistent with structure 37 . Similarly, hydroboration of copaene, followed by oxidation of the resulting alcohol, gave copaone (38) which, on treatment with sodium methoxide in deuteromethanol, was equilibrated to a mixture of copaone and its C-4 epimer, and incorporated three deuterium atoms. Ketol 37 , by treatment with formic acid and hydrolysis of the resulting formate, was converted to a phenol (39), and the position of the ethylenic linkage in copaene was inferred from a consideration of the mechanism of formation of this

compound. Finally, in a series of oxidations, diol 36 was converted to a keto-acid, characterised as 40 . Thus the first cyclobutanoid structure for copaene was proposed, and the known stereochemical relationship of (-)-α-copaene to the cadinenes[46] allowed allocation of its absolute stereostructure (3).

This structure was confirmed by Kapadia et al.[47] in connection with the structural determination of (+)-mustakone (41), a sesquiterpenoid ketone obtained from Cyperus rotundus Linn. and directly related to copaene. Oxidation of copaene with t-butyl chromate yielded mustakone, and hydrogenation over pre-reduced platinum oxide in glacial acetic acid resulted in the uptake of 1 mole of hydrogen.

β-Copaene was first obtained from Valencia orange oil by Hunter et al.[48] This group, however, originally described the compound as "β-ylangene", in accordance with their earlier report on "α-ylangene",[49] a description subsequently corrected[50] to α-copaene. β-copaene was found to isomerise to α-copaene in the presence of sulphuric acid, and uptake of one mole of hydrogen was reported in a hydrogenation of β-copaene over platinum oxide.

α-Ylangene was isolated from Schizandra chinensis in 1963 by Motl et al.,[51] who proposed three possible structures containing a cyclopropane ring. In 1965, however, the demonstration[45] of the cyclobutanoid nature of

copaene, led the Czech group to revise their original findings[52] and to conclude that one antipode of ylangene was represented by the structure 4. The absolute configuration of (+)-α-ylangene was later established as 4 by Ohta et al.,[53] in a study of its acid-catalysed isomerisation to the amorphene system, described below.

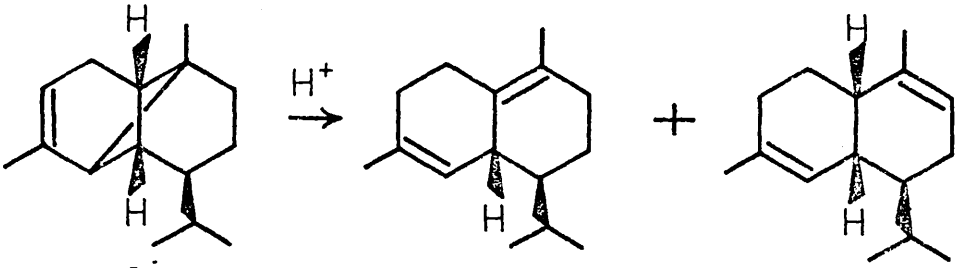
β-Ylangene, first reported in 1966 as a constituent of Swedish sulphate turpentine,[54] was also obtained from the wood of Pinus silvestris L.[55] and its structure established in a partial synthesis from the α-isomer.

An elegant total synthesis of the copaene/ylangene system, achieved by Heathcock et al. in 1966,[56] finally confirmed the structures of the copaenes and ylangenes.

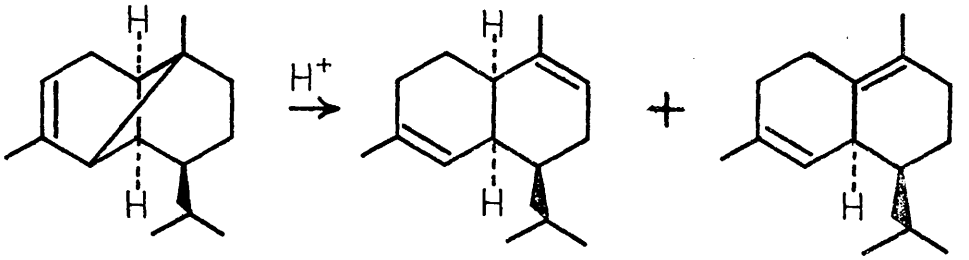
Sesquiterpenoids isolated more recently include (+)-copadiene (42), from Cyperus rotundus Linn.,[57] and (+)-copaborneol (43), from the wood of Pinus silvestris L. and from Swedish sulphate turpentine.[58] The relationship of these compounds to copaene is obvious.

Biogenetic Relationships

It is of interest to consider a number of biogenetic speculations regarding the copaene and ylangene classes. Büchi et al.,[45] in describing the absolute stereostructure of copaene, suggested the ion 44 as its immediate precursor. This would necessitate Markownikoff attack on



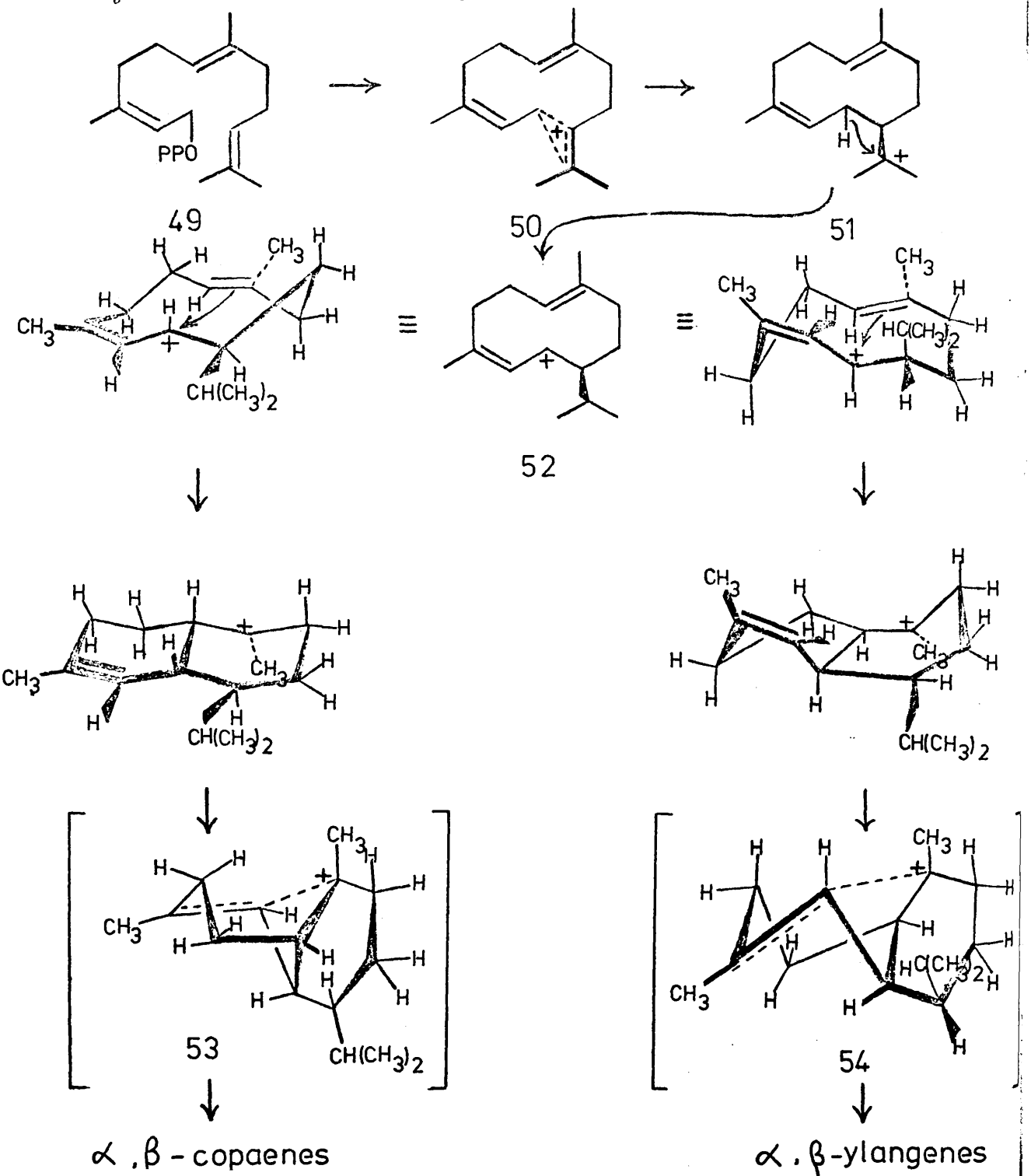
3 45 46



4 47 48

Figure 2.

3D Representation of Mechanism proposed by Ohta *et al.* (in 2D) for Biogenesis of Copaenes and Ylangenes, showing Transition States 53 and 54 , respectively, involved in formation of cyclobutanoid structures.



the ethylenic linkage in the formation of copaene. Ohta et al. supported this postulate: from a study of the behaviour of copaene and ylangene in the presence of mineral acid,[53] they inferred a possible biogenetic relationship between these compounds and their analogues of bicyclic structure.

On treatment with mineral acid, (-)-α-copaene (3) isomerised to a mixture comprising (+)-δ-cadinene (45) and (-)-α-muurolene (46), while (+)-α-ylangene (4) gave two new compounds, (-)-α- (47) and (+)-δ- (48) amorphene (see Table 1). These facts, together with a consideration of cadalene types found in nature, prompted Ohta et al. to propose a mechanism for the biogenesis of copaene and ylangene. They suggested that the precursors of these compounds must be either identical or closely related, and postulated the involvement of cation 51 (Fig. 2) which may be derived[59] from cis-farnesyl pyrophosphate (49) by way of the non-classical carbonium ion 50 . Cation 51 , by a 1,3-hydride shift, could be converted to the common intermediate 52 . From this intermediate, via the bicyclic transition state 53 , the copaenes could be formed by elimination of a proton; similarly, the ylangenes may be produced from intermediate 52 via the transition state 54.

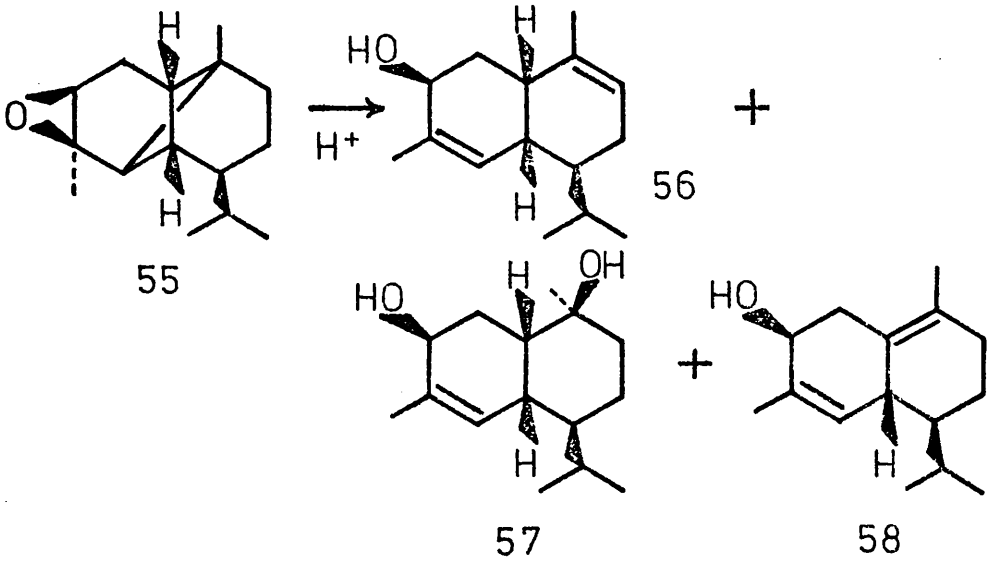
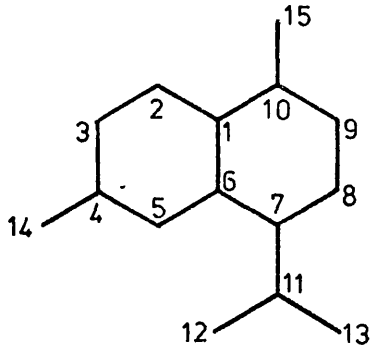

H
O
H
55
HO
H
H
H+
56
+
HO
H
OH
H
57
HO
+
H
58

Table 1

Classification of Cadinenes, Muurolenes, Amorphenes and Derivatives, as relevant to Introduction.



Numbering of skeleton based on principle formulated by Barton et al.[111]

A. Cadinenes: 1α-H, 6β-H; 7β-isopropyl

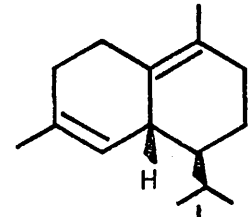
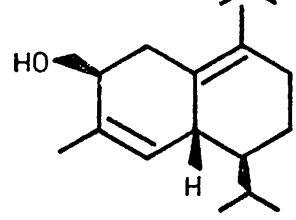
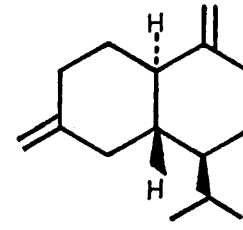
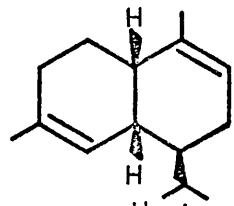
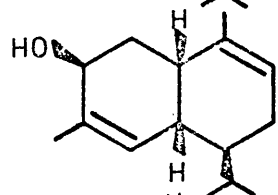
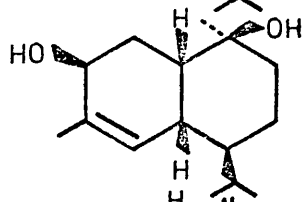
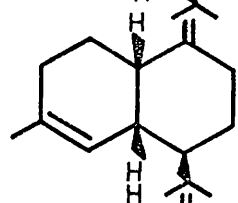
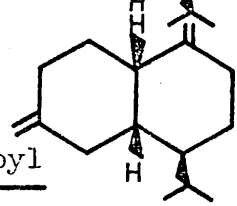
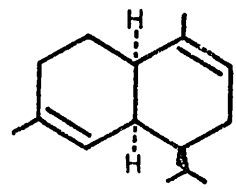
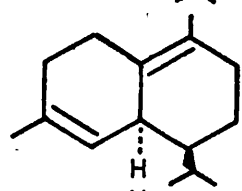
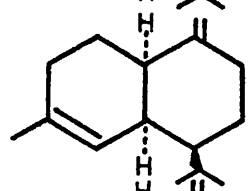
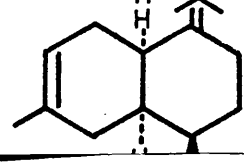
Name	No.	Unsaturation	Formula
δ-cadinene	45	$\Delta^{1(10),\ 4(5)}$	
3β-hydroxy-δ-cadinene	58	$\Delta^{1(10),\ 4(5)}$	
ε-cadinene	60	$\Delta^{4(14),\ 10(15)}$	

Table 1, cont'd

B. Muurolenes: 1β-H, 6β-H; 7β-isopropyl

Name	No.	Unsaturation	Formula
α-muurolene	46	$\Delta^{4(5),9(10)}$	
3β-hydroxy-muurolene	56	$\Delta^{4(5),\ 9(10)}$	
3β-hydroxy-T-muurolol	57	$\Delta^{4,\ 5}$	
γ-muurolene	59	$\Delta^{4(5),\ 10(15)}$	
ε-muurolene	61	$\Delta^{4(14),\ 10(15)}$	

C. Amorphenes: 1α-H, 6α-H; 7β-isopropyl

Name	No.	Unsaturation	Formula
α-amorphene	47	$\Delta^{4(5),\ 9(10)}$	
δ-amorphene	48	$\Delta^{1(10),\ 4(5)}$	
γ-amorphene	62	$\Delta^{4(14),\ 10(15)}$	
γ_2-amorphene	63	$\Delta^{3(4),\ 10(15)}$	

Bicyclic Terpenoids Related to Copaene and Ylangene

Among recent workers in this field, Ohloff *et al.*[60] have studied the isomerisation of 3,4-epoxy-(-)-α-copaene (55) on treatment with weak mineral acid. In accordance with the results of Ohta *et al.*,[53] the epoxide was found to yield compounds of the α-muurolene and δ-cadinene series (see Table 1): (-)-3β-Hydroxymuurolene (56), (-)-3β-hydroxy-T-muurolol (57) and (-)-3β-hydroxy-δ-cadinene (58) were the products of this re-arrangement.

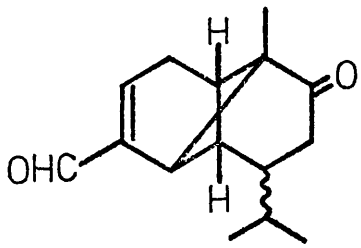
Thus it is evident that the copaenes may be described as tricyclic muurolenes, and the ylangenes, similarly, as amorphenes. In this context, a brief account of the history of the muurolene and amorphene groups is of relevance.

The presence of "muurolene" was reported by Aschan in 1929[61] in a commercial extract of pine stumps. In 1964, Pentegova[62] obtained "muurolene" from the resin of the common pine. In neither of these cases, however, was the compound fully characterised. The structures of α- and γ-muurolene, major sesquiterpenoids of the wood of Scots pine (*Pinus silvestris* L.) and of Swedish sulphate turpentine, were first elucidated by Westfelt in 1966[63] and, independently and almost simultaneously, by Zabza *et al.*[64] in an examination of sesquiterpenoids from the turpentine of *Pinus silvestris* of Polish origin. Both (-)-α- and

(-)-γ-muurolene were found to be 1-epi-cadinenes, with the absolute stereostructures 46 and 59 respectively. In addition, Westfelt[63,65] revised the structure of a compound investigated by Sykora in 1958,[66] for which structure 60 had been postulated and the name "ε-cadinene" adopted. A cis-(61) rather than a trans-ring junction was now proposed and, in consequence, the original name was changed to (+)-ε-muurolene.

"Amorphene" was first reported in 1904[67] as a constituent of the oil from Amorpha fruticosa L. fruits, but at that time its characterisation was not attempted. Motl et al., however, in a re-examination of the oil in 1966,[68] postulated the structures of two new bicyclic hydrocarbons, (stereoisomeric with the cadinenes) for which the names γ- and γ_2-amorphene were proposed: (-)-γ-amorphene was assigned structure 62 and, (-)-γ_2-amorphene, more tentatively, structure 63. The structures of the (-)-α-(47) and (+)-δ-(48) isomers, first obtained by the acid-catalysed isomerisation of (+)-α-ylangene, were elucidated in 1968 by Ohta et al.[53]

The natural occurrence of the muurolenes and amorphenes is thus of particular relevance to the copaene and ylangene groups, and it is interesting to observe that the first structural elucidations of these bicyclic compounds were reported after the structures of their tri-



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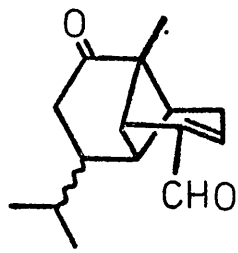

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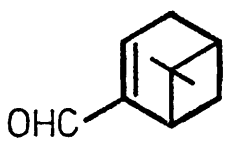

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cyclic counterparts had been largely established.

The importance of the copaenes and ylangenes will, it is felt, be appreciated more fully when considered in the context of terpenoids in general; it is with the intention of illustrating the variety of interesting cyclobutanoid structures within the larger field that this brief review has been presented.

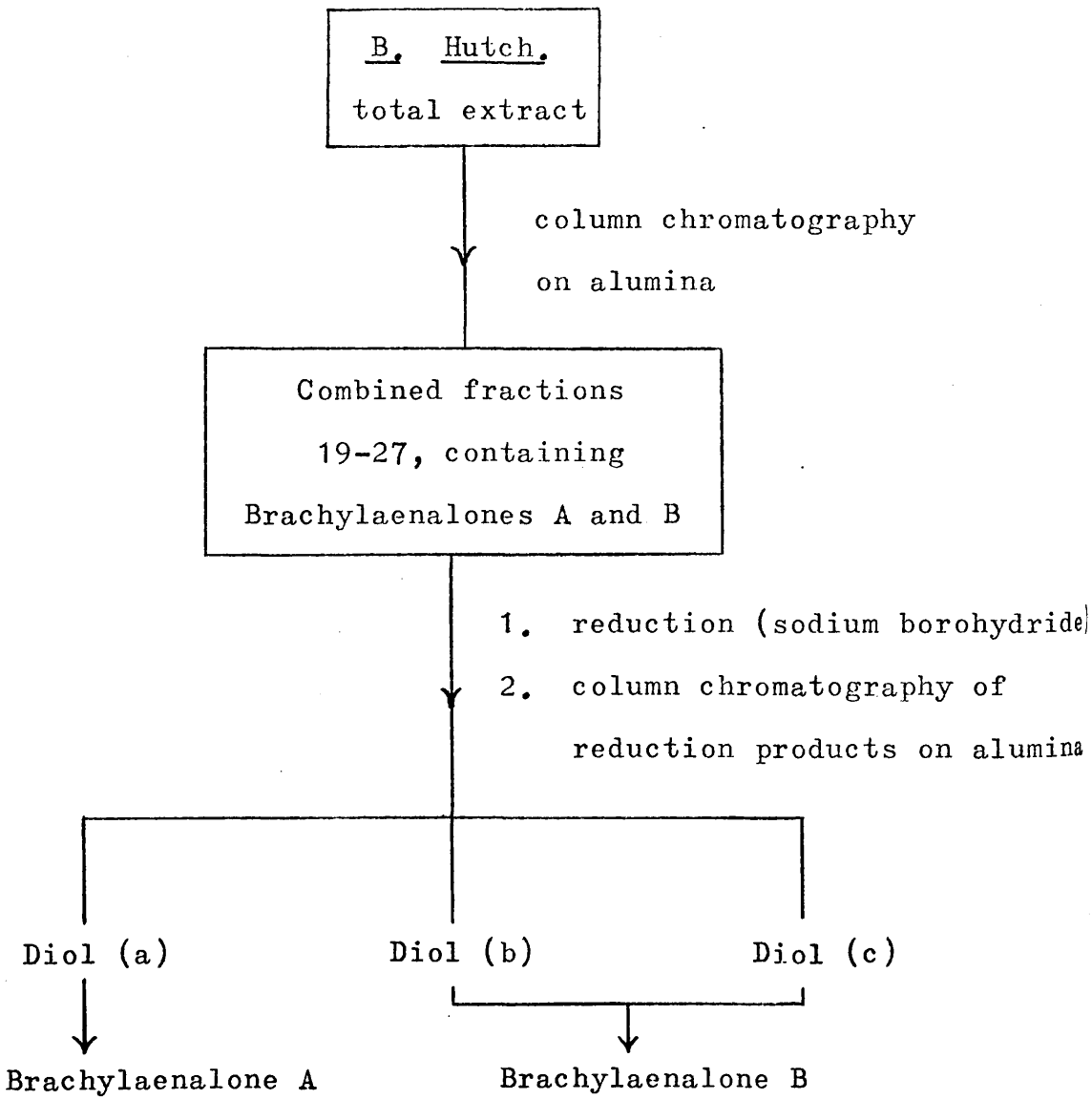
Sesquiterpenoids of Brachylaena Hutchinsii

In the present work, the partial structures (1, 2) proposed in this Department[69] for two new sesquiterpenoid ketoaldehydes are re-examined and revised in the light of further evidence. The compounds designated as Brachylaenalones A and B were isolated from the heartwood of Brachylaena hutchinsii, a hardwood indigenous to East Africa and also known as Muhuhu. The tree, a member of the Compositae, is found at altitudes of up to 6,500 feet and yields a hard, dense timber which is used to manufacture flooring blocks.[70] The principal literature references to Brachylaena hutchinsii[71-73] describe the essential oil obtained by steam-distillation. The oil, described as reminiscent of cedarwood and vetiverwood oil in fragrance, was found to comprise a predominance of sesquiterpenoid alcohols and ketones, but a more detailed investigation of its constitution has not been reported. The evidence

presented by Brooks and Campbell for the structures of the Brachylaenalones, isolated as major constituents of the essential oil from extraction of the heartwood, depended largely on an NMR analysis of the two epimers and a comparison with the apparent monoterpenoid analogue, myrtenal (13). The ketoaldehydes were correlated with their products of partial reduction, three diol isomers. It seemed necessary to extend the evidence on which the structural assignments were based, and to consider in some detail stereochemical assignments within the ketoaldehyde and diol series. It was with this intention that the present work was undertaken, during which, in consequence of a refinement in analytical procedure, the occurrence of the fourth expected diasteromeric diol was established. In addition, an investigation of the composition of the steam-volatile essential oil, using modern techniques of separation and analysis, provided an interesting comparison with that found in the essential oil from extraction of the heartwood.

Figure 3

Isolation of Pure Diols and Ketoaldehydes from Total Extract of *Brachylaena hutchinsii*



Part 1: Heartwood of Brachylaena hutchinsii - The Constitution of the Brachylaenalones

1.1 Discussion

Isolation of the Brachylaena Diols

In their initial investigation of the heartwood of Brachylaena hutchinsii, Brooks and Campbell[69] reported that extraction with ethyl acetate afforded an oil which represented about 20% by weight of the wood. Careful chromatography of this oil on alumina yielded two isomeric ketoaldehydes (Brachylaenalones) and the apparently analogous ketoalcohols as intractable mixtures, and only partial success was achieved in the attempted resolution of these isomers by further chromatography. A more efficient procedure for obtaining the individual Brachylaenalones was found to be the chromatographic separation of their products of reduction, reported to be three epimeric diols, and subsequent oxidation of these to the respective ketoaldehydes. This procedure was employed in the present work for obtaining the diols and their corresponding homogeneous ketoaldehydes (Fig. 3). Reduction of the total extract before column chromatography was found to offer no advantage.

The heartwood, finely powdered and extracted in a Soxhlet apparatus with ethyl acetate, afforded a brown oil,

Table 2

Useful Fractions Collected in Column Chromatography of Brachylaena Hutchinsii Extract

Fractions combined on the basic of TLC	Eluent	Content
1 - 6	5% ether-petrol[a]	See Part 2 of thesis
7 - 10	" " "	
17 - 18	25% " "	"Ketols"
19 - 27	" " "	Brachylaenalones
30 - 32	50% " "	"Ketols"
33 - 38	100% " "	

[a] light petroleum, 60 - 80°C.

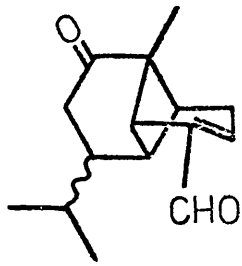
Table 3

Useful Fractions Collected in Column Chromatography of Ketoaldehyde Reduction Mixture (13 g.)

Fractions combined on the basis of TLC	Eluent	Weight	Content
9	25% ether-petrol[a]	387 mg	Rel. pure mono-ol
10-11	" " "	811 mg	Mono-ol, slightly less pure
28-34	50% " "	1·12 g	Rel. pure diol (b)
39-43	70% " "	254 mg	Rel. pure diol (a)
44-49	" " "	1·40 g	Pure diol (a)
50-53	" " "	319 mg	Rel. pure diol (a)
59-61	100% " "	376 mg	Pure diol (c)
62-63	10% ethyl acetate-ether	112 mg	Rel. pure diol (c)

[a] light petroleum, 60-80°C

Overall recovery for column = 63% (run over 5 days).



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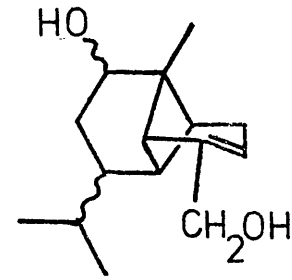
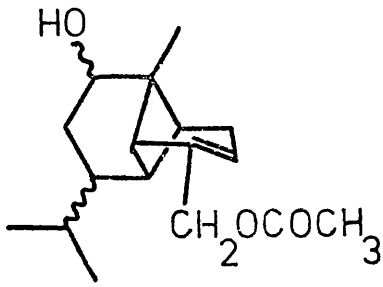


64-66

comprising about 15% by weight of the wood. Chromatography of 100 g of this oil on alumina resulted in its separation into fractions which were combined on the basis of TLC, as detailed in Table 2. Fractions 19-27 (19 g) were shown to contain the Brachylaenalones A and B, of postulated structures 1, 2, and on treatment with sodium borohydride this combined fraction was reduced to a mixture of diols (a), (b) and (c) (64-66) from which, on standing, 600 mg of crystalline isomer (c) was isolated. 13 G of the remainder was subjected to column chromatography on alumina, and useful fractions were combined on the basis of TLC (Table 3). A satisfactory separation of diols (a) - (c) was thus achieved. Ketoaldehyde A was obtained by Sarett oxidation of diol (a), and isomer B by similar treatment of diols (b) or (c). The purities of all compounds were estimated by TLC, GLC (SE-30 and OV-1) and by IR examination.

High resolution IR spectra of the Brachylaenalones (carbon tetrachloride solution) revealed differences in the main positions of absorption in the region 2800 - 3000 cm^{-1}: isomer A exhibited bands due to ν_{C-H} at 2953, 2920, 2868 and 2813 cm^{-1}, while the corresponding bands in the spectrum of isomer B appeared at 2956, 2927, 2870 and 2815 cm^{-1}. Absorption frequencies in the carbonyl region were identical, with bands at 1717 (ketone), 1686 (conjugated aldehyde) and



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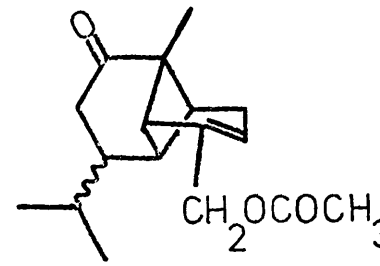
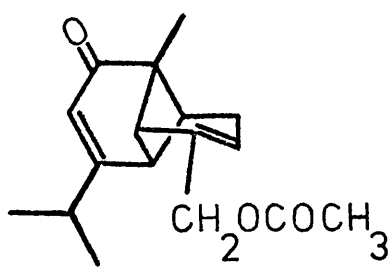
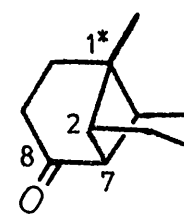


68

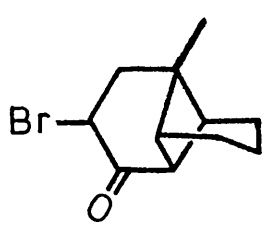


69



*ref. 56

70



71

1627 (conjugated C=C) cm^{-1}. In addition, slight differences in the relative intensities of absorption were evident throughout both spectra (for example, see experimental section).

In order to confirm that the stereochemical difference between the Brachylaenalones lay in the configurations of their isopropyl groups, it was decided to attempt the introduction of unsaturation α, β to the ketone, thus eliminating the possibility of isomerisation at the β-carbon. Corresponding α, β-unsaturated ketones from both series should then be identical. In the preparation of compounds of this type, it was necessary to protect other reactive sites in the molecule. Selective acylation, subsequent oxidation of the primary monoacetates and attempted preparation of α- β-unsaturated ketoacetates appeared to be a suitable reaction sequence for investigation.

Preparation of Ketoacetate (68) from Diol (c)

Treatment of diol (c) with acetic anhydride/pyridine under carefully selected conditions gave an approximately 9:1 ratio of diol monoacetate (67): starting material, as judged by TLC. A small amount of diacetate was also observed. Pure monoacetate was obtained by preparative TLC of the mixture, and its homogeneity was verified by TLC and by GLC on the phase OV-1. The IR spectrum of the monoacetate (carbon tetrachloride solution) had bands at

3617 (ν_{O-H}), 1743 ($\nu_{C=O}$ of acetate), 1235, 1225, 1039 and 1023 (ν_{C-O} of alcohol and ester) cm^{-1}.

Oxidation of the monoacetate with chromium trioxide/pyridine (Sarett[74]) resulted in conversion to the corresponding ketoacetate (68). The presence of a negligible amount of diol monacetate in the product was suggested by TLC and a further impurity of R_f value higher than the ketoacetate was also observed in apparently low concentration. GLC examination of the crude product on OV-1 afforded further proof of its purity. The IR spectrum (carbon tetrachloride solution) had characteristic bands at 1744 ($\nu_{C=O}$ of acetate), 1720 ($\nu_{C=O}$ of ketone), 1689 ($\nu_{C=C}$), 1226 and 1023 (ν_{C-O} of ester) cm^{-1}. There was no absorption above 3100 cm^{-1}. The mass spectrum of the ketoacetate had the required molecular ion, m/e 273; m/e 43 was the base peak of the spectrum.

On this evidence, it was decided to proceed to the next stage in the reaction sequence without further purification of the ketoacetate.

Attempted Preparation of α, β-unsaturated Ketoacetate (69)

Bromination with bromine in acetic acid was first investigated.

Heathcock *et al.*,[56] in reporting the successful bromination of 1-methyltricyclo [4·4·0·0·^{2,7}] decan-8-one (70) under these conditions, found that this compound under-

went acid-catalysed bomination more slowly than the bromoketone 71. Thus when compound 70 was treated with one equivalent of bromine in glacial acetic acid containing a catalytic amount of hydrogen bromide, and the reaction was quenched immediately, the dibromo derivative was found to be present to a considerable extent. If, on the other hand, the reaction was allowed to proceed for several hours at room temperature after all the bromine had been consumed, the monobromo compound (71) was obtained in almost quantitative yield. No cleavage of the cyclobutane ring under these conditions was reported.

The presence of a double bond allylic to the cyclobutane ring in compound 68 was expected to add a degree of complication. It was not surprising, therefore, that in the attempted bromination of the ketoacetate by this method, a mixture of products was obtained.

TLC examination of the mixture indicated two major components, of lower polarity than that of the starting material (R_f 0·65 and 0·58 in 80% light petroleum-ethyl acetate; cf. 0·47). These were separated by careful preparative TLC and their IR spectra were obtained. Both components exhibited bands at 1732 cm^{-1} (carbon tetrachloride solution), but these absorptions differed markedly in intensity. It was evident that the component of higher R_f value, present to the lesser extent, was not the required

product as the absorbance of its C=O band was much lower than would have been expected. The second component, however, was very similar to the ketoacetate in this respect. The single maximum in the carbonyl region of the spectrum was explained by postulating that bromine had entered a pseudo-equatorial position adjacent to the ketone, thus raising the carbonyl stretching frequency to a point where it coincided with that of the acetate.[75] The 'shape' of the region 2800 - 3000 cm^{-1} in the spectrum of the main product was distinctly different from that of the corresponding region in the spectrum of the starting material. Moreover, corresponding frequencies in this region were not identical.

This component comprised about 25% of the total product. As a liquid film, its IR spectrum showed a sharp peak of medium intensity at about 755 cm^{-1} which was consistent with the presence of a C-Br bond, although a little higher in frequency than anticipated. A study of the IR spectra of brominated sterols has indicated[76] that equatorial substitution leads to bands in the range 750 - 700 cm^{-1}, and axial substitution to absorption in the region 690 - 590 cm^{-1}. C-O absorption occurred at the expected frequencies.

On the basis of the above evidence, therefore, it seemed likely that the α-bromoketoacetate was present as a

major component in the product mixture. Attention was therefore directed towards increasing the selectivity of the reaction. The method of Glazier,[77] reported to be effective in the selective α-bromination of ketones, gave a mixture of products similar to that afforded by the previous reaction. It was felt that the various possible modes of cleavage of the cyclobutane ring in the presence of strong mineral acid could account for the complexity of the product and, indeed, treatment of the ketoacetate with HBr/acetic acid in a control experiment was found to result in its immediate decomposition to a complex mixture.

Alternative methods of bromination were therefore considered. The apparent stability of the ketoacetate in pyridine, and its stability to mild heat, suggested the use of pyridinium hydrogen bromide perbromide. This reagent, however, was found to offer no advantage: in a reaction in which an equimolar amount of the freshly-prepared pyridinium complex was added to a warm solution of the ketoacetate, a mixture of products was again obtained. TLC comparison of this mixture with that obtained in the previous attempted bromination reaction indicated a striking similarity of composition.

The reaction was repeated in the presence of acetamide, which would remove the HBr released in the reaction. In

this case, some simplification of the product mixture was effected: major components of R_f 0·32 and 0·73 in 80% light petroleum-ethyl acetate were observed, with the latter in predominance, constituting more than 25% of the total product. Preparative TLC afforded this component in a pure state, as confirmed by GLC on SE-30, and its IR spectrum (liquid film) was obtained. It was notable that the corresponding IR spectra of this compound and the major product of the bromine/acetic acid experiment were almost identical. The mobilities of these compounds on silica gel, however, were different, suggesting an isomeric relationship between them.

The IR spectrum (liquid film) of the other major component of the last reaction showed bands at 1740 ($\nu_{C=O}$ of acetate; cf. starting material) and 1669 cm^{-1}. The latter frequency was consistent with the presence of an α, β-unsaturated ketone, but the UV data obtained for this compound indicated that it was not the required α, β-unsaturated ketoacetate (69). Absorption at 230 nm (ε, 7,650) was recorded, in contrast to the theoretical value of 239 nm, for which a higher intensity of absorption would have been expected.

Attention was therefore focused once again on the predominant component of the mixture, that of higher R_f value. An investigation of the effect of lithium chloride/

dimethylformamide treatment[78] of this compound, in an attempt to dehydrobrominate, established that it was resistant to these conditions (see experimental section). The stability of the compound was incongruous: it was purified by sublimation, and a mass spectrum and microanalysis were obtained. These data indicated that bromine was not present in the molecule, but were consistent with the structure of dioctyl phthalate, a plasticiser. The IR spectrum previously obtained for the compound was also explainable in terms of this proposed structure.

The purity of the ketoacetate was re-examined. As before, TLC indicated the presence of a small amount of diol monoacetate, but a direct comparison between the impurity of higher R_f value and dioctyl phthalate now suggested that these compounds were identical. Similarly, a re-examination of the ketoacetate by GLC under conditions of temperature-programming on the phase SE-30, where OV-1 had been used previously under isothermal conditions, revealed a major impurity of retention time much greater than that of the ketoacetate; the retention time of this compound was identical with that of dioctyl phthalate.

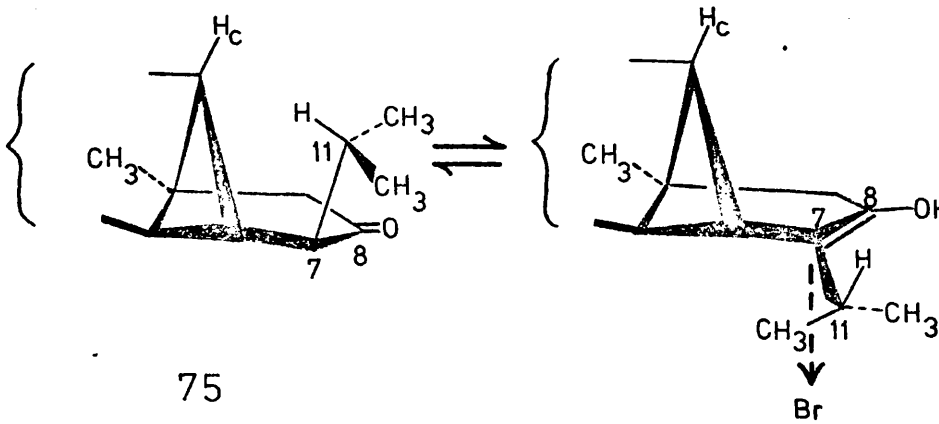
A similar examination of a sample of the diol monoacetate confirmed its homogeneity.

Preparative TLC afforded the ketoacetate as 70% by weight of the crude material, and the major by-product as

20%. Examination of the ketoacetate by GLC on SE-30 and OV-22 confirmed its purity. The second component also appeared as a single peak on SE-30. Analysis on OV-22, however, suggested the presence of a small concentration of a lower alkyl phthalate, inseparable from dioctyl phthalate on TLC, as the retention index of the only peak observed on this phase was lower than that predicted for dioctyl phthalate. IR and mass spectra of the material of higher R_f value were shown to be identical with corresponding spectra of the major components obtained from each of the attempted brominations. A portion of the ketoacetate impurity was sublimed and submitted for NMR examination. A consideration of the IR and NMR spectra of the compound confirmed its proposed identity with dioctyl phthalate. Insufficient quantities of the material were available to allow a detailed analysis.

The above sequence of experiments has been reported in this way as it later proved to be of direct relevance to a subsequent structural revision of the Brachylaena diols and ketoaldehydes, and formed a suitable basis for further work. It was evident from the results that at no time was an α-bromoketoacetate prepared successfully in major quantity, if at all. Consequently, it appeared necessary to re-investigate the structures proposed by Brooks and Campbell[69] for the corresponding ketoaldehydes, as no

Figure 4



75

obvious difficulty would have been envisaged in the introduction of bromine α to the ketone.

In the event, a revised position was proposed for the ketone, α to the isopropyl group (75; see later), and the failure of the ketoacetate to brominate could then be explained by consideration of the stereochemistry of the molecule.

α-Bromination of a ketone proceeds by preliminary formation of its enol and, in the case of compound 75, two modes of enolization are possible. It is evident, however, that enol formation as illustrated in Figure 4 will result in the release of steric congestion caused by the interaction of the isopropyl group with H_c as carbon atoms 8, 7 and 11* tend towards coplanarity; preferential enolization therefore occurs in this manner, with bromine expected to approach the plane of atoms constituting the enol function at an axial position (Fig. 4), according to the principle of perpendicular attack.[79] Substitution of bromine at C-7, however, would be difficult to achieve, as considerable steric interference would be experienced between an isopropyl group and a bromine atom substituted at the same position. This may explain the lack of success in attempts to prepare an α-bromoketoacetate. It should be added, however, that substitution on the other side of the ketone would have been expected to occur to a small extent: no

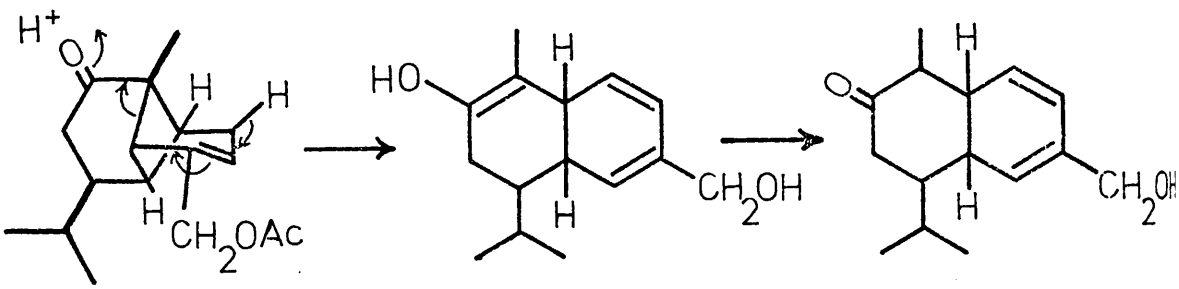
* accepted numbering convention

compounds of this type were obtained.

The failure of a series of experiments in which the purified ketoacetate was treated with 2,3-dichloro-5,6-dicyano-1,4-benzoquinone (DDQ)[80] in various solvent systems, in an attempt to prepare the α, β-unsaturated ketone directly, may also be readily understood in the context of the later structural revision.

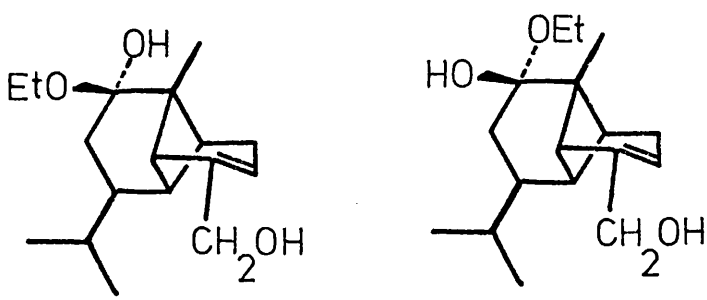
Finally, reference must be made once again to the dioctyl phthalate present in the ketoacetate. The origin of this compound was obscure, as at no time were materials expected to contain plasticiser used in the experiments. Moreover, although a plastic vessel was used in the procedure for washing apparatus, care was exercised in finally rinsing the apparatus with copious quantities of water and acetone of 'AnalaR' grade, to remove possible contaminants from the plastic. It was concluded that the dioctyl phthalate had arisen from organic solvents used in the experiments, particularly in extraction procedures where large volumes of solvents were concentrated.

Figure 5



68

$\lambda^{EtOH}_{calc.}$ 269 nm



72 , 73

Effect of Mineral Acid on Ketoacetate 68: Preparation of Diastereomeric Ketols 76 and 77: Structural Revisions in the Keto Series

A study of the behaviour of ketoacetate 68 in the presence of mineral acid was undertaken, with the intention of examining the possibility of stereochemically-controlled opening of the cyclobutanoid ring to a bicyclic system of known relative configuration, as exemplified in Fig. 5. Previous work had indicated that the ketoacetate decomposed in the presence of 50% HBr/glacial acetic acid to a multi-component mixture: its behaviour under milder conditions was therefore investigated.

A solution of ketoacetate 68 in absolute ethanol was acidified to 0·6 N with concentrated HCl. The acidic solution was shaken well and the reaction was monitored by GLC. 15 Minutes after the addition of acid, a small portion of the reaction mixture was examined on 1% OV-17 at 150°: no change in the constitution of the starting material was evident. After 1½ hours, further HCl was added to give a 1·2 N solution and the reaction mixture was again examined: two products of similar retention indices were observed, comprising about 10% of the mixture and at retention times slightly lower than that of the ketoacetate. 3½ Hours after the initial addition of HCl, the reaction was

half-way to completion and, after 21 hours, no starting material remained. GLC examination of the products on 1% OV-1 at 160° showed two components of almost identical retention indices ($I^{160°}_{OV-1}$ ~1790) and of lower molecular weight than that of the starting material. On 0·5 % XE-60, some separation of these components was observed ($I^{160°}_{XE-60}$ 2285 and 2295) and, on this phase, the retention index of the product of lower polarity (present in slight predominance) was almost identical to that of the starting material. TLC (70% light petroleum-ethyl acetate) confirmed the absence of starting material (R_f 0·32) and indicated two products of different mobilities (R_f 0·18 and 0·26); examination on silver nitrate-impregnated layers afforded no improvement in the separation of these components.

A UV spectrum of the product mixture showed no significant absorption, suggesting that cleavage of the cyclobutanoid ring had not occurred. It was considered possible that these compounds represented hemiketals (72, 73). On repeating the reaction in the presence of aqueous ethanol, however, identical results were obtained, invalidating this explanation of the products.

The crude product was treated with acetic anhydride/dry pyridine and again examined by TLC: a single spot was

observed, corresponding in R_f value to the original ketoacetate. GLC examination on OV-1 and XE-60 appeared to indicate that the ketoacetate had been regenerated. The behaviour of ketoacetate 68 in base was investigated. In the presence of a dilute solution of sodium hydroxide, the ketoacetate immediately reacted to form products apparently identical to these obtained from treatment with HCl (TLC; GLC on OV-1 and XE-60).

These observations indicated that in the treatment of ketoacetate 68 with HCl, simple hydrolysis had resulted. Similarly, hydrolysis had occurred in the presence of sodium hydroxide. No simple explanation, however, was evident for the formation of two products.

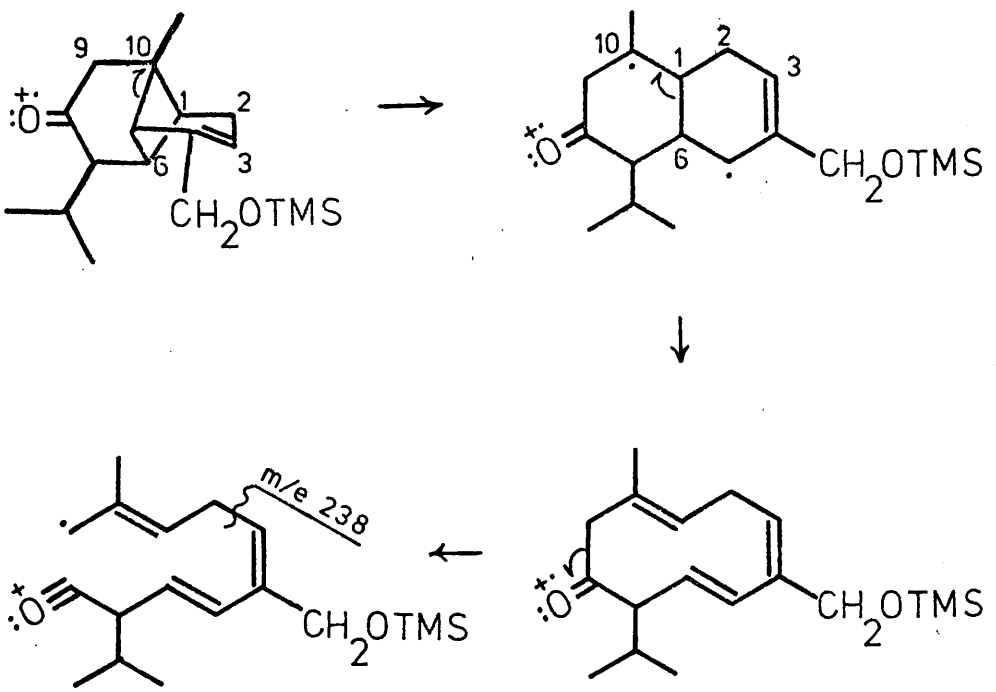
Treatment of ketoacetate 68 in absolute ethanol with HCl was repeated on a larger scale, and the reaction products were separated by careful preparative TLC. The purity of each component was verified by TLC and by GLC on 1% OV-1 and 0·5% XE-60 at 160°. The product of R_f value 0·18 was correlated with the peak of retention index 2295 on XE-60, and the product of R_f 0·26, similarly, with the peak of retention index 2285. IR spectra (carbon tetrachloride solutions) indicated that both compounds were ketols: in the spectrum of the more polar component, prominent absorptions were recorded at 3617 (ν_{O-H}) and 1718 cm^{-1}

($\nu_{C=O}$); corresponding peaks in the spectrum of the component of lower polarity occurred at 3611 and 1718 cm^{-1}. Mass spectra were almost identical, with molecular ions at m/e 234 and base peaks m/e 119 and 91: the outstanding difference between the spectra was the appearance of a peak at m/e 166 in the spectrum of the component of higher R_f value, completely absent in the spectrum of the second component (see below).

Each ketol in turn was treated with acetic anhydride/dry pyridine to convert it to its corresponding ketoacetate, and the purities of the products were estimated by TLC and GLC. TLC data of the ketoacetates were identical, as were corresponding retention indices on OV-1 and XE-60. Mass spectra of the derivatives were obtained. The spectra were almost identical, with molecular ions at m/e 276 and base peaks at m/e 43: once again, however, the important difference lay in the peak of m/e 166, present only in the spectrum of the acetate obtained from the less polar ketol. Attempts to propose a plausible mechanism for the formation of this fragment from the less polar ketol and from its acetate were unsuccessful, and it was therefore considered possible that an impurity, present in both samples, was responsible for these peaks. On the other hand, the peak at m/e 166 in the mass spectrum of the less polar ketol

Figure 6

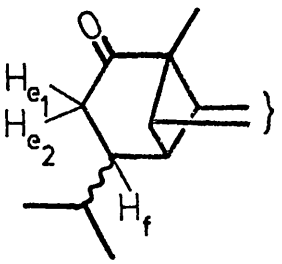
Proposed Mass Spectral Fragmentation of Trimethylsilyl Derivative of Less Polar Ketol‡



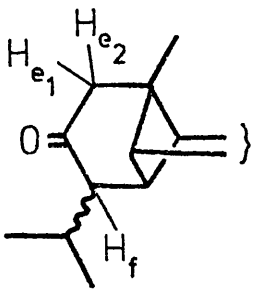
‡ Anticipates later structural revision (see p.33 et seq.).

corresponded to a loss of 68 mass units from the molecular ion [a small metastable peak at about m/e 117 (calculated 117·3) supported this loss], and an identical loss from the molecular ion was observed in the spectrum of the trimethylsilyl derivative of this compound (although the expected metastable peak was not evident in this spectrum). From the results of on-column deuteration of the latter compound (see p. 39), it was concluded that C-9 was lost in the fragmentation process and, to account for this loss, the mechanism shown in Figure 6 was proposed. This mechanism was considered unsatisfactory, in so far as it required the unfavourable breakage of the vinylic 2,3 bond: no other mechanism, however, appeared viable.

The similarity of the physical data of the ketols prompted the postulate that they were diastereomers. It seemed probable that the original tricyclic structures had remained intact in the formation of the ketols (as suggested by their lack of conjugation) and that inversion at some centre had occurred on hydrolysis of ketoacetate 68 (since two apparently epimeric ketoacetates, one of which was identical with ketoacetate 68, were produced on acetylation of the ketols). The isopropyl centre appeared to be the only point at which inversion could reasonably be considered to have occurred but, for this to be possible, it



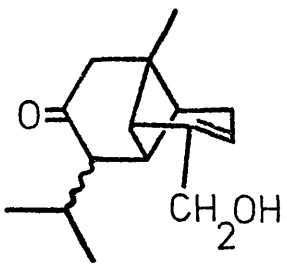
74



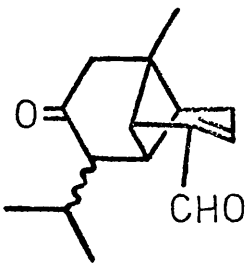
75

was necessary for the ketone and isopropyl group to have been in adjacent positions. A structural revision of ketoacetate 68 and related compounds of the keto series was therefore tentatively proposed, and the NMR spectra (M.M. Campbell[81]) of the Brachylaenalones were re-examined, as it was largely on the basis of these spectra that the original structures (1, 2) of the Brachylaenalones had been postulated.[69] Assuming the structures of the Brachylaenalones and related Brachylaena ketones to belong to known classes of sesquiterpenoids, only revised partial structures 75 appeared plausible. The spectra were therefore reconsidered in relation to structures 75 and the original partial structures 74, and it soon became evident that the spectra were in fact readily explainable in terms of the new structure 75.

An important point emerged in respect of the protons designated as H_{e_1}, H_{e_2} and H_f. Protons H_{e_1} and H_{e_2} appeared at τ 7·49 in the spectra of the Brachylaenalones, and the coupling constants between these protons and H_f in both spectra were recorded as $J_{e_1f} = J_{e_2f} = 0$. In the spectrum of isomer A, H_{e_1} and H_{e_2} did not exhibit geminal coupling, as these were equivalent protons. The corresponding protons in Brachylaenalone B were non-equivalent, due to different steric and anisotropic effects in this



76 , 77



78 , 79

isomer, and an extreme 'AB' system in which 'δ_A' → 'δ_B' was observed. The lack of significant further splitting of these peaks in either spectrum was explained in terms of 'facile interconversion of ring conformers', resulting in each case in a 'time-averaged configuration' in which the protons H_{e_1} and H_{e_2} became equivalent on the NMR time scale and subtended a dihedral angle of 90° with the vicinal proton H_f. In the present work, however, examination of models showed this theory to be untenable: at no time in the interconversion of conformers would the protons H_{e_1} and H_{e_2} become magnetically equivalent, as the ring structure was not sufficiently flexible; in addition, the time-averaged conformation of these protons would subtend dihedral angles of about 0° and 120° with H_f in each molecule, resulting in appreciable further coupling. In terms of partial structures 75, no significant coupling would have been anticipated between protons H_{e_1} and H_{e_2} and proton H_f: from the point of view of the NMR spectra of the Brachylaenalones, then, partial structures 75 were acceptable.

The revised structures of the ketols were thus tentatively designated as 76 and 77, and their behaviour in mineral acid and in base could then be appreciated as enolisation (slow in mineral acid; rapid in base), result-

ing in epimerisation of the isopropyl group. It followed that interconversion of the Brachylaenalones, of revised structures 78 and 79, should also be possible, by treatment of one isomer with acid or base. Indeed, Brachylaenalone B was found to epimerise rapidly in base to a mixture of isomers. The percentage composition of the mixture 30 minutes after the addition of base was recorded: isomer A/isomer B = 55/45. Epimerisation in mineral acid was observed to be slow. These properties of the Brachylaenalones were not observed by M.M. Campbell,[81] possibly because of the identical mobilities of the isomers on silica gel.

Fractions from column chromatography of the heartwood of Brachylaena hutchinsii (Table 2) were investigated as a possible natural source of the diastereomeric ketols. An IR spectrum of combined fractions 30-32 was almost identical with the spectra of the known ketols, and TLC and GLC (0·5% XE-60) suggested that this material comprised principally the ketol of R_f value 0·26. Fractions 33-38 appeared to contain both known ketols, but this combined fraction was considered to be too complex to provide a convenient source of pure ketols. It was decided to prepare first the pure ketol of higher R_f value by careful preparative TLC of fractions 30-32, to obtain a mixture of both ketols by base treatment of this pure isomer, and then

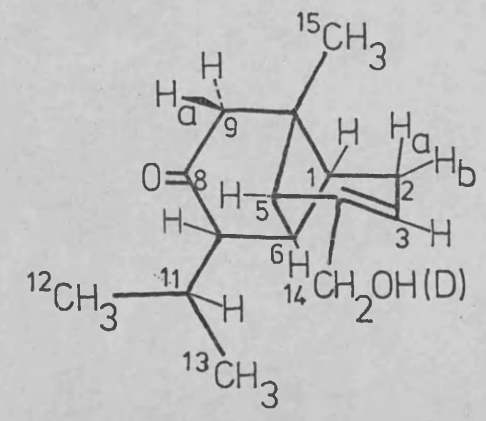
to prepare both pure ketols by careful preparative TLC of the mixture.

Both pure ketols were successfully prepared by this method (although not in crystalline form), and microanalysis of each isomer after sublimation was consistent with the required molecular formula, $C_{15}H_{22}O_2$. Measurements of the sign and amplitude of the Cotton effect[82] exhibited by each ketol were recorded: the compound of lower polarity showed a small, negative Cotton effect, while for the other isomer a small, positive Cotton effect was observed. [An ambiguity in the interpretation of the results precluded the assignment of relative stereochemistry to the isopropyl group in either compound.]

220 MHz NMR spectra of the ketols in carbon tetrachloride solution were obtained. Considerable 'crowding' of peaks in the region 7·6 - 8·1 τ was evident in both spectra and, even on an expanded scale, after exchange of the hydroxylic proton with deuterium, the spectra were too complex to allow detailed interpretations. Spin decoupling experiments were not possible as the spectrometer was not equipped with a frequency lock at that time. Tentative analyses were, however, performed where possible by correlations of the spectra with those of the corresponding Brachylaenalones and Brachylaena diols (see later): the results are detailed in Table 4. In the spectrum of the

Table 4

Chemical Shift Data (220 MHz) of the Ketols (76,77)* after deuterium exchange of the hydroxylic proton. Splitting Patterns** and Observed Coupling Constants‡ are noted, where these are simple.



76, 77

Proton(s)	Ketol of Lower Polarity		Ketol of Higher Polarity	
	Chemical Shift (τ)	Observed Splitting Patterns and Coupling Constants (J)	Chemical Shift (τ)	Observed Splitting Patterns and Coupling Constants (J)
H_1	~7·77		8·02?	broad
H_{2a}, H_{2b}	7·68	m	uncertain	
H_3	4·50	s, broadened	4·48	s, broadened
H_5	~7·77		uncertain	
H_6	8·04	d; J_{6-7} = 1·5 Hz	8·07?	d; J = 2 Hz (J_{6-7}?)
H_7	7·95		uncertain	
H_{9a}, H_{9b}	7·61	d; virtually equivalent protons with small δ/J ratio	7·61	s
H_{11}	~7·95		uncertain	
$3H_{12}$	9·03	d	9·04	d
$3H_{13}$	9·19	d; J_{12-13} = 7 Hz	9·18	d; J_{12-13} = 7 Hz
$2H_{14}$	6·10	s, broadened	6·12	s, broadened
$3H_{15}$	9·15	s	9·17	s

* Protons numbered according to number of C atom (accepted convention) to which attached; geminal protons at C-2 and C-9 are denoted by a, b.

** s = singlet; d = doublet; m = multiplet.

‡ Dihedral angles are indicated in Table 11.

more polar ketol, which proved more amenable to interpretation, certain points require clarification. H_1 and H_5 were observed as an asymmetric cluster of peaks at $\tau \sim 7{\cdot}77$. The appearance of this region of the spectrum can be explained by consideration of the interactions of these protons with each other and with the rest of the molecule. Significant transannular coupling was expected first between protons H_1 and H_5 (*cf.* $J_{1-5} = 6{\cdot}5$ Hz in the spectra of the Brachylaenalones) and, in view of the small δ/J ratio involved, this was expected to result in an AB quartet in which the satellite peaks were very small. Further allylic coupling of H_1, however, to protons H_{2a} and H_{2b} (with which it subtends equal angles) would afford a triplet, which would again be split by W-(4σ-) coupling of H_1 to H_3 ($\sim 1{\cdot}5$ Hz). Proton H_5 was expected to appear as an approximate doublet, due to W-coupling with H_3 ($\sim 1{\cdot}5$ Hz) and further (negligible) coupling to H_6 ($\varphi_{5-6} \rightarrow 90°$). The complexity of this region of the spectrum can thus be readily appreciated.

In the spectrum of the ketol of higher polarity, proton H_6 appeared as a doublet of 2 Hz. This coupling was assumed to be J_{6-7}; as H_7 could not be identified with certainty, however, the 'A' part of this AB system has not been assigned.

A spectrum of the less polar ketol in benzene was recorded and, in this solvent, peaks which had appeared in the region 7·6-8·1 τ in carbon tetrachloride were observed to lie over a wider range, 7·6-8·4 τ. Spin decoupling experiments, however, were again considered necessary.

The discovery by Hinckley[83] of the effectiveness of complexes of paramagnetic lanthanide ions as shift reagents in NMR spectrometry, followed by more recent investigations of others,[84] has suggested another method in which the ketol spectra might be conveniently simplified. This is an area in which future work is intended.

Attention was directed towards a proof of the revised structure proposed for the ketols. Deuterium exchange of the protons α to the ketone appeared to be a suitable method of distinction between partial structures 74 and 75. If the ketols were represented by partial structures 75, then a maximum of three deuterium atoms could be introduced whereas, in partial structures 74, only two α hydrogen atoms were available for exchange. Two methods of deuteration were investigated: in the first experiment, sodium was added to a solution of the less polar ketol in perdeuteromethanol (CD_3OD), and the reaction was followed by repeated NMR examination; in the second case, deuteration was

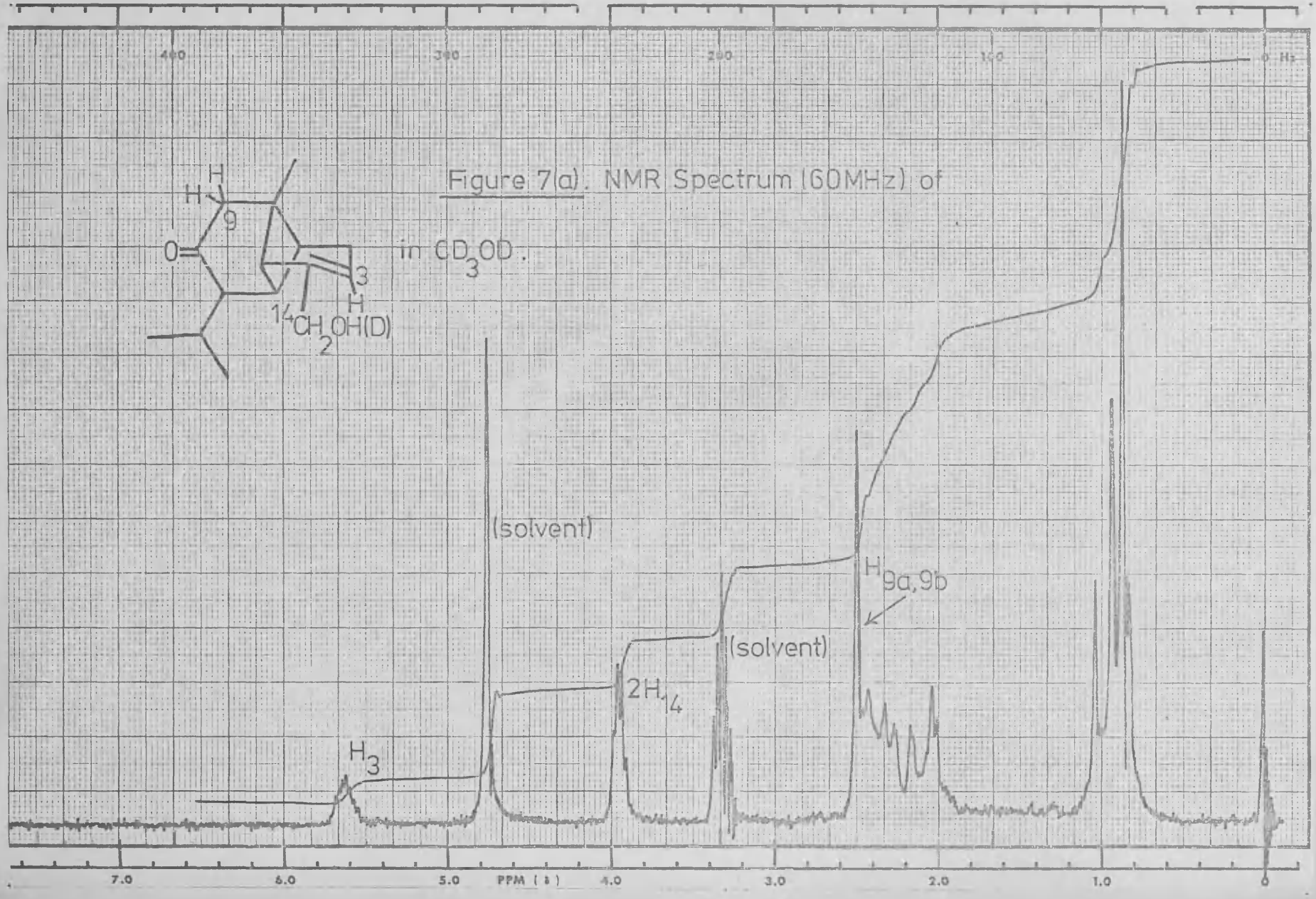


Figure 7(a). NMR Spectrum (60MHz) of

in CD_3OD.

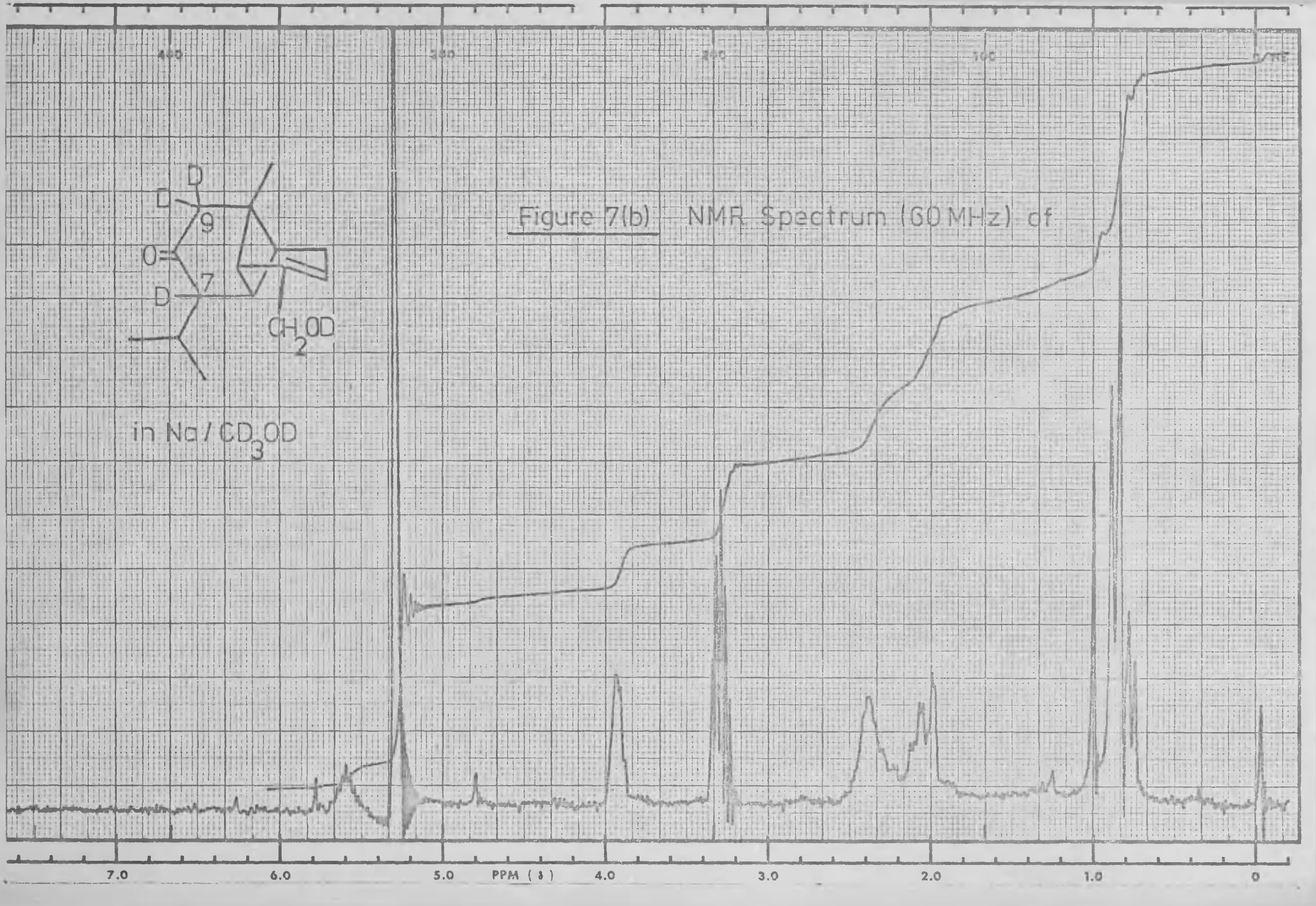


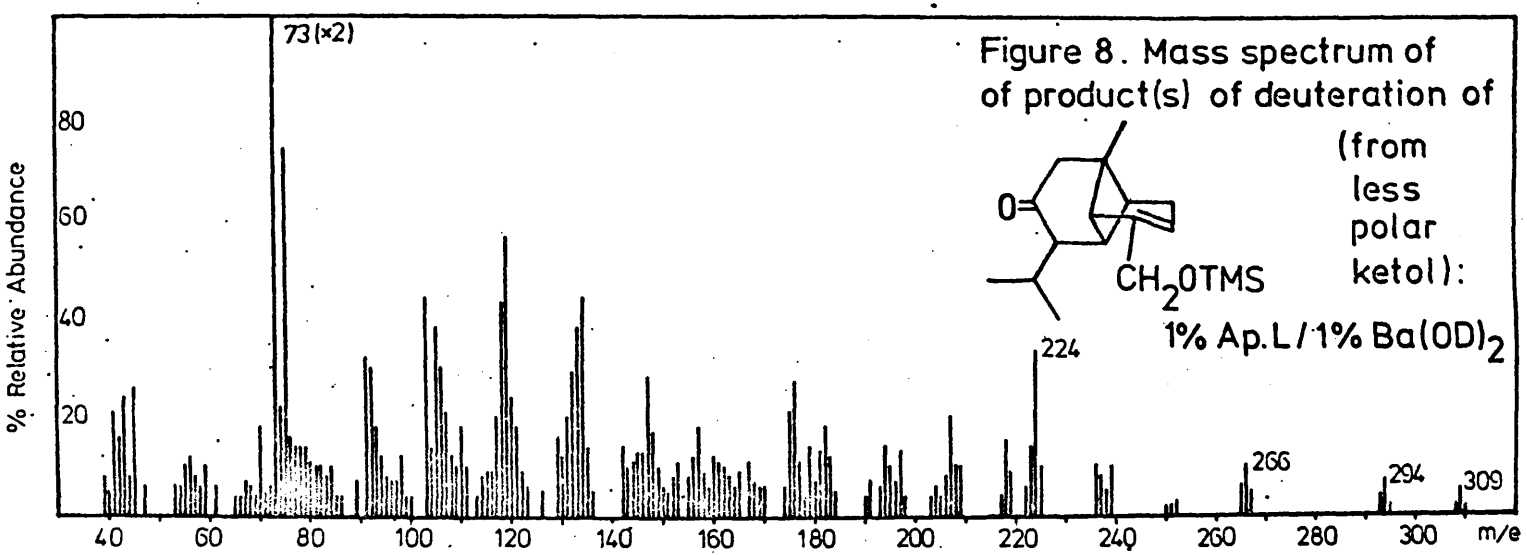
Figure 7(b) NMR Spectrum (60 MHz) of

effected on a GLC column, and the products were analysed directly by combined GC-MS.

A 60 MHz spectrum of the ketol of lower polarity in perdeuteromethanol was obtained, and the cluster of peaks which had appeared in 7·6-8·1 τ in the corresponding 220 MHz spectrum was observed to lie over the range 7·5-8·1 τ. The solution was removed from the NMR tube, sodium was added and the process of deuterium exchange was monitored by repeated NMR examination. After 10 minutes, the reaction was complete, and an integral of the spectrum showed that 3 protons had been removed in the region 7·5 8·1 τ (Fig. 7). The sharp singlet at τ 7·51, due to the geminal protons at C-9, had completely disappeared but, as epimerisation of the isopropyl group had occurred in the presence of base, it was not possible to analyse the remainder of this region of the spectrum. GLC examination of the products on XE-60 confirmed the presence of both isomers.

The trimethylsilyl derivative of the less polar ketol was prepared for the second experiment, and mass spectra of the products of deuteration of this compound on a 1% Apiezon L/1% barium deuteroxide column* were obtained by way of GC-MS and the following procedure. Deuterium oxide

* prepared by G. Anthony


73(×2)
% Relative Abundance
80
60
40
20
224
266
294
309
40
60
80
100
120
140
160
180
200
220
240
260
280
300
m/e
Figure 8. Mass spectrum of product(s) of deuteration of
O
CH2OTMS
(from less polar ketol):
1% Ap.L/1% Ba(OD)2

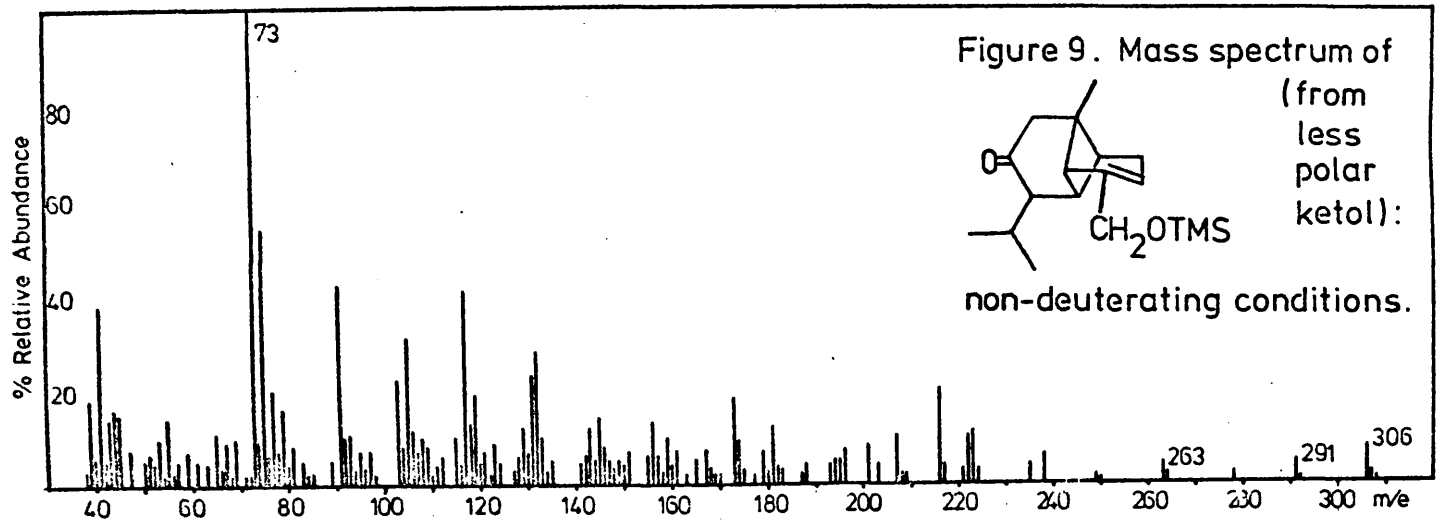
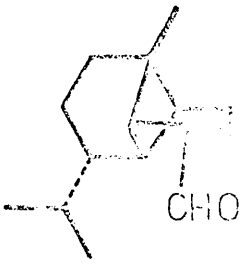


Figure 9. Mass spectrum of (from less polar ketol): non-deuterating conditions.



80

was first injected into the column, followed by androstan-17-one, and mass spectra of the deuterated steroid were recorded. This sequence was repeated until the highest possible intensity of the molecular ion corresponding to a maximum level of deuteration (+ 2D) was attained. The ketol trimethylsilyl derivative was then injected, and mass spectral scans were taken at several positions of the broad peak observed on GLC. Mass spectra of mixtures of compounds with varying degrees of deuteration were thus obtained and, from the results, it was evident that a maximum of three deuterium atoms could be incorporated. The spectrum of a mixture in which the trideutero compound predominated is shown in Fig. 8. Exchange of three active hydrogen atoms is represented by the most intense molecular ion at m/e 309, while the peak at m/e 308 corresponds to a compound in which two of the available atoms have been replaced. A mass spectrum of the corresponding non-deuterated ketol trimethylsilyl derivative is shown in Fig. 9 for comparison.

This evidence strongly supported the revised partial structure 75, in which deuterium exchange had occurred in the positions adjacent to the ketone. The possibility of exchange of the allylic hydrogen atoms at C-2, however, was also recognised.

'Copaene-aldehyde' (80), kindly supplied by Dr. E. Klein (Dragoco, Holzminden) in connection with work dis-

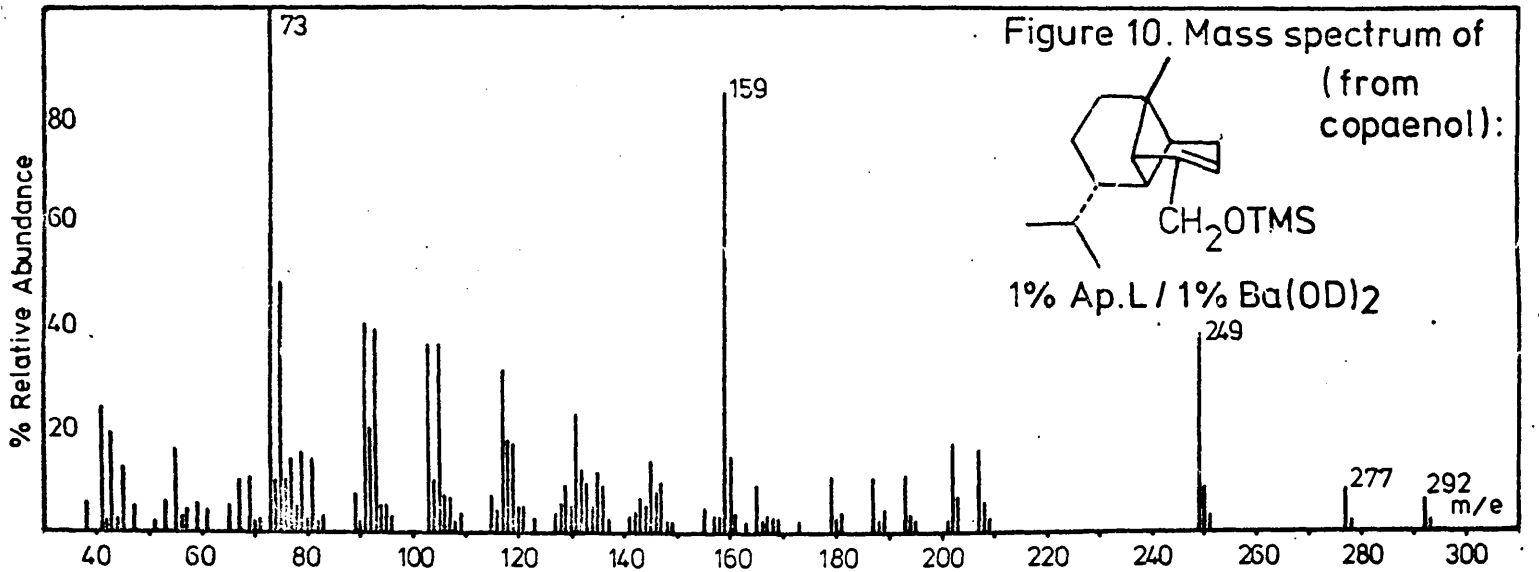


Figure 10. Mass spectrum of (from copaenol):

cussed later in the thesis, was reduced to copaenol by treatment with lithium aluminium hydride, and the trimethylsilyl derivative of the alcohol was prepared. This was subjected to conditions of deuteration identical to those employed in the last experiment, and mass spectra were obtained as before. No incorporation of deuterium was achieved in this case. It followed therefore, that deuterium exchange in the ketol derivative had occurred on account of the presence of the ketone. The mass spectra obtained in this experiment were identical, corresponding to the spectrum of the trimethylsilyl derivative of copaenol, illustrated in Fig. 10.

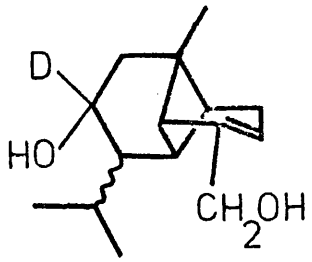
The Fourth Diastereomeric Diol. Stereochemical Relationships in the Diol Series

The products of oxidation and reduction of ketols 76 and 77 were investigated in an attempt to correlate them with the Brachylaenalones and Brachylaena diols respectively. An important factor in this work was the use of the phase XE-60 for analyses of the Brachylaenalones and Brachylaena diols, first demonstrated during this sequence of experiments.

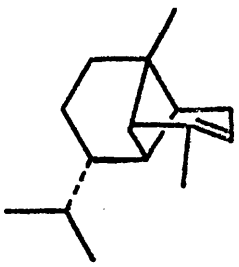
Sarett[74] oxidation of the ketol of lower polarity resulted in its quantitative conversion to Brachylaenalone A, as estimated by TLC and by GLC on OV-1 and XE-60. Similarly, on oxidation of the more polar ketol, Brachylaenalone B was produced. The ketols were therefore said to belong to the 'A' and 'B' series respectively, and were described as 'Brachylaena ketols A and B'.

Ketol B was treated with sodium borohydride, and the products of the reaction were examined by TLC and GLC (OV-1 and XE-60): Brachylaena diols (b) and (c) were obtained in approximately equal proportions. Similar reduction of ketol A gave two reaction products, one of which was identified as diol (a), by TLC and GLC (OV-1 and XE-60) comparisons with an authentic sample. The second component had an R_f value (0·40) identical with that of

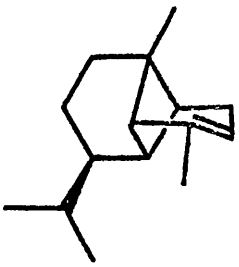
diol (a) in 100% ether, and a retention index on XE-60 slightly lower than that of diol (a). Its mass spectrum (GC - MS; 1% OV-225) had a molecular ion at m/e 236 (see p. 44). The compounds were separated by preparative GLC on 1% OV 225 and their IR spectra (chloroform solutions) were recorded. These showed identical positions of absorption, although differences in the relative intensities of peaks in the region 2800-3000 cm^{-1} were observed. The spectra were similar to the corresponding spectra of diols (b) and (c). It therefore seemed reasonable to assume that in the reduction of ketol A, diastereomeric diols with identical isopropyl but different secondary hydroxyl configurations had been produced, and that the fourth, expected diol, designated as isomer (d), had been obtained. The reason for the apparent absence of this isomer in fractions collected during the initial column chromatographic separation of the Brachylaena diols (Table 3) was not clear. However, since the mobilities of diols (a) and (d) on silica gel (100% ether) were identical, and the phase XE-60 had not been employed in the GLC analyses of these fractions, it was recognised that diol (d) might have been present as an undetected constituent of fractions containing diol '(a)'. Indeed, examination on XE-60 of fraction 48 from column chromatography (Table 3), previously believed to contain pure diol (a), revealed the



81, 82



3



4

presence of diols (a) and (d) in equal proportions. Similar analyses of fractions collected before and after fraction 48 during column chromatography of the Brachylaena diols indicated that diol (d) had been retained on the column slightly longer than diol (a). Chromatographic data of the complete series of diols (TLC; GLC on XE-60) are indicated below.

Table 5

	Brachylaena Diols			
	(a)	(b)	(c)	(d)
$R_f(Et_2O)$	0·40	0·50	0·30	0·40
$I^{156°}_{XE-60}$	2390	2345	2400	2335

Mass spectra of the Brachylaena diols (reproducible: see Appendix 2) were compared. Differences in the relative intensities of peaks above m/e 90 were observed: in particular, differences in base peaks were recorded (Table 12). The predominant fragments of the spectra are presented in Table 6. Ketol A was treated with lithium aluminium deuteride to produce a mixture of diols (a) and (d), deuterated on C-8 (81, 82). Attempts to separate these isomers by automatic preparative GLC on 1% OV 225 yielded disappointing results, due to an instrumental fault.

Table 6

Major Peaks in Mass Spectra of Brachylaena Diols, Deuterated Diol (d) (p.44), Copaene and Ylangene [Relative abundances(%) indicated in brackets]

m/e	Brachylaena Diols				Deuterated Diol (d)	Copaene	Ylangene
	(a)	(b)	(c)	(d)			
250							
	218(15)	218(5)	218(15)	218(35)	219(35)	204(15)	204(5)
200		200(10)					
	187(25)	187(40)					
		176(30)			176(55)		
	175(40)	175(30)	175(30)	175(60)			
					158(45)	161(90)	161(60)
	157(35)	157(20)	157(30)	157(50)			
150							
			147(30)	147(40)	146(55)		
	145(85)	145(85)	145(30)	145(60)			
	135(85)	135(75)	135(100)	135(95)	135(100)		
	131(85)	131(100)	131(35)	131(70)	132(55)		
	119(70)		119(35)	119(70)	119(55)	119(100)	119(85)
	105(100)	105(65)	105(55)	105(100)	105(70)	105(100)	105(100)
100							
	93(60)			93(75)		93(55)	93(80)
	91(90)	91(70)	91(55)	91(100)	91(75)	91(45)	91(55)
	79(60)	79(40)		79(60)	79(50)		
75							

Only deuterated diol (d) was obtained in a pure state. The mass spectrum of this compound was also obtained, and the major peaks in this spectrum and in the corresponding spectra of copaene (3) and ylangene (4) are shown in Table 6, to allow comparisons with the fragmentation patterns of the diols. It should be noted that the fragments at m/e 105 and 119 in the spectra of the diols and of their parent hydrocarbons are probably based on the tropylium ion (at m/e 91), with added methylene groups. Peaks at m/e 91, 105 and 119 also occur in the spectrum of deuterated diol (d): if, therefore, these fragmentation processes do not involve migration of the carbinyl hydrogen (deuterium) atom on C-8, it can be concluded that the fragments do not contain C-8.

An outstanding feature of the diol spectra is the appearance in the spectra of diols (a) and (b) of an intense peak at m/e 187, present only in very low intensity in the spectra of diols (c) and (d). This peak corresponds to a total loss of CH_5O_2 from the molecular ion: a consideration of the structure of the molecule confirms that no other combination of atoms can be reasonably envisaged. Loss of two oxygen atoms must necessarily involve the loss of two separate fragments, in view of the distance between these atoms in the molecule. A tentative mechanism of fragmentation is proposed in Figure 11. In

Figure 11

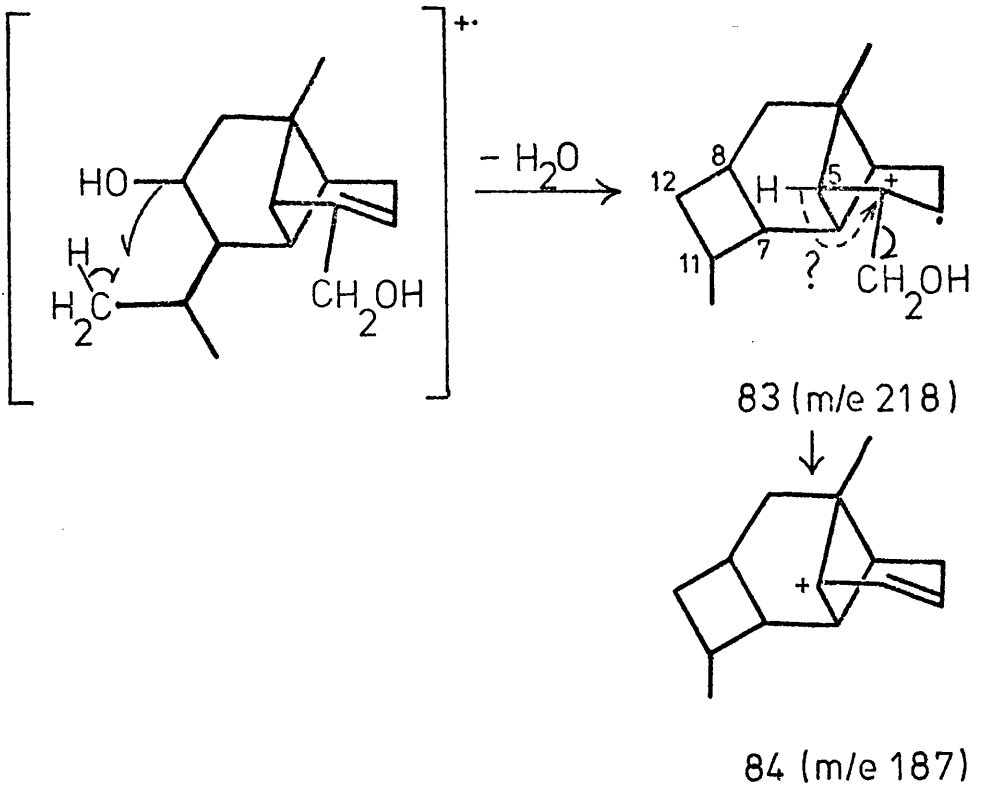
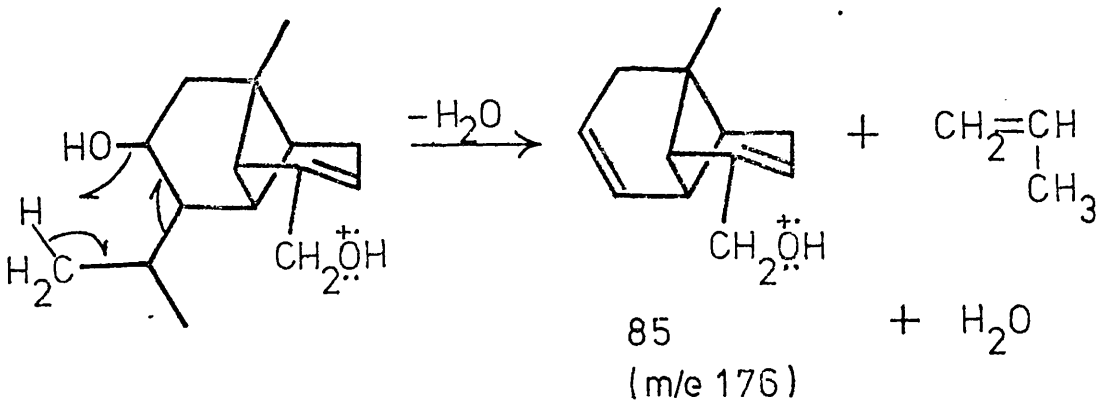


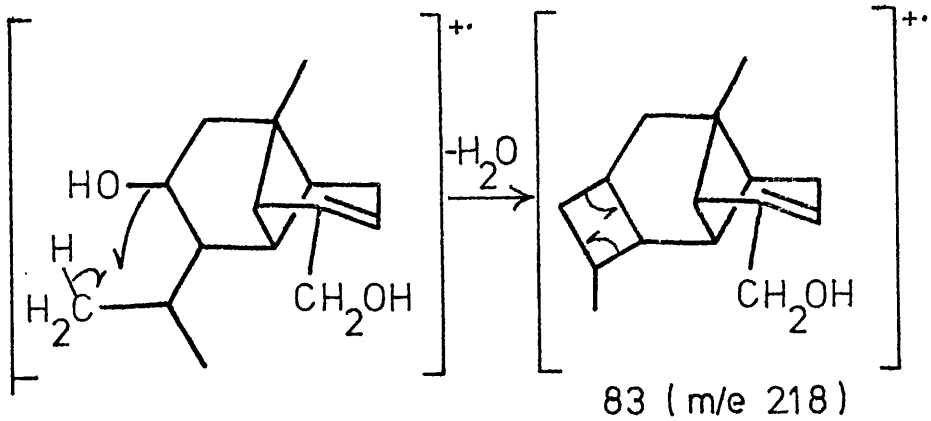
Figure 12

i)

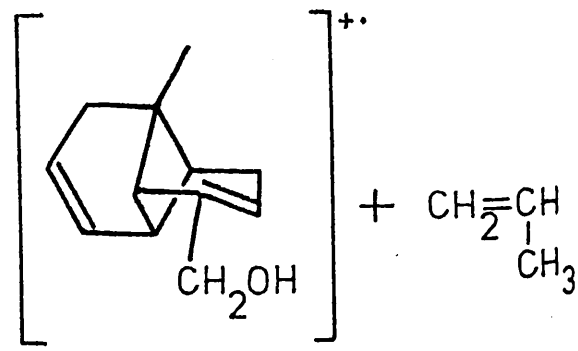


85
(m/e 176)

ii)



83 (m/e 218)



85 (m/e 176)

the initial stage, elimination of the secondary hydroxyl group as water, a cyclobutanoid ring is formed, as shown. The subsequent stage involves elimination of a fragment containing C-14 and the primary hydroxyl*. This very unfavourable vinylic cleavage is not without precedent, eg.[85] but would probably be followed by a one-hydrogen transfer, for example from C-5.

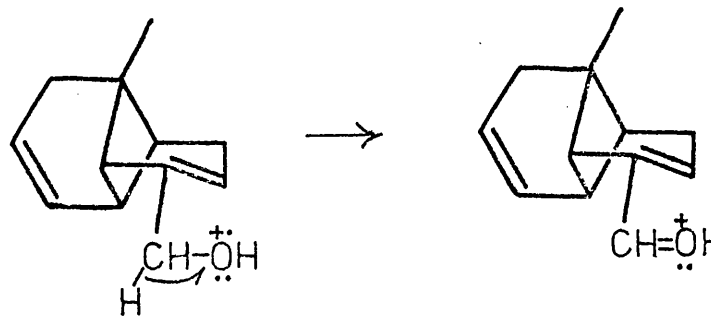
Another spectral feature worthy of note is the marked variation in the intensity of the peak at m/e 176, particularly intense in the spectrum of diol (b). Possible mechanisms of formation of this fragment are shown in Figure 12. Mechanism (i) is consistent with the spectrum observed, as a strong metastable peak at m/e 131·3, corresponding to the transition m/e 236 → 176, is evident in the spectrum; by contrast, no metastable peak appears at m/e 105·4, corresponding to the transition m/e 218 → 176: mechanism (i) is therefore proposed to account for the peak at m/e 176.

Prominent peaks occur at m/e 175, 157, 145, 135 and 131 in the spectra of all the diols. Mechanisms of formation of these fragments are postulated in Figure 13. The fragment of m/e 175 probably arises by elimination of a hydrogen atom from the ion of m/e 176, to give ion 86

* There is very little published information on the fragmentation of allylic alcohols.

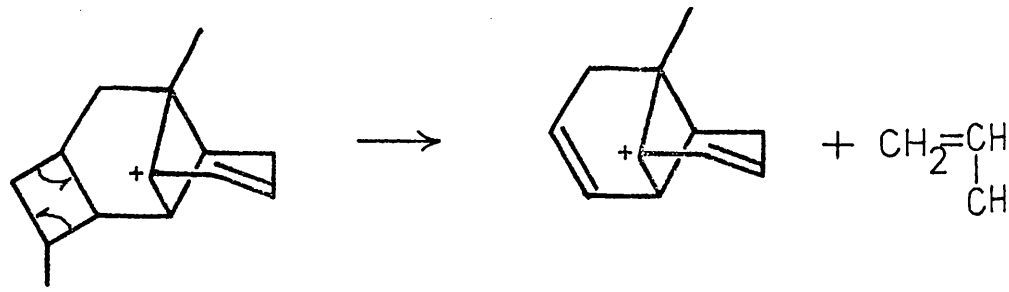
Figure 13

i)



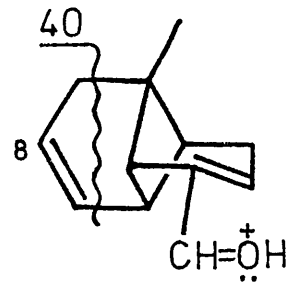
85 (m/e 176) 86 (m/e 175)

ii)



84 (m/e 187) 87 (m/e 145)

iii)



86

[Fig. 13(i)] from which, by further loss of the elements of water, the fragment of m/e 157 is formed. A prominent metastable peak at about m/e 141 (calculated m/e 140·9) in the spectrum of diol (c) supports the latter transition. The fragment of m/e 145 could arise either by elimination of formaldehyde from ion 86, and subsequent hydrogen transfer similar to that shown in Figure 11, or by loss of CH_3-CH=CH_2 from ion 84, as illustrated in Figure 13(ii). The presence of metastable peaks at about m/e 112·5 (calculated m/e 112·5) in the spectra of all the diols implicates the second mechanism: no metastable peaks at m/e 120·1, corresponding to the first transition, were observed in the spectra. Loss of C_3H_4 from the fragment of m/e 175 would produce a peak at m/e 135. Cleavage of ion 86 as indicated in Figure 13(iii) is supported by the occurrence of an identical peak in the mass spectrum of C-8 deuterated diol (d). The required metastable peaks at m/e 104 were not, however, evident in the spectra. Finally, the peak at m/e 131 was explainable in terms of an isopropenyl tropylium ion.

Since a complete series of diols was available, an investigation of relative stereochemistry within the series was undertaken, and the applicability of the dibenzoate chirality rule[86] in this context was considered. The rule, an extension of the benzoate sector rule[87], was said to

provide a method for determining the chiralities of optically active glycol dibenzoates in cases where a dipole interaction was found to occur between the benzoate chromophores, resulting in a strong $\pi \rightarrow \pi^*$ Cotton effect. The method was reported to be applicable to all molecules containing two interacting benzoate chromophores, and was not confined to α-glycol derivatives.

In the present work, the dibenzoate and di-p-chlorobenzoate derivatives of diol (b) were first prepared. The compounds were purified by preparative TLC and examined by IR (liquid film). The spectrum of diol (b) dibenzoate showed the required absorptions at 1710 ($\nu_{C=O}$ of ester), 1275-55 and 1120-1100 (both broad; ν_{C-O} of esters) and 724 (monosubstituted benzene rings) cm^{-1}. Absorptions around 1600 cm^{-1} associated with aromatic rings ($\nu_{C=C}$) were also observed; these peaks, however, were present only in very low intensity. The spectrum of the di-p-chlorobenzoate showed absorptions at 1715 ($\nu_{C=O}$ of ester), 1597 and 1495 ($\nu_{C=C}$ of benzene rings), 1290-70 and 1120-1095 (both broad; ν_{C-O} of esters), 866 (para-disubstituted benzene rings) and 775 (ν_{C-Cl}) cm^{-1}. Mass spectra of the compounds were obtained. In the spectrum of diol (b) dibenzoate, the expected molecular ion at m/e 444 was observed, together with peaks at m/e 322 and 200, corresponding to losses of ester fragments together

with two hydrogen radicals. The base peak of the spectrum appeared at m/e 105 but, on reducing the electron voltage (from 70 to 15 e.v), a spectrum possessing a base peak at m/e 200 was obtained. The spectrum of the di-p-chlorobenzoate showed the required molecular ions at m/e 512, 514 and 516, due to the presence of the ^{37}Cl isotope. The relative intensities of these ions were as expected. Peaks corresponding to successive losses of the elements of p-chlorobenzoic acid (156 a.m.u.) were observed at m/e 356 and 200. The base peak of the spectrum (at 70 e.v.) appeared at m/e 139 ($ClC_6H_4CO^+$).

Measurements of the ORD and CD effects of these compounds were then recorded*. In respect of the ORD curves, no significant rotation could be detected at a concentration such that light could be transmitted by the solution: the CD curves, however, were found to be more suitable for analysis. With diol (b) dibenzoate, a positive Cotton effect was observed at 231 nm (methanol; $\Delta\varepsilon$ +2·47). This corresponds to the $\pi \rightarrow \pi^*$ intramolecular charge-transfer transition of the benzoate chromophore. In the case of interacting benzoate chromophores, two Cotton effects, of the same amplitude but of opposite

* By courtesy of Professor W. Klyne and Dr. P. M. Scopes, Westfield College (University of London)

signs, are expected, the first at about 230 nm and the second around 220 nm. In the CD curve of diol (b) dibenzoate, no Cotton effect was observed at about 220 nm: it was therefore assumed that the benzoate groups were too remote from each other to interact (a further positive Cotton effect was recorded below 205 nm, but neither the dibenzoate chirality rule nor the benzoate sector rule is concerned with this transition). Application of the benzoate sector rule alone, then, was possible. The corresponding CD curve obtained from the di-p-chlorobenzoate of diol (b) showed a positive Cotton effect (at 244 nm in methanol) of greater amplitude ($\Delta\epsilon$ 3·87) than that recorded for the dibenzoate. It appeared, therefore, that the diol di-p-chlorobenzoates were more suitable derivatives for CD analyses.

The corresponding di-p-chlorobenzoates of diols (a), (c) and (d) were prepared. An IR spectrum (liquid film) of the ester of diol (c) was found to be very similar to that of the corresponding derivative of diol (b). IR spectra of the esters prepared from diols (a) and (d) were not recorded, as insufficient quantities of material were available. Mass spectra of diols (a), (c) and (d) p-chloro dibenzoates were obtained. The derivative of diol (c) afforded a spectrum which was very similar to that of the di-p-chlorobenzoate of diol (b), with the base

peak at m/e 139. In the mass spectra of diols (a) and (d) di-p-chlorobenzoates, however, the molecular ions were invisible, and no traces of peaks at m/e 356 were evident. The base peaks of these spectra appeared at m/e 105. The results of microanalyses of diols (b) and (c) di-p-chlorobenzoates were consistent with the proposed identities of the compounds, but microanalyses of the corresponding derivatives of diols (a) and (d) were not obtained, as insufficient quantities of material were available.

CD measurements of the di-p-chlorobenzoates of all four diols in hexane were recorded. The results are tabulated below. As no dipole interaction of the benzoate

Table 7

Diol from which derivative was prepared	Cotton Effects (around 240 nm)	
	$\Delta\varepsilon$	λ (nm)
(a)	+1·34	246
(b)	+4·17	243
(c)	-4·40	242
(d)	-1·52	239

chromophores was evident in the CD curve of any diol, only data relevant to the Cotton effects at about 240 nm were

Figure 14

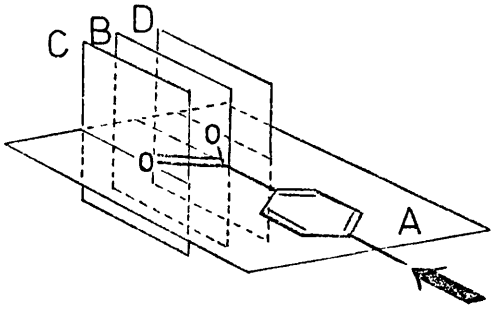


Figure 15

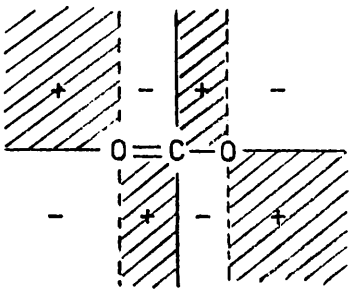


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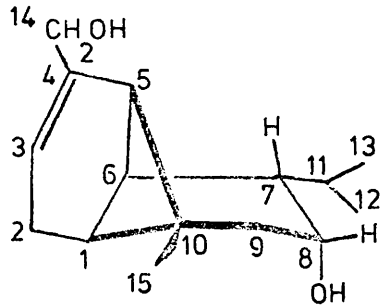
Figure 16

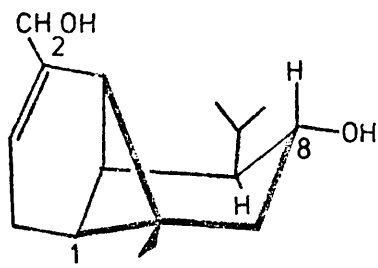
Hypothetical Conformations of the Brachylaena Diols (Relative Configurations at C-7 and C-8 are indicated).

i) α- hydroxyl
α-isopropyl



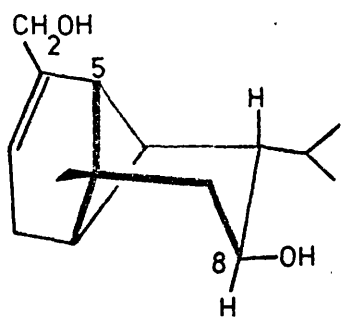
Carbon atoms 6-10 almost planar

ii) α-hydroxyl
β-isopropyl



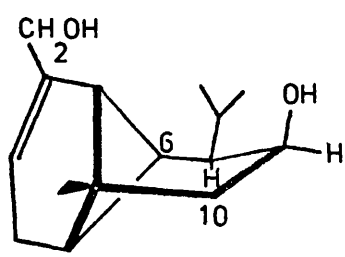
Chair, C-1 → C-8

iii) β-hydroxyl
α-isopropyl



Chair, C-5 → C-8

iv) β-hydroxyl
β-isopropyl

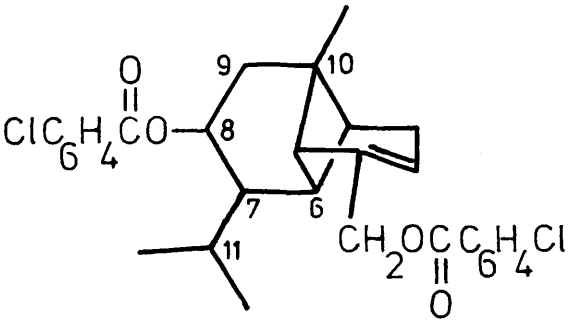


Carbon atoms 6-10 almost planar.

considered, and the applicability of the benzoate sector rule to the complete series of diastereomers was examined. In their original exposition of the rule, Harada et al.[87] postulated that the magnitude and sign of the Cotton effect around 240 nm exhibited by the p-chlorobenzoate of a secondary alcohol could be predicted by consideration of a model of the compound. By viewing the benzoate of the secondary alcohol from its para-position (Fig. 14), the rotatory contributions of the α,β and β,γ bonds could be estimated by reference to an empirical rule which stated that bonds falling in the shaded and unshaded areas in Figure 15 make, respectively, positive and negative contributions to the sign of the Cotton effect around 240 nm. Models of the diols were constructed and their preferred conformations were considered (Fig. 16). It was considered unlikely that in the preparation of di-p-chlorobenzoates, these conformations would be affected. In each case, the preferred conformation of the benzoyloxy group at C-8 was assumed to be that in which it lay staggered between the carbinyl hydrogen atom and C-7. The secondary benzoate was viewed as described above, and the signs of the contributions of α,β and β,γ bonds to the Cotton effect exhibited by each isomer were assessed (Table 8). In cases where a bond was found to lie close to the plane of symmetry, and its contribution was therefore considered

Table 8

Benzoate Sector Rule: Predictions of Signs of Cotton Effects in Diol Di-p-Chlorobenzoate Series (opposite)



Configurations at C-7 and C-8		Rotatory Contributions of α, β Bonds		Estimated Rotatory Contributions of β, γ bonds			Cotton Effect
Isopropyl	Hydroxyl	8,9*	8,7	9,10	7,11	7,6	(Total)
α	α	-3	0	-2	-δ	+1	-4-δ
α	β	+3	0	+2	+1	-δ	+6-δ
β	α	-3	0	-2	-1	+δ	-6+δ
β	β	+3	0	+2	+δ	-1	+4+δ

* C-C bond between positions 8 and 9

to be small, the notation 'δ' was used. In addition, since the contributions of σ-bonds would decrease with increasing distance from the ester, nominal contributions varying from 1 to 3 were assumed: these are represented in Table 8 by numbers following the signs. The sums of the individual contributions to the Cotton effect of each isomer appear in the last column of the Table. A rough correlation of the actual results with those predicted, according to the signs and magnitude of Cotton effects, yielded the following assignments.

Table 9

Brachylaena Diols	Relative Configurations at C-7 and C-8, as deduced from results of Circular Dichroism	
	Isopropyl	Hydroxyl
(a)	β	β
(b)	α	β
(c)	β	α
(d)	α	α

These results, however, are inconsistent with the fact that the configurations of Brachylaena diols (a) and (d) at C-7 (isopropyl moieties) are known to be identical, but opposite to those possessed by diols (b) and (c).

While the reason for this discrepancy is not clear, it is recognised that a degree of complication might have arisen from the presence of the primary p-chlorobenzoate and the double bond at C-3, 4. In addition, the effect of the cyclobutanoid structure is not known. In respect of these results, however, it should be noted that if only the signs of the observed Cotton effects are considered, and compared with those predicted on theoretical grounds, no inconsistency need arise in the two possible interpretations of the results (Table 10).

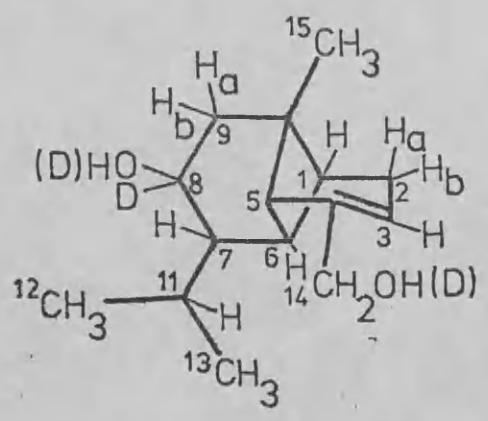
Table 10

Brachylaena Diols (two possible assignments)		Relative Configurations at C-7 and C-8, as deduced from results of Circular Dichroism (signs only)	
		Isopropyl	Hydroxyl
(a)	(b)	β	β
(b)	(a)	α	β
(d)	(c)	β	α
(c)	(d)	α	α

A further experiment is proposed, in which the double bonds of the diols are first reduced, the di-p-chlorobenzoates of the reduction products prepared, and the diesters then partially hydrolysed to afford a series of

Table 11

Chemical Shift Data* (100 MHz) of Deuterated Diol (d) (below), together with Observed Coupling Constants after exchange of the hydroxylic proton. Approximate Dihedral/'Allylic'‡ Angles in a model of the compound are also shown.



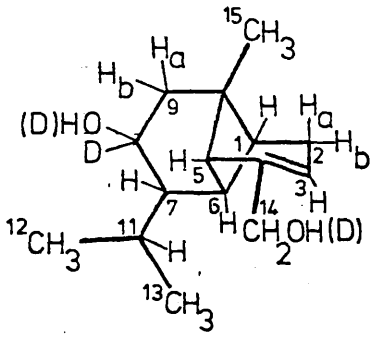
* See Figure 17

**s = singlet; d = doublet;
q = quartet; m = multiplet.

‡θ_x = the angle which H_x subtends with the plane of the allylic system.

Proton(s)	Chemical Shift (τ) and Observed Splitting Pattern**	Coupling Constants (J)	Measured Dihedral (φ)/ 'Allylic' (θ)‡ Angles
H_1	8·38(d, further split, broadened)	J_{1-5} = 6 Hz (W) $J_{1-2a} = J_{1-2b}$ = 3 Hz	$\varphi_{1-2a} = \varphi_{1-2b} = 60°$
H_{2a}	~ 7·73(m, broad)	Coupling to H_1, H_3;	$\varphi_{2a-1} = \varphi_{2b-1} = 60°$
H_{2b}		$2H_{14}$ (homoallylic)	$\varphi_{2a-3} = \varphi_{2b-3} = 60°$ $\theta_{14} = \theta_2 = 60°$
H_3	4·48(m)	J_{3-5} = 2 Hz (allylic) J_{3-1} = 1 Hz (W)	$\theta_5 = 0$
H_5	7·36 (d, further split)	J_{5-1} = 6 Hz (W) J_{5-3} = 2 Hz (allylic)	$\theta_5 = 0$
H_6	8·23(s)	—	[$\varphi_{6-1} = \varphi_{6-5} = \varphi_{6-7} = 90°$]
H_7	8·09(d)	J_{7-11} = 5 Hz J_{7-9b} = 0·5 Hz (W)	freely rotating bond
H_{9a}	8·32(d?)	J_{9a-9b} = 9 Hz (gem.)	
H_{9b}	8·59(d, broadened)	J_{9b-9a} = 9 Hz (gem.) J_{9b-7} = 0·5 Hz (W)	
H_{11}	—	—	
$3H_{12}$	8·96(d)	J_{12-11} = 6 Hz	freely rotating bond
$3H_{13}$	9·05(d)	J_{13-11} = 6 Hz	freely rotating bond
$2H_{14}$	~6·05(m)	J_{14-2} = 2 Hz (homoallylic) J_{14-3} = 1·5 Hz (allylic)	
$3H_{15}$	9·17(s)	—	

Figure 17. NMR Spectrum (100 MHz) of C-8 Deuterated Diol (d) [$CDCl_3 + D_2O$]



compounds possessing free primary hydroxyl functionalities and secondary p-chlorobenzoates. Unambiguous stereochemical assignments, consistent with known data, might then be obtained by consideration of the CD curves of these compounds.

100 MHz NMR spectra of the Brachylaena diols in deuterochloroform were recorded. These were complex, and considerable 'crowding' of peaks in the region 7-9 τ was evident. In addition, very little similarity was observed between the spectra in this region. It was decided first to examine an NMR spectrum of C-8 deuterated diol (d) (see p. 44), and then, by a direct comparison of this spectrum with that of the corresponding non-deuterated diol, to attempt to derive a complete NMR analysis of the latter compound. A 100 MHz NMR spectrum of deuterated diol (d) was obtained (Fig. 17), and its main features are summarised in Table 11, together with dihedral and 'allylic'* angles relevant to the observed couplings, as estimated from a model of the compound. Dihedral angles associated with coupling across four σ-bonds ('W'-coupling) are all approximately 0°, with the exception of those involved in the coupling between protons H_7 and H_{9b}, described later.

* See note in Table 11.

Allylic and homoallylic systems require the specification of one and two 'allylic' angles, respectively. Certain characteristics of the spectrum, and the results of spin decoupling experiments (examples illustrated in Appendix 3), are indicated in detail below.

The splitting pattern of proton H_1 (8.38 τ) was obscured by overlap with proton H_{9a} in the spectrum: on simplification of the splitting pattern of the latter proton in decoupling experiments (p. 58), however, proton H_1 became evident as a doublet (J_{1-5} = 6 Hz), further split by vicinal coupling of 3 Hz to the magnetically similar protons H_{2a} and H_{2b}. No coupling between protons H_1 and H_6 was observed, as the dihedral angle between these protons was about 90°. Double irradiation on H_1 resulted in collapse of the H_5 doublet to a broad singlet. In addition, some sharpening of the broad multiplet ascribed to protons H_{2a} and H_{2b} was noted. Back irradiation on H_2 resulted in considerable sharpening of the H_1 doublet.

Irradiation on H_2 ($\sim$ 7.73 τ) also caused collapse of the six-line pattern at 6.05 τ to a doublet (J_{14-3} = 1.5 Hz), and a marked sharpening of the diffuse peak ascribed to vinylic proton H_3 was recorded.

Allylic coupling of proton H_3 (4.48 τ) to proton H_5 (7.36 τ) (J_{3-5} = 2 Hz) was confirmed by double irradiation on H_3, which resulted in the appearance of H_5 as a sharp doublet. The reason for this coupling is not clear, as

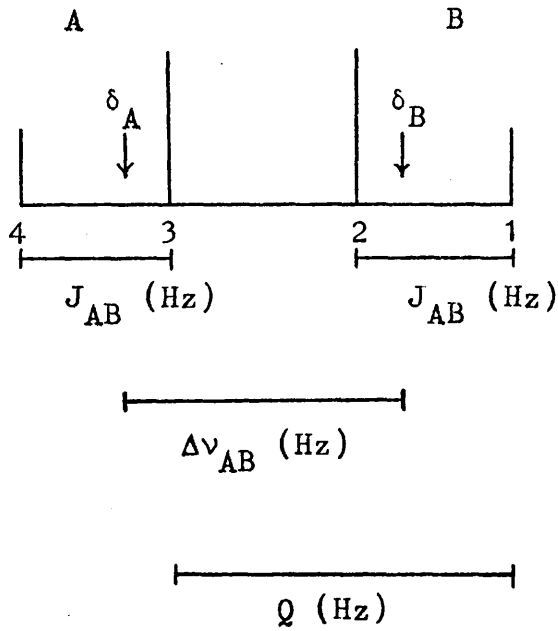
the 'allylic' angle involved is 0°, as indicated by examination of a model of the compound. The coupling of proton H_3 to proton H_1 (W-coupling) was measured during double irradiation at 7·73 τ (i.e. on protons H_2: see above). The splitting pattern of H_1 was then considerably simplified, leaving only coupling to H_5 (J_{1-5} = 6 Hz) and to H_3. This latter coupling was estimated as 1 Hz.

The one-proton doublet at 8·09 τ was ascribed to proton H_7, coupled to vicinal proton H_{11}. No coupling was observed between protons H_7 and H_6, as the dihedral angle between these protons was 90°. Proton H_{11} was further coupled to methyl protons $3H_{12}$ and $3H_{13}$ ($J_{12-11} = J_{13-11}$ = 6 Hz) and, consequently, was not observed in the spectrum.

Isochronous protons $2H_{14}$ appeared at about 6·05 τ as a six-line pattern, due to homoallylic coupling of these protons to the magnetically-similar protons $2H_2$, producing a triplet (J_{14-2} = 2 Hz), which was split by further coupling of protons $2H_{14}$ to vinylic proton H_3 (J_{14-3} = 1·5 Hz). Double irradiation at 6·05 τ resulted in a pronounced sharpening of the broad peak ascribed to protons $2H_2$. Back irradiation confirmed this result (as indicated above) and, by removing the coupling of $2H_{14}$ to $2H_2$, left a doublet corresponding to the coupling of protons $2H_{14}$ to protons H_3. Similarly, on double irradiation at 4·48 τ, the multiplet at 6·05 τ collapsed to a triplet

Figure 18

Notation for a Perturbed 'AB' System (δ/J small)



which represented the coupling of protons $2H_{14}$ to protons $2H_2$.

Signals were observed at 136 and 145 Hz, representing the higher field ('B') proton of a perturbed 'AB' system (small δ/J ratio) involving geminal protons H_{9a} and H_{9b} and, from the equations

$$' \quad I_2/I_1 = (Q + J)/(Q - J) \quad '$$

and

$$' \quad \Delta\nu = \sqrt{(Q^2 - J^2)} \quad '$$

(Fig. 18), the positions of the signals representing the lower field ('A') proton, and the chemical shifts of both protons (Table 11) were calculated. These results were to some extent confirmed by double irradiation on the higher field proton, which resulted in the collapse of some peaks in the region where the lower field proton was expected to occur, although the complexity of this region obscured the effect. Further coupling of both geminal protons was evident: in the case of proton H_{9b} (higher field), further splitting of 0·5 Hz was recorded and, in the case of proton H_{9a} (lower field), the low intensities of the observed peaks indicated appreciable further coupling. According to a model of deuterated diol (d), these interactions were most likely to involve proton H_7 (distorted W-coupling).

The NMR spectrum of C-8 deuterated diol (d) was com-

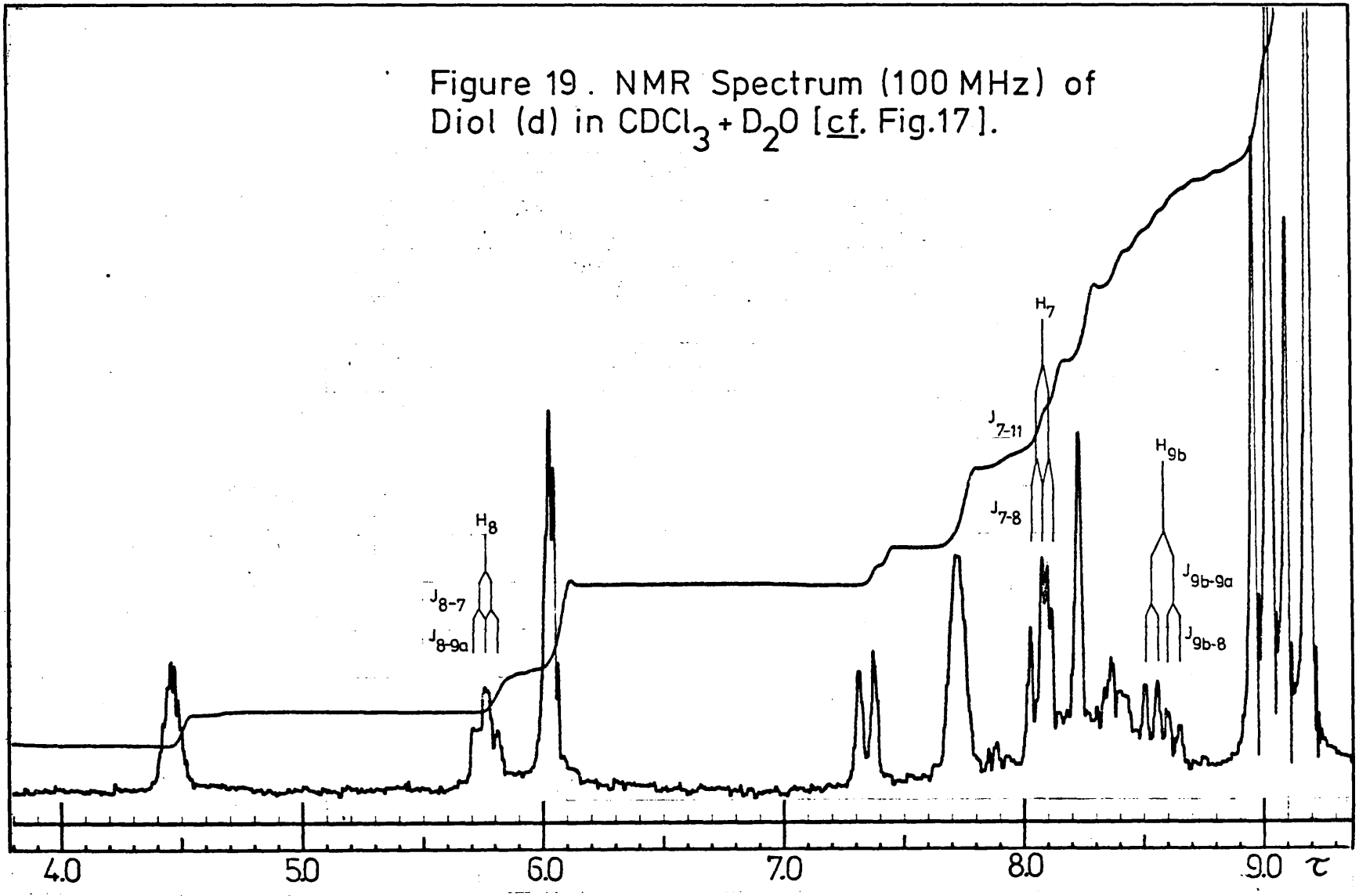


Figure 19. NMR Spectrum (100 MHz) of Diol (d) in $CDCl_3 + D_2O$ [cf. Fig.17].

pared with the corresponding spectrum of a sample of non-deuterated diol (d) (Fig. 19). In the second spectrum, carbinyl proton H_8 was observed as a broadened triplet at 5·76 τ and, as expected, peaks ascribed to protons H_7 and H_{9b} in the last spectrum were further split. The splitting pattern of proton H_{9a} appeared to be unchanged, although this result was uncertain in view of the considerable overlapping of peaks in the region of this proton.

Proton H_{9b} exhibited coupling of 5 Hz to vicinal proton H_8, and thus appeared in this spectrum as a four-line pattern. Proton H_7 was evident as a multiplet, due to further coupling of about 5 Hz to proton H_8. The exact splitting pattern of this proton was not clear: if, however, the peak at 8.11 was considered to have arisen from proton H_{11} (cf. p.57), then the remaining three-line pattern could be explained by postulating coupling of H_7 to H_8 (J_{7-8} = 5 Hz), resulting in splitting of the original doublet (J_{7-11} = 5 Hz) into a four-line pattern in which the inner lines had coalesced.

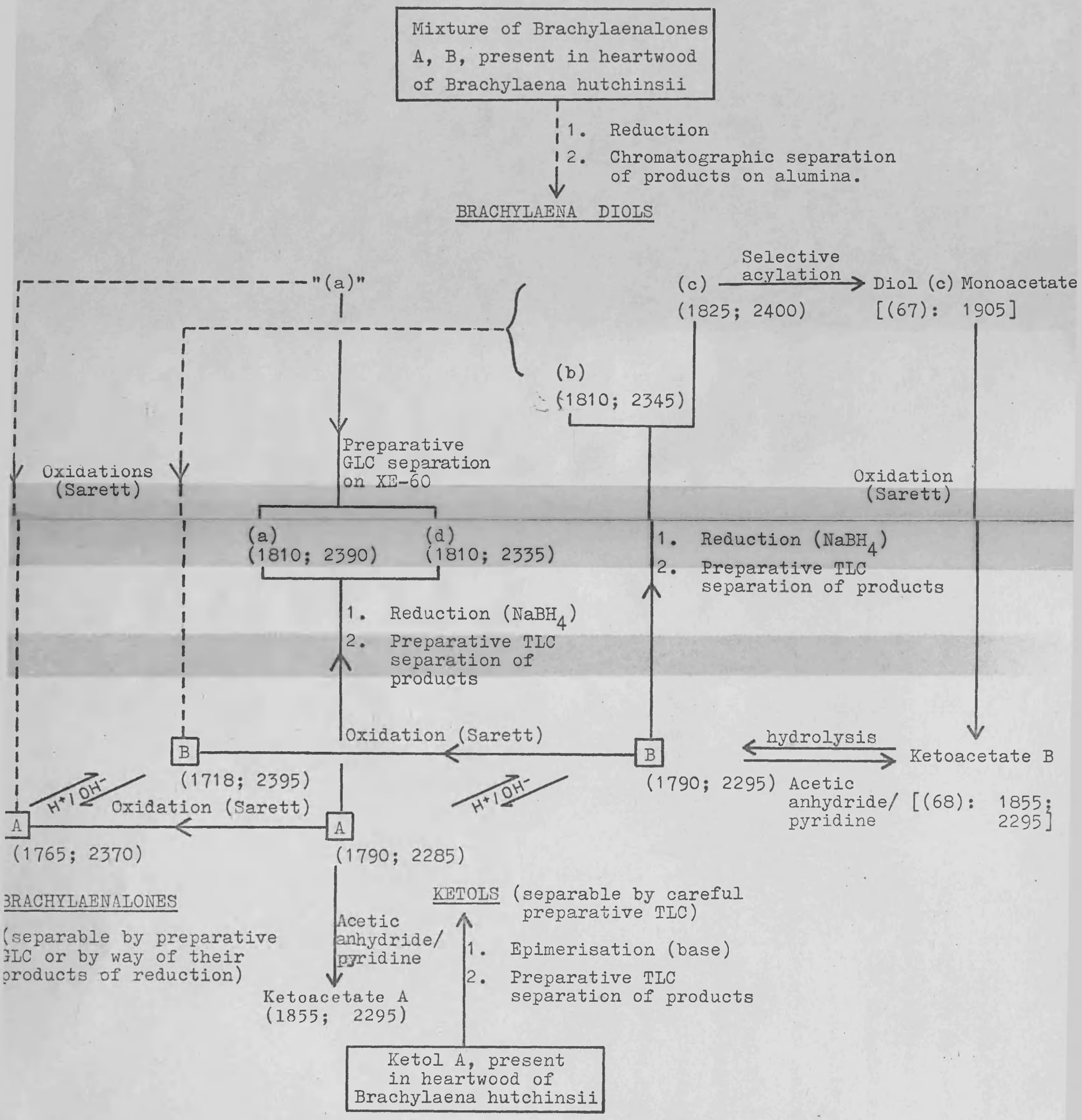
The apparent triplet representing carbinyl proton H_8 could be readily understood in terms of its coupling to protons H_7 and H_{9a}. Once again, coalescence of the inner lines of the expected quartet had occurred. Slight broadening of the triplet was assumed to have arisen on account of coupling to vicinal proton H_{9a} (J_{8-9a} = ca. 0·5 Hz).

Examination of models showed that these couplingsto

proton H_8 were consistent with either α,α or β,β secondary hydroxyl/isopropyl configurations: the first possibility was favoured, as this was in agreement with the results of CD, as represented in Table 10. In a model of the Brachylaena diol possessing α-secondary hydroxyl/α-isopropyl configurations, the following dihedral angles were recorded: $\varphi_{7-8} = 20°$; $\varphi_{8-9\alpha} = 95°$; $\varphi_{8-9\beta} = 20°$. These angles would be expected to afford the following approximate coupling constants: $J_{7-8} = 7$ Hz; $J_{8-9\alpha} = 0$ Hz; $J_{8-9\beta} = 7$ Hz. Comparisons of actual coupling constants with the predicted values indicated that the β-proton on C-9 was proton H_{9b} (8·59 τ), as further splitting of this proton was observed on the introduction of proton H_8.

Figure 20

Correlations Achieved* within the Brachylaena Series: Retention Indices (OV-1 and XE-60, respectively)‡ are indicated in brackets



* Correlations achieved also by M.M. Campbell[81] are shown as dotted lines.

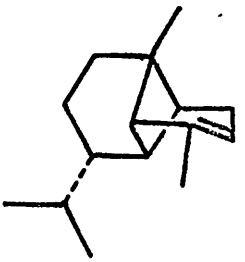
‡ For Diol (c) Monoacetate (67), retention index on OV-1 only is indicated.

Table 12

Physical Properties of Brachylaena Diols and Diol Di-p-Chlorobenzoates

	Free Brachylaena Diols						Diol Di-p-Chlorobenzoates			
Diol	m.pt.	Optical Rotation	R_f (Et_2O)	$I^{156°}_{OV-1}$	$I^{156°}_{XE-60}$	Mass Spectral Base Peak(s)*	Cotton Effect(hexane) $\Delta\varepsilon_{max}$	λ_{max}(nm)	R_f (90% light petroleum-ethyl acetate)	Mass Spectra Base peak*
(a)	-	$[\alpha]_D^{25}$ = -52°	0·40	1810	2390	m/e 105	+1·34	246	0·48	m/e 105
(b)	106-108·5°	$[\alpha]_D^{28}$ = +15°	0·50	1810	2345	m/e 131	+4·17	243	0·50	m/e 139
(c)	137-138·5°	$[\alpha]_D^{28}$ = -27°	0·30	1825	2400	m/e 135	-4·40	242	0·55	m/e 139
(d)	-	$[\alpha]_D^{25}$ = -31°	0·40	1810	2335	m/e 91,105	-1·52	239	0·48	m/e 105

* at 70 e.v.



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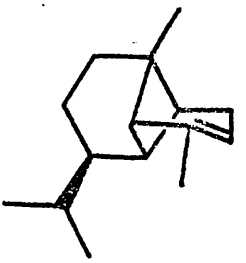

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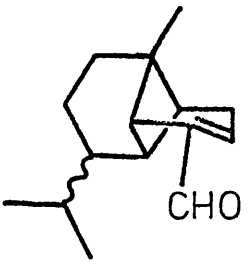


88

Hydrogenation Experiments within the Brachylaena Series of Compounds

Hydrogenation experiments performed within the Brachylaena series of compounds were of two types: in the first instance, attention was directed towards a possible correlation of the Brachylaenalones with their expected parent hydrocarbons, copaene (3) and ylangene (4), and reductions of the ketoaldehydes by the Wolff-Kishner method, and of one Brachylaenalone bis-ethylenethioacetal over Raney nickel catalyst are described; in the second part of this section of the thesis, the products of hydrogenation of a Brachylaena diol over Adams catalyst are compared with the results of similar treatment of ylangene.

Wolff-Kishner Reductions of the Brachylaenalones

Wolff-Kishner reduction of Brachylaenalone A was first attempted according to a modified Huang-Minlon[88] procedure in which the reaction was performed in a Carius tube to minimise losses of volatile products. A solution of the ketoaldehyde in anhydrous diethylene glycol was treated with potassium hydroxide pellets and excess anhydrous hydrazine, and the reaction mixture was maintained at 210° for 5 hours in a sealed tube. The

Figure 21. Products from Wolff-Kishner Reduction of Brachylaenalone A. 3% SE-30. Temperature programmed from 90°, 4°/min. (1 cm = 1 min.).

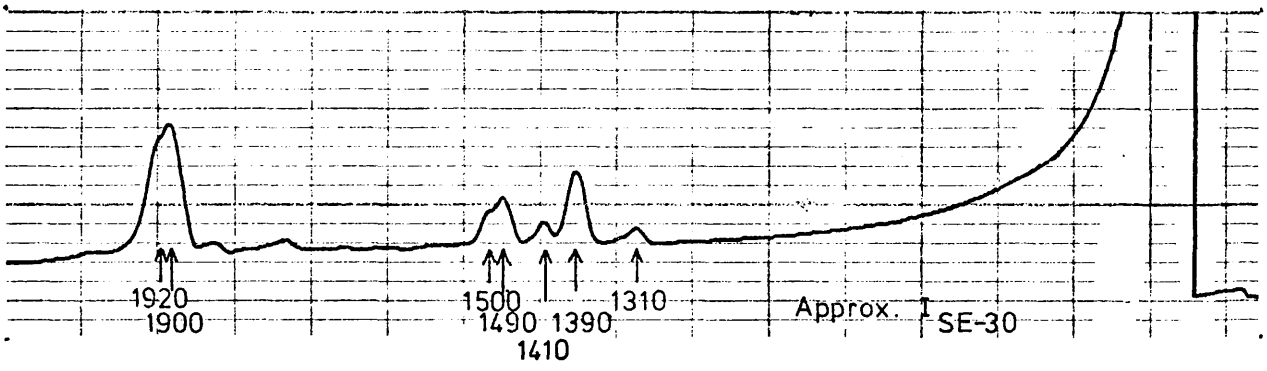
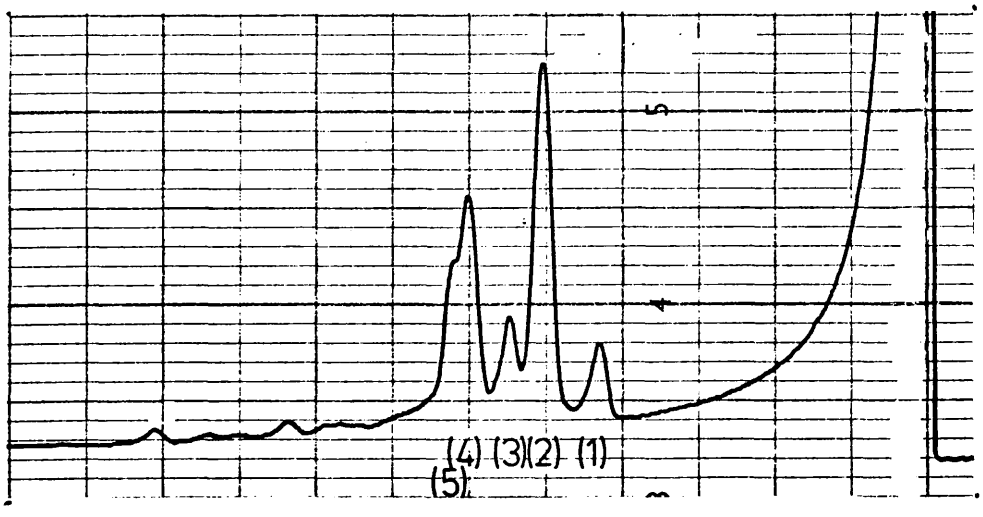


Figure 22. Hydrocarbon Fraction obtained by Preparative TLC Separation of products of Wolff-Kishner Reduction (above). 3% SE-30. Temperature programmed from 120°, 2°/min.



pale yellow solution of reaction products was diluted with water, and extracted with ether to yield a yellow gum which, on examination by TLC, was found to comprise four major components of R_f values (90% light petroleum-ethyl acetate) 0.12, 0.25, 0.44 and 0.80 (*cf.* copaene and ylangene, ~0.80); no starting material remained. GLC on 3% SE-30 under conditions of programmed temperature indicated products of retention indices 1310, 1390, 1410, 1490, 1500, 1900, 1920, with the components of I_{SE-30} 1900 and 1920 in predominance (Fig. 21). The latter components occurred at retention indices approximately equivalent to those expected for copaenal or ylangenal (88): coinjection of these aldehydes with the product mixture on the phase SE-30, however, established that the major products did not correspond to these standard compounds. The retention indices of copaene and ylangene on SE-30 under conditions identical to the above were found to be both 1390, suggesting that these hydrocarbons were present as minor constituents of the total product.

Preparative TLC of the product mixture (90% light petroleum-ethyl acetate) afforded the hydrocarbon fraction of R_f 0.80, which was examined by GLC on SE-30 and found to comprise principally peaks of retention indices 1310-1500, described as peaks 1-5 in Figure 22. An IR spectrum (liquid film) of the fraction confirmed that it

was composed solely of hydrocarbons. Coinjection of copaene and ylangene with this material on SE-30 supported the postulate that these hydrocarbons were present in the fraction. Mass spectra of the compounds represented by peaks 1-5 were obtained by combined GC-MS, yielding the following results.

Peak 1 afforded a spectrum with a molecular ion at m/e 190: the base peak of the spectrum appeared at m/e 105 (methyl tropylium ion). These data were consistent with a 14-carbon bicyclic structure containing two double bonds. The mass spectrum obtained from peak 2 was almost identical to the corresponding spectrum of either copaene or ylangene; small differences in the relative abundances of corresponding fragments in the spectra were observed. From peak 3, a mass spectrum with a molecular ion at m/e 206 was recorded: the base peak of this spectrum was observed at m/e 163 ($M^{+\bullet}$ - isopropyl radical), and the presence of a bicyclic sesquiterpene containing one double bond was assumed. Peaks 4 and 5 yielded spectra representing mixtures of compounds of mocleular weights 204, 206 and 208: the base peaks of these spectra all appeared at m/e 163.

Examination of this hydrocarbon fraction at 100° on the phase 5% Bentone 34/5% dinonyl phthalate, known to separate copaene and ylangene, revealed the possible

presence of both these hydrocarbons in the ratio copaene: ylangene = 2:3. In addition, two peaks of equal intensities, apparently corresponding to the single peak 1 on SE-30, were recorded on the Bentone phase, suggesting the presence of diastereomers. Other single peaks observed on SE-30 appeared as single peaks also on Bentone 34/dinonyl phthalate. Retention times on the latter phase are shown on page115.

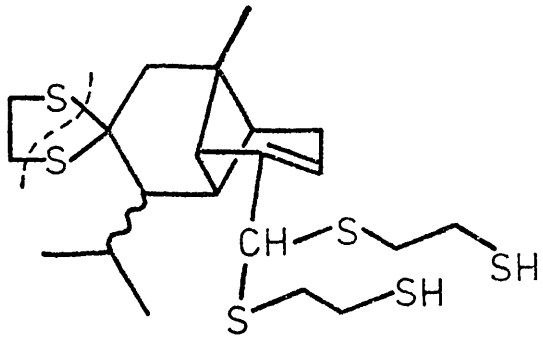
An estimation of peak areas in the chromatogram of the above hydrocarbon fraction on SE-30 indicated that copaene and ylangene were present as about 6% by weight of the total mixture of products obtained in the reduction.

Similar reduction of Brachylaenalone B at a lower temperature, in the presence of higher molar proportions of anhydrous hydrazine, resulted in the formation of products of high molecular weights thought to correspond to azines (on account of their retention data on OV-1 and XE-60), and a very poor yield of a copaene/ylangene mixture was recorded. Wolff-Kishner reduction of Brachylaenalone B at 120^{o} with sodium methoxide as base, according to the method of Moffett and Hunter,[89] did not afford any improvement in the yields of the required products.

Preparation of the Bis-Ethylenethioacetal Derivatives of the Brachylaenalones

It was decided to prepare the bis-ethylenethioacetal derivatives of Brachylaenalones A and B, and to attempt reductions of these compounds over Raney nickel. As these reactions should not normally involve epimerisation of the isopropyl groups, it was hoped thus to prepare copaene from one experiment and ylangene from the other, in higher yields than in the previous reaction.

Ethanedithiol was added carefully, in portions, to a boron trifluoride etherate solution of Brachylaenalone A, of high purity as estimated by GLC on 0.5% XE-60 at 145°, and the reaction was monitored by TLC. After 2 hours, no starting material remained. TLC examination (80% light petroleum-ethyl acetate) showed two main products of R_f values (0.78 and 0.59) consistent with the formation of both mono- and bis-ethylenethioacetals. The solution was neutralised and extracted with ether to yield an oil. The two main components were separated by careful preparative TLC, and the purities of the resultant compounds were estimated by analytical TLC. The corresponding derivatives of Brachylaenalone B were obtained in a manner parallel to the above. Identical mobilities on silica gel of the compounds thought to represent the respective bis-thioacetals of the Brachylaenalones were



89

recorded. Reproducible differences, however, were observed in the mobilities of the postulated monothioacetals: (80% light petroleum-ethyl acetate) derivative from Brachylaenalone A, 0.59; derivative from Brachylaenalone B, 0.52.

Mass spectra of all these derivatives (introduced into the LKB 9000 mass spectrometer by way of the Direct Inlet) were obtained. The identities of the proposed monothioacetals were supported by molecular ions occurring in the spectra of these compounds at m/e 308. Isotope peaks at m/e 309 and 310 were also observed. The mass spectra of the proposed bis-thioacetals showed molecular ions at m/e 384, as required, but were complicated by the presence of impurities, giving rise to prominent ions at m/e 418 and 478 in both spectra. These peaks are explainable in terms of structures such as 89 (molecular weight 478), and the fragments at m/e 418 may then be understood in terms of losses of C_2H_4S from these structures, as illustrated.[90] That these impurities did not exist in high concentration in the samples, however, was suggested by the IR spectra (liquid films) of the bis-ethylenethioacetals, in which no absorption was observed in the regions 2600-2550 cm^{-1} (ν_{S-H}). In the corresponding IR spectra of the monothioacetals, prominent peaks were observed at 1668 cm^{-1}, indicating

the presence of α,β-unsaturated aldehydes. The low values of these frequencies were due to the fact that the spectra were recorded from liquid films.

Reduction of Brachylaenalone A Bis-Ethylenethioacetal

Reduction of the bis-ethylenethioacetal of Brachylaenalone A by treatment with Raney nickel catalyst, grade W2,[91] was then attempted. A solution of the bis-thioacetal in absolute ethanol was treated with excess Raney nickel catalyst, and the mixture was refluxed for 4½ hours. The catalyst was then removed by filtration through cotton wool and Celite 535, and the resultant clear solution was examined by GLC on 1% OV-1 under conditions of programmed temperature: a considerable complexity of products, of retention indices ranging approximately from 1300 to 1700, was observed. In particular, as the majority of peaks in this chromatogram appeared in the range I_{OV-1} 1300-1500, ring-opening to the decalin system was inferred. Since ring-opening of the original tricyclic structure could have occurred by a variety of mechanisms, a complex pattern of products was then expected. The earliest peak in the chromatogram appeared at a retention time consistent with that of copaene and ylangene on this phase: examination on 5% Bentone 34/5% dinonyl phthalate, however, revealed that neither of these hydrocarbons was present.

Mass spectra of the products were obtained by GC-MS.

GLC peaks in the region I_{OV-1} 1300-1500 were found to represent hydrocarbons of molecular weights 204 and 206. Base peaks in the mass spectra of the compounds of lower molecular weight occurred at m/e 105 (methyl tropylium ion), while the spectra of the compounds of higher molecular weight all possessed base peaks at m/e 163 ($M^{+\bullet}$ - isopropyl radical). The mass spectra of two later GLC peaks, appearing at about I_{OV-1} 1550 and 1650, represented compounds of molecular weights 202 and 198, respectively; ie. sesquiterpene hydrocarbons, possibly bicyclic, with varying degrees of unsaturation. Base peaks in the spectra of these compounds appeared at m/e 202 ($M^{+\bullet}$ - isopropyl radical) and m/e 183 ($M^{+\bullet}$ - methyl radical), respectively.

The reduction was repeated on a second portion of Brachylaenalone A bis-ethylenethioacetal, in the solvent system acetone : benzene = 3:1. The presence of acetone would result in deactivation of the catalyst: benzene was required to overcome the difficulty arising from the low solubility of the starting material in acetone. A large number of products, however, similar to that produced in the last reaction, was obtained in this case.

It was decided to abandon attempts to correlate the Brachylaenalones with their expected parent hydrocarbons by this method.

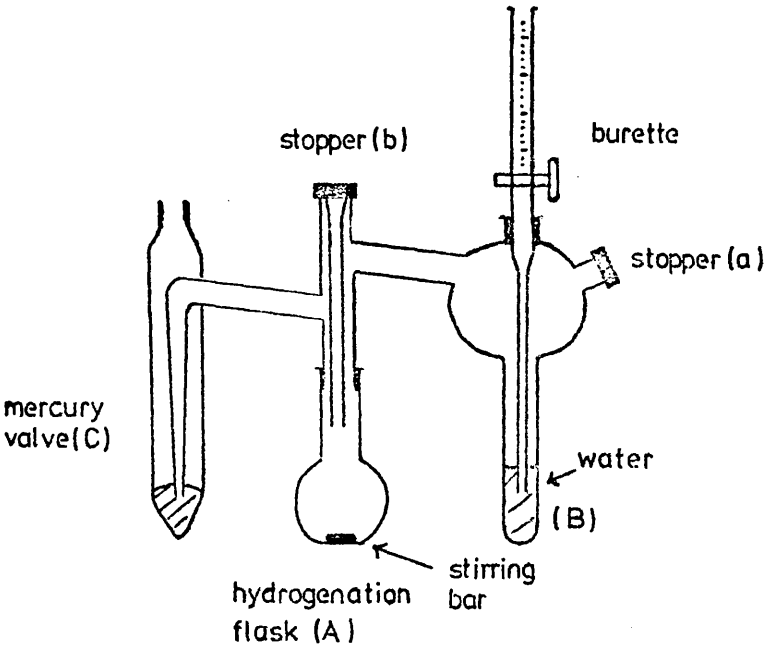


Figure 23. Apparatus for hydrogenation (C.A. Brown[92])

Reductions over Adams Catalyst: General Remarks

The apparatus used in the following small-scale reductions over Adams catalyst was a modification of that devised by C.A. Brown.[92] The model employed in the present work (Fig. 23) was built in the Department of Chemistry, University of Glasgow, and utilises commercially available hydrogen rather than hydrogen produced *in situ* from sodium borohydride as in the original paper. The following general procedure was adopted for the experiments.

A known quantity of platinum oxide was placed in the hydrogenation flask (A), together with a small volume of glacial acetic acid and a stirring bar. The flask was attached to the apparatus. A burette was filled with water, and its stopcock was opened until water had displaced the air in its tip. The burette was inserted into compartment B of the apparatus, which contained a little water, and all ground glass joints were sealed with silicone grease. The apparatus was then flushed with hydrogen, introduced by way of a fine needle passing through a silicone rubber stopper (a) in a side-arm of the apparatus; during this procedure, air (and hydrogen) was displaced via a mercury valve (c). After about 45 minutes, the hydrogen lead was carefully removed, and the closed system was stirred until no further movement

of the mercury in the capillary tube of the valve was observed (about 50 minutes). The mercury in the capillary tube was adjusted to a convenient level, and its position was marked: the pressure in the apparatus could be increased by careful addition of water from the burette, or decreased by removal of hydrogen through stopper (a) with a syringe. A known weight of the sample to be reduced was dissolved in a measured volume of glacial acetic acid, and this solution was injected directly into the reaction flask through stopper (b). The reaction mixture was stirred continually throughout the reduction. The behaviour of the mercury in the capillary of the valve provided a sensitive indication of the rate of absorption of hydrogen, and the end of the reaction was indicated when the level of mercury remained constant over a reasonable period. This level was then adjusted to its original position by addition of water from the burette, and the volume of water required was noted. Another sample could then be injected, and the above procedure repeated. Atmospheric pressure and temperature were recorded. The quantity of hydrogen absorbed (m.moles) was calculated as follows.

$$\text{m.moles hydrogen} = \frac{273P(V_w + V_l)}{22.4 \times 760\ (T + 273)}, \text{ where}$$

P = Atmospheric pressure (mm mercury) - partial pressures

of water and acetic acid at the temperature of the reaction.

V_w = Volume of water added from the burette (ml).

V_l = Volume of sample injected (ml).

T = Temperature (^oC).

Reductions of Ylangene (4) over Adams Catalyst

It was first necessary to purify the sample of ylangene available‡. Preparative TLC (100% light petroleum) afforded ylangene (R_f 0.71) of high purity, as estimated by GLC on 1% OV-1 and 0.5% XE-60, under conditions of programmed temperature.

A solution of pure ylangene in glacial acetic acid was reduced over Adams catalyst in the manner described above. A calculation indicated that exactly one molar proportion of hydrogen had been consumed in the reduction. Repetition of the experiment yielded an identical result. The suspension was filtered through cotton wool, and examined by GLC on OV-1. The filtrate was then adjusted to about pH 8 by addition of concentrated aqueous sodium

‡ Kindly supplied by Professor V. Herout, Czechoslovak Academy of Science, Institute of Organic Chemistry and Biochemistry, Prague.

bicarbonate, saturated with sodium chloride, and extracted with ether. Traces of acetic acid were removed from the combined, dried, concentrated extracts by azeotropic distillation with benzene. Examination of the resultant oil by GLC on OV-1 yielded results which were in agreement with those obtained from the acetic acid solution before work-up: a single peak of $I^{88^{\circ}}_{OV-1}$ 1392 was observed (contrast ylangene, 1352 under identical conditions). Analysis on the phase 5% Bentone 34/5% dinonyl phthalate at 100° also indicated a single, homogeneous peak: a mixture of diastereomers was not evident on this phase. A single product, apparently homogeneous on the phase Carbowax 20M at 160°, was also reported by G.L.K. Hunter et al.[49] from the hydrogenation of ylangene over palladium-on-carbon catalyst, under conditions of high temperature and pressure. The above results may, however, be contrasted with the findings of N.H. Andersen et al.[93] and L. Westfelt,[55] who obtained mixtures of two products (diastereomers) from reductions of copaene over platinum oxide in acetic acid at room temperature and pressure. The latter workers employed the following GLC phases and column temperatures in examinations of the products. Andersen: Carbowax 20M, 165°; Apiezon L, 155°; SF-96, 170°; DEGS, 175°. Westfelt: E 301, 100°.

Mass spectral examination of the product (via GC-MS, using a column of OV-1) confirmed its molecular weight as 206; other prominent peaks in the spectrum occurred at m/e 191, 177, 163 (100%), 149, 135, 121, 107, etc., corresponding to successive losses of methylene radicals. It was not possible to determine from the spectrum whether the cyclobutanoid structure had been opened or the trisubstituted double bond had been reduced in the experiment: an IR spectrum of the product (liquid film), however, showed negligible absorption in the region of 785 cm^{-1}, indicating that hydrogenation of the double bond had been effected (for intensity of absorption exhibited by starting material in this region, compare Büchi *et al.*[45]).

Reductions of Brachylaena Diol (c) over Adams Catalyst

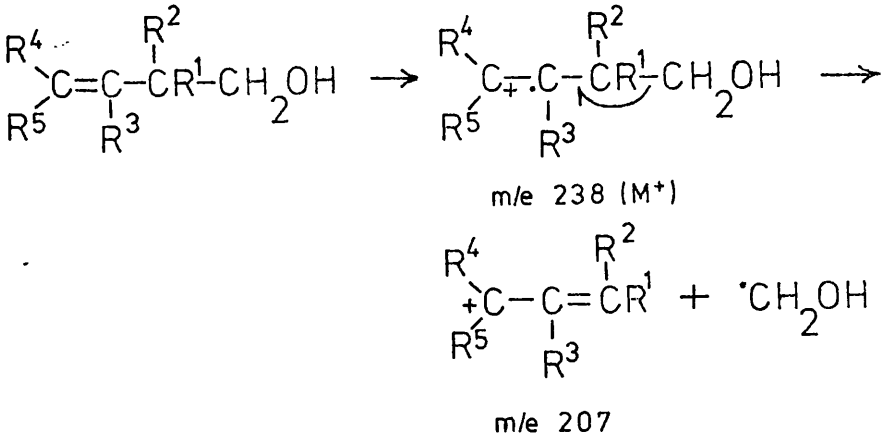
The procedure adopted for the following work was identical to that employed in the hydrogenations of ylangene described above. Brachylaena diol (c) of high purity, as estimated by GLC on 1% OV-1 and 0.5% XE-60, was reduced as its solution in glacial acetic acid: a calculation indicated that 0.89 molar proportions of hydrogen had been consumed. A further solution of diol (c) in acetic acid was injected into the reaction flask: on this occasion, the rate of hydrogen uptake was much slower

than that recorded during the previous experiment, and the reduction was terminated before its completion. The reaction mixture was examined by GLC on the phase XE-60: five main peaks were observed. Water was added to the solution, which was then neutralised by addition of dilute aqueous sodium hydroxide, saturated with sodium chloride and extracted with ether, to yield a partially crystalline oil. The product was again examined on XE-60 under conditions of programmed temperature*, affording a chromatogram identical to that of the reaction mixture prior to the extraction procedure. Peaks of the following retention times (minutes) were recorded (the percentage of the total mixture represented by each peak is indicated in brackets): 7.9 (5%); 8.8 (20%); 20.1 (8%; corresponding in retention time to the starting material); 20.9 (8%); 22.2 (50%); cf. retention time of product of similar reduction of ylangene, approximately 1.5 minutes under conditions identical to the above.

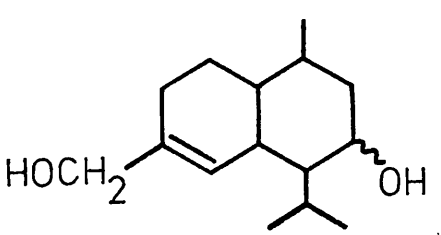
The total product mixture was examined by GC-MS on 1% OV 225. The major peak (t_R 22.2 minutes on XE-60) was found to represent a diol of molecular weight 238. Significant fragments above m/e 150 in the mass spectrum of this compound are indicated in Table 13. The fragment at m/e 207 corresponds to a loss of

* 83° → 190°, 2°/minute.

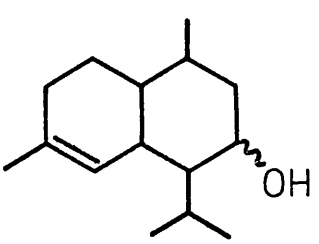
Figure 24



$R^1 - R^5$ = H or alkyl



90



91

Table 13

m/e	Relative Abundance (%; m/e 41 = 100%)	Proposed Nature of Fragment‡	Metastable Peak (where necessary to support proposed transition)
238	2	M	
223	12	M - $^{\bullet}CH_3$	
220	9	M - H_2O	
207	7	M - $^{\bullet}CH_2OH$	
205	7	m/e 223 - H_2O	m* 188.5
190	35	m/e 205 - $^{\bullet}CH_3$	
189	36	m/e 207 - H_2O	m* 172.6
177	40	m/e (205 → 191 - $^{\bullet}CH_2$	m* 158.8
159	73	m/e 177 - H_2O	m* 142.8

[‡ For convenience, the molecular ion ($M^{+\bullet}$) is denoted by 'M'.]

$^{\bullet}CH_2OH$ from the molecular ion, and is satisfactorily explainable in terms of the presence of a β,γ-unsaturated primary alcohol (Fig. 24). On the other hand, losses of $^{\bullet}CH_2OH$ were also observed in the mass spectra of the Brachylaena diols (p. 45), which comprise α,β-unsaturated primary alcohols. Indeed, both the retention index (XE-60) and the mass spectrum of this material were consistent with a structure such as 90, corresponding to

ring-opening of Brachylaena diol (c) to the decalin system.

The peak of t_R 8.8 minutes on XE-60 afforded a mass spectrum representing a mono-ol of molecular weight 222. Prominent fragments in this spectrum are shown in Table 14. The retention index and mass spectrum of

Table 14

m/e	Relative Abundance (%)	Proposed Nature of Fragment	Metastable Peaks (where necessary to support proposed transition)
222	2	M	
207	10	M - $^{\bullet}CH_3$	
204	18	M - H_2O	
189	12	m/e 204 - $^{\bullet}CH_3$	m* 175.1
179	23	m/e 207 - C_2H_4	no m* observed
	or	m/e (207 → 193) - $^{\bullet}CH_2$	
161	100	m/e 179 - H_2O	m* 144.8
105	65	methyltropylium ion	

this compound suggested structure 91, which would result from hydrogenolysis of the primary alcohol in structure 90.

The spectrum obtained from the peak of t_R 7.9 minutes on XE-60 was very similar to the latter spectrum, indicating that this compound was possibly a diastereomer of the previous mono-ol.

Of the remaining peaks in the chromatogram, that of t_R 20.1 minutes afforded a mass spectrum which was identical with the corresponding spectrum of Brachylaena diol (c), thus confirming the presence of starting material in the mixture. Finally, the peak of t_R 20.9 minutes on XE-60 was found to represent a mixture of compounds, possibly mono-ols, of molecular weights 222 and 220: the presence of minor amounts of further compounds of the same retention time, however, was evident from the spectra obtained.

Hydrogenation Experiments: Summary of Main Results

Wolff-Kishner reduction of Brachylaenalone A afforded a mixture of products (Fig. 21), from which a hydrocarbon fraction (Fig. 22) was separated by preparative TLC. Examination of this fraction by GLC (SE-30; Bentone 34/dinonyl phthalate) and GC-MS indicated the presence of copaene and ylangene as 6% by weight of the total product, and in the ratio copaene:ylangene = 2:3.

Reduction of the bis-ethylenethioacetal of Brachylaenalone A over Raney nickel catalyst, grade W2,

afforded a considerable complexity of products comprising neither copaene or ylangene: the retention indices (OV-1) of the majority of constituents of this mixture suggested that ring-opening of the original structure to the decalin system, according to a variety of possible mechanisms, had occurred. This postulate was supported by the results of GC-MS examination of the products. A second reduction over Raney nickel, in the presence of acetone to deactivate the catalyst, afforded a similar mixture of products.

Hydrogenation of ylangene over Adams catalyst yielded a single product, as judged by GLC (OV-1 and Bentone 34/dinonyl phthalate), and uptake of exactly one molar proportion of hydrogen was observed. Mass spectrometry confirmed that the molecular weight of the product was two mass units higher than that of the starting material, and an IR spectrum (liquid film) of the product indicated that reduction of the trisubstituted double bond of ylangene had occurred.

Similar hydrogenation of Brachylaena diol (c) over Adams catalyst afforded a mixture of products which was examined by GLC (XE-60) and GC-MS. The presence of some starting material (8% by weight of the mixture) was evident, and the retention index and mass spectrum of the predominant product (50%) were consistent with a

structure such as 90, which would arise from ring-opening of the original diol to the decalin system. Corresponding data of the other major constituent (20%) of the mixture suggested structure 91, which would result from ring-opening of Brachylaena diol (c) and hydrogenolysis of the primary alcohol.

Figure 25(a)

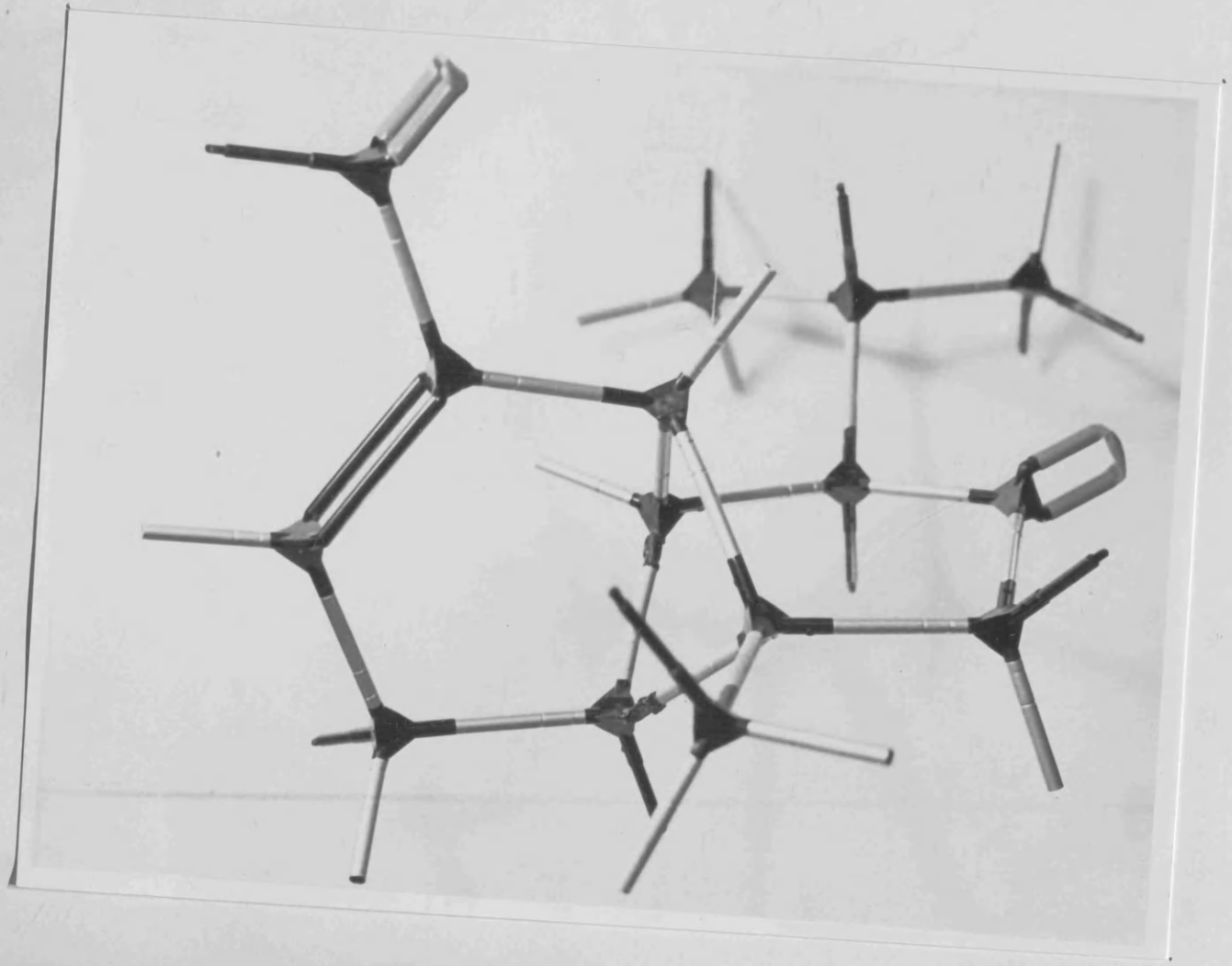
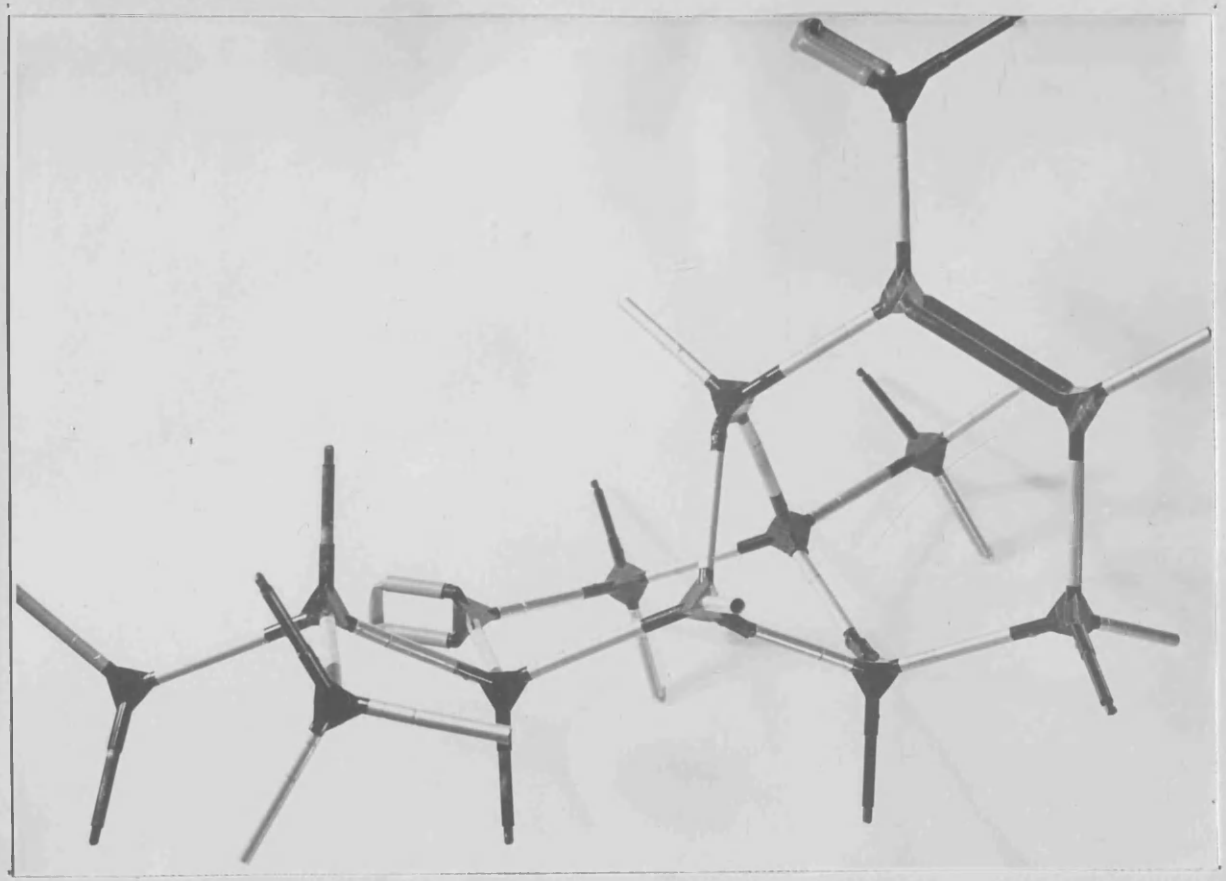
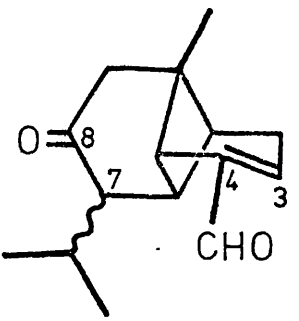


Figure 25(b)





78, 79

1.2. Conclusions

The evidence detailed in Part 1 of this thesis is presented in strong support of revised structures 78, 79 for the Brachylaenalones. The photographs shown in Figure 25 depict a model (Fieser) of the diastereomer with β-isopropyl, as viewed from different angles. According to the results of NMR analysis of C-8 deuterated Brachylaena diol (d) (p. 55), considered in conjunction with the results of CD analyses of the di-p-chlorobenzoates of Brachylaena diols (a)-(d) (p. 47), the 'A' series may be tentatively designated as the α-isopropyl series, and the 'B' series as the β-isopropyl series: the configurations of the Brachylaena diols may than be represented as follows, according to Table 10 (for the conformation of these compounds, see Figure 16).

Diol (a): α-isopropyl, β-sec-hydroxyl
Diol (b): β-isopropyl, β-sec-hydroxyl
Diol (c): β-isopropyl, α-sec-hydroxyl
Diol (d): α-isopropyl, α-sec-hydroxyl

Investigations into the effectiveness of complexes of paramagnetic lanthanide ions as shift reagents in NMR analyses of Brachylaena ketols A and B would provide a valuable extension to the above work. In addition,

successful reductions of the 3,4 double bonds of the Brachylaena diols, followed by formation of the di-*p*-chlorobenzoates of these compounds and selective hydrolyses of the primary esters, would afford a series of compounds from whose CD data unequivocal configurational assignments at C-7 and C-8 might possibly be derived.

A promising area for future work lies in the attempted preparation of crystalline derivatives suitable for X-ray structural determination from compounds within the Brachylaena series. It was hoped that the diol di-*p*-chlorobenzoates might have been useful in this context: to the present time, however, attempts to prepare crystalline samples of these compounds have been unsuccessful.

1.3. Experimental Procedure

General Remarks

Column chromatography was carried out on Woelm neutral alumina, deactivated with water to grade III. Merk "Kieselgel HF_{254}" was used for analytical thin layer chromatography (TLC) on 0.25 mm layers, and also for preparative work on 0.75 or 1.00 mm layers. Spots were detected by charring with ceric sulphate-sulphuric acid or by spraying with iodine and, in preparative TLC, bands were located by the method of examining separate 'lanes'. In the latter case, plates were checked for uniform running by observing suppression of the fluorescence of "HF_{254}" under brief irradiation.

Analytical gas-liquid chromatography (GLC) was carried out either on Pye Argon chromatographs (at constant temperature) or, more generally, on a Varian Aerograph Model 204 dual column gas chromatograph fitted with hydrogen flame-ionisation detectors (at constant or programmed temperature). Packings were prepared by the method of Horning *et al.*[94] Acid-washed and silanised Gas Chrom P, sieved to 100-120 mesh size (Applied Science Laboratories, Inc.), was used as the solid support. In indicating the stationary phases used, reference is made in Table15 to the dimensions of columns and to the

instruments on which they were employed.

Table 15

Abbreviation	Phase	Column Dimensions (length x I.D.)	Chromatograph
SE-30	Methylsiloxane polymer	4' x 4 mm	Pye Argon
OV-1	Methylsiloxane polymer (equivalent to SE-30)	10' x 3 mm	Aerograph 204
OV-17	50% phenyl methyl-siloxane polymer	4' x 4 mm	Pye Argon
OV-22	65% phenyl methyl-siloxane polymer	7' x 3 mm	Aerograph 204
QF-1	50% trifluoropropyl methylsiloxane polymer	4' x 4 mm	Pye Argon
XE-60	25% cyanoethyl methyl-siloxane polymer	7' x 3 mm	Aerograph 204
B34/DNP	Bentone 34/ dinonyl phthalate	4' x 4 mm	Pye Argon
"Carbowax" 20M	Polyalkylene glycol; M.W. 20,000	7' x 3 mm	Aerograph 204

The following additional stationary phases have been used in connection with combined gas chromatography-mass spectrometry (LKB 9000) and preparative GLC (Pye 105 preparative chromatograph).

Table 15 (ctd.)

Abbreviation	Phase
Ap. L.	"Apiezon L"
OV-210	50% trifluoropropyl methylsiloxane polymer (equivalent to QF-1)
OV-225	25% phenyl-25% cyanopropyl methyl-siloxane polymer (equivalent to XE-60).

Reagents used throughout were of BDH 'AnalaR' grade. 'Light petroleum' refers to the fraction of boiling point 60-80°, unless otherwise stated.

Melting points were recorded on a Kofler block. Optical rotations were measured in chloroform on a Perkin-Elmer 141 polarimeter.

Ultraviolet spectra were measured on an automatic recording instrument (Unicorn SP 800). Routine infrared spectra were obtained on a Unicam SP 200 or on a Perkin Elmer 257 model, while high resolution spectra were

measured on a Perkin Elmer 225 spectrometer. Mass spectra were normally measured by combined gas chromatography - mass spectrometry on an LKB 9000 instrument, and occasionally on an AEI MS 9 spectrometer. Nuclear magnetic resonance spectra were recorded on a Perkin Elmer R 10 spectrometer, a Varian T 60 model (60 MHz spectra), a Varian HA 100 model equipped with a spin decoupler (100 MHz spectra), or on a Varian HR 200 spectrometer (220 MHz)*.

As some of the reactions described in the experimental section were performed on a micro scale, characterisation of the products in these instances relies primarily on GC-MS data, rather than on elemental analyses. In the extraction of an aqueous reaction mixture with ether, the organic material was partitioned at least four times between the aqueous portion and ether, 1:2 v/v. The combined, concentrated ether extracts were washed several times with small portions of brine and dried over anhydrous magnesium sulphate: solvents were then completely removed in vacuo, usually below 45°. This procedure was generally followed, unless otherwise indicated.

* S.R.C. NMR Service, Imperial Chemical Industries Ltd. Petrochemical and Polymer Laboratory, Runcorn, Cheshire.

Thanks are due to Mr. B.S. Middleditch and Miss J. Johnston (GC-MS), Miss I. Ninian (Preparative GLC), Dr. J.S. Roberts (MS), Mrs. F. Lawrie and Mrs. A. McNiven (IR), Mr. J. Gall (NMR) and to Mr. J.M.L. Cameron and his assistants for microanalyses. Brachylaena hutchinsii wood was a gift from Dr. E.M. Wallace (Robinson, Dunn and Co.).

Finally, I gratefully acknowledge the assistance provided in the extraction of the heartwood of Brachylaena hutchinsii by the late Mr. G. Milmine and his staff.

Extraction of Brachylaena hutchinsii heartwood: Chromatography of the Heartwood Oil. Preparation of the Brachylaena Diols.

Dried, powdered heartwood of Brachylaena hutchinsii (2·4 kg) was extracted at room temperature for 2 days with ethyl acetate (23 litres). Evaporation of the solvent in vacuo at 30° afforded a brown oil (380 g), part of which (100 g) was chromatographed on alumina (4 kg) by gradient elution. 500 ml fractions were taken, and combined on the basis of TLC (Table 2, facing p.17). Fractions 19-27 (19·5 g) were shown to contain the Brachylaenalones, by comparison of IR and TLC data with those of authentic samples (provided by M.M. Campbell), and a portion of this combined fraction (9·7 g) was reduced by stirring with excess sodium borohydride in methanol (50 ml) at room temperature for 12 hours. Water (400 ml) was added, the mixture was extracted (x 3) with ether (400 ml) and the combined ethereal extracts, evaporated under reduced pressure to 250 ml, were washed (x 3) with small portions (50 ml) of brine. The solution was dried over anhydrous magnesium sulphate, concentrated in vacuo and, from the concentrate on trituration with light petroleum (40-60°), white crystals of diol (c) (300 mg) were obtained, of high purity, as estimated by TLC. The reaction was repeated on the remainder of the

fraction with similar results, and the crystalline diol which precipitated (230 mg) was added to that from the first experiment. Recrystallisation from ether gave material of m.p. 137-138·5°, $[\alpha]_D^{28}$ -27° (c, 0·7 in $CHCl_3$).

A portion (13 g) of the remaining reduction mixture was chromatographed on alumina (520 g) under conditions of gradient elution. 80 ml aliquots were collected, yielding the results detailed in Table 3 (facing p.17). The purity of each fraction was assessed by TLC, and the diols were identified by comparisons of their IR and TLC data with those of authentic compounds (M.M. Campbell). Diols (a) and (b), obtained as oils, crystallised from ether: diol (a) had m.p. 110·5-112·5°, $[\alpha]_D^{28}$ -55° (c, 1·0 in $CHCl_3$); diol (b) m.p. 106-108·5°, $[\alpha]_D^{28}$ +15° (c, 1·0 in $CHCl_3$).

I.R. absorption of the diols, ν_{max}($CHCl_3$; soluble with difficulty): 3620 (s), 1040 cm^{-1} (s); mass spectra: M^+ 236 ($C_{15}H_{24}O_2$ requires 236), different base peaks (see p.44); 100 MHz. NMR spectra were recorded (see p.55); chromatographic data are indicated below: retention indices[95] are quoted for OV-1, rather than for SE-30, as the former phase was found to afford a superior separation of diol (c) from diols (a) and (b).

Table 16

	R_f (ether)	$I^{156°}_{OV-1}$	$I^{156°}_{XE-60}$*
diol (a)	0·40	1810	2390
diol (b)	0·50	1810	2345
diol (c)	0·30	1825	2400

Preparation of the Brachylaenalones (1, 2) from the Brachylaena Diols (Sarett Oxidation[74])

Diol (c) [123 mg ex fraction 48, Table 3 (facing p.17)] was dissolved in dry pyridine (2 ml) and added in portions to a stirred suspension of chromium trioxide (2·3 g) in dry pyridine (23 ml). The reaction mixture was stirred for 30 minutes, water (350 ml) was added and the aqueous solution was extracted with ether to afford an oil (100 mg). TLC (ether): one product; GLC (3% SE-30, 176°) showed that the oil comprised ketoaldehyde A in 80% purity, together with a minor amount of starting material and two components of slightly higher retention times than the main product. Preparative TLC (ether) afforded pure Brachylaenalone A. Brachylaenalone B was obtained by similar treatment of diols (b) and (c).

High resolution IR spectra of the Brachylaenalones were obtained and compared (see p.17). $\frac{\varepsilon_{1686\ cm^{-1}}}{\varepsilon_{1717\ cm^{-1}}}$:

* this phase became available later (see p.44)

isomer A, 0·90; isomer B, 0·73; mass spectra: M^+ 232 ($C_{15}H_{20}O_2$ requires 232); 100 MHz NMR spectra were recorded (complex; cf. M.M. Campbell[81]); chromatographic data are indicated below.

Table 17

	R_f (ether)	$I^{125°}_{SE-30}$	$I^{156°}_{OV-1}$	$I^{156°}_{XE-60}$ *
Brachylaenalone A	0·70	1725	1765	1370
Brachylaenalone B	0·70	1760	1780	2395

The ketoaldehydes were found to decompose to a multicomponent mixture on standing in air at room temperature.

Preparation of Diol (c) Monoacetate (67)

Conditions were carefully selected to achieve complete conversion of diol (c) to its monoacetate, with formation of a minimum amount of diacetate. In a trial experiment, the diol was recovered unchanged from a reaction in which it was stirred with acetic anhydride (1·1 mole) in dry pyridine at 0°C. The reaction was repeated at room temperature and monitored by TLC: after 5 minutes, TLC indicated complete formation of mono-

* this phase became available later (see p. 44).

acetate alone, but on carefully neutralising the reaction with dilute mineral acid, the ester was hydrolysed to starting material. The reaction was repeated with addition of water in place of dilute acid in the extraction procedure: under these conditions, monoacetate of high purity was obtained.

The reaction was then attempted on a larger scale. To a solution of diol (c) (580 mg) in dry pyridine (15 ml), dry acetic anhydride (0·24 ml; 1·1 mole) was added slowly, and the reaction mixture was stirred at room temperature. TLC (ether) indicated complete conversion of diol (c) to the diol monoacetate (R_f 0·71) after 20 minutes. A negligible quantity of a component thought to correspond in R_f value (0·86) to the diacetate was also observed. Water (200 ml) was added and the reaction mixture was extracted with ether. Analytical TLC of the resultant oil (600 mg) now indicated that the reaction had been reversed to some extent during the extraction procedure. Preparative TLC (ether) afforded the monoacetate as an oily solid (180 mg) and GLC (1% OV-1, 150°; 3% OV-22, 197°) confirmed the purity of the product: $I_{OV-1}^{150°}$ 1905; ν_{max} (CCl_4): 3617(m), 2955(s), 2920(s), 2872(s), 2828(m), 1743(s), 1235(s), 1225(s), 1039(s), 1023 cm^{-1} (s); M^+ 278 ($C_{17}H_{26}O_3$ requires 278);

Diol (c) monoacetate was found to decompose on standing in air at room temperature. [Found: C, 73.34%; H, 9.27%; $C_{17}H_{26}O_3$ requires C, 73.35%; H, 9.42%.]

Preparation of Ketoacetate (68) from Diol (c) Monoacetate (67) (Sarett Oxidation)

A solution of diol (c) monoacetate [(67); 137 mg] in dry pyridine (10 ml) was added slowly to a stirred suspension of chromium trioxide (1·29 g) in dry pyridine (12 ml). The reaction mixture was stirred for 30 minutes. Water (300 ml) was added and the aqueous portion was extracted with ether to afford the ketoacetate as a colourless oil (125 mg). The purity of the product (R_f 0·47 in 80% light petroleum-ethyl acetate) was estimated by TLC: a neglible amount of starting material (R_f 0·20) was observed, and a component (R_f 0·73) of lower polarity than the ketoacetate, in apparently low concentration. GLC data of the ketoacetate: $I^{150°}_{OV-1}$ 1855, $I^{160°}_{OV-17}$ 2150; ν_{max} (CCl_4): 2956(s), 2924(s), 2880(s), 2820(m), 1744(s), 1720(s), 1689(m), 1226(s), 1023 cm^{-1} (m); mass spectrum (most abundant ions): m/e 276 [M^+; 16% ($C_{17}H_{24}O_3$ requires 276)], 233 (10%), 216 (14%), 132 (41%), 117 (76%), 105 (31%), 91 (43%), 43 (100%).

Bromine/acetic acid treatment of Ketoacetate 68

A solution of bromine in acetic acid (1·45 g/22·3 ml)

was prepared and, from this stock solution, a molar quantity of bromine (130 µl) was added slowly to a solution of ketoacetate 68 (14 mg) in glacial acetic acid (22 µl) containing a catalytic amount (2-3 µl) of 50% hydrobromic acid. The reaction mixture was left for $3\frac{1}{2}$ hours with occasional shaking, added to water (60 ml) and the organic products were extracted with ether. A dark brown oil (22 mg) was obtained. TLC (80% light petroleum-ethyl acetate) indicated a multi-component mixture, with two major products (R_f 0·65 and 0·58). These components (1·4 and 5·7 mg, respectively) were obtained by preparative TLC of the mixture and examined by IR. Component of higher R_f, ν_{max} (CCl_4): 2955(s), 2925(vs), 2754(s), 1732(m), 1260-90(m), 1122(w), 1074 cm^{-1} (w). Component of lower R_f, ν_{max} (CCl_4): 2960(s), 2930(m), 2873(m), 1732(s), 1270-90(s), 1122(m), 1074 cm^{-1} (m); ν_{max} (liquid film): 750 cm^{-1} (m).

Treatment of Ketoacetate 68 with Cupric Bromide/Dioxan (Glazier reaction[77])

Ketoacetate 68 (12 mg) was dissolved in dioxan (1 ml) and the mixture was refluxed for 40 minutes in the presence of cupric bromide (19 mg). Water (50 ml) was added and the reaction mixture was extracted (x 4) with an ether-ethyl acetate mixture (100 ml; 10:1, V/V). The combined

extracts were concentrated, washed with brine, dried and solvents were evaporated to yield an oil (26 mg). TLC (80% light petroleum-ethyl acetate) indicated a complex mixture of products, suggesting that this reaction offered no improvement on the previous method. The IR spectrum (liquid film) of the crude product was very similar to that of the main product from the last reaction.

Stability of Ketoacetate 68 to mild heat; its instability to strong mineral acid (50% hydrobromine acid/glacial acetic acid)

Ketoacetate 68 (1 mg) in glacial acetic acid (10 ml) was warmed in a water bath to 55°: TLC (80% light petroleum-ethyl acetate) of the solution after 5 minutes indicated no change in the constitution of the ketoacetate. The solution was cooled to room temperature and 50% hydrobromic acid/glacial acetic acid (2·5 ml) was added: the reaction mixture immediately turned deep pink; on re-examination by TLC, a complex mixture of products was observed, which remained unaltered on raising the temperature of the solution to 85°.

Pyridinium Hydrobromide Perbromide treatment of Ketoacetate 68

The reagent was freshly prepared according to the method of Fieser.[112] Ketoacetate 68 (11 mg) in glacial

acetic acid (750 µl) was treated with 1 equivalent (13 mg) of the pyridinium complex at 55° for 2 minutes, water (10 ml) was added and organic material was extracted as usual with ether. TLC examination (80% light petroleum-ethyl acetate) of the reaction products indicated a complex mixture, similar to those obtained from previous bromination reactions. The experiment was repeated in the presence of acetamide, to remove the hydrobromic acid produced in the reaction.

A solution of ketoacetate 68 (10 mg) and acetamide (4 mg: about 2 equivalents) in glacial acetic acid (750 µl) was warmed to 60°. 1 equivalent (12 mg) of pyridinium hydrobromide perbromide in glacial acetic acid (250 µl) was added at once, and the mixture was shaken well. A fine, white precipitate (acetamide hydrobromide) was observed almost immediately. After 20 minutes at 66°, the reaction mixture was added to water (10 ml) and extracted with ether to yield an oil which, on examination by TLC (80% light petroleum-ethyl acetate) was found to comprise two major components (R_f 0·73 and 0·32). These were separated by preparative TLC (7·4 and 1·8 mg, respectively) and examined by IR. Component of higher R_f, ν_{max} (liquid film): 1726(s), 1275-91(s), 1133(s), 1081(s), 758 cm^{-1} (m); a Beilstein test proved inconclusive. Component of lower R_f, ν_{max} (liquid film): 1740(s), 1669(s), 1227-53(s),

1042(m); $EtOH_{max}$ 230 nm (ε 7,650).

Lithium Chloride/Dimethylformamide treatment[78] of main compound from preceding experiment

To a solution of the main compound obtained from the last experiment (7·4 mg) in dimethylformamide (100 µl), lithium chloride (2·2 mg) was added, and the solution was refluxed at 80° under nitrogen for 3 hours. TLC (80% light petroleum-ethyl acetate; 100% benzene) indicated no change in the constitution of the starting material. Water (10 ml) was added and the reaction mixture was extracted with ether to afford a light brown oil (7·0 mg). Examination of GLC (I_{SE-30} 2570) and IR (liquid film) confimed the presence of starting material alone. The compound was purified by fractional sublimation, and submitted for microanalysis: found: C, 73·61%; H, 9·92%; required for $C_{17}H_{23}O_3Br$: C, 57·46%; H, 6·52%; required for $C_{24}H_{38}O_4$: C, 73·69%; H, 9·79%. Mass spectral analysis of the pure compound confirmed the absence of bromine: m/e 390 [M^+ ($C_{17}H_{23}O_3Br$ requires 354); 1%], 279 (32%), 167 (47%), 149 (100%), 113 (16%), 112 (13%), 104 (10%), 83 (11%), 71 (28%), 70 (26%), 57 (42%), 43 (32%); m* 100 (279 → 167); there was a notable absence of other peaks in the spectrum. These data were consistent with the structure of dioctyl phthalate.

The Impurity in the Ketoacetate (68)

A direct TLC comparison (80% light petroleum-ethyl acetate) between the major component from the most recent bromination experiment and the ketoacetate impurity of R_f 0·73 suggested that these compounds were identical, a postulate further supported by GLC (3% SE-30, temperature programming to 210°).

The ketoacetate was purified by preparative TLC (80% light petroleum-ethyl acetate), yielding its major impurity as 20% by weight of the crude material. The purity of this component was assessed by GLC on 3% SE-30 and 3% OV-22: in each case single components were observed: $I^{210°}_{SE-30}$ 2570, $I^{210°}_{OV-22}$ 1880. An IR spectrum of the material was obtained and found to be identical with that of the main compound from the last bromination experiment. Similarly, mass spectra were identical. A 60 MHz NMR spectrum of the ketoacetate impurity showed signals at τ: 2·4 (2H, quartet, J = 4 c/s), 5·85 (3H, doublet, J = 5 c/s), and absorption in the region 8·4 - 9·3 τ; insufficient material was available to afford a more useful spectrum. These data supported the proposed identity of this compound with dioctyl phthalate. Only the retention index recorded on OV-22 appeared anomalous. This was explained by postulating the presence of a small amount of a lower alkyl phthalate in the sample.

DDQ[80] treatment of purified Ketoacetate (68)

Ketoacetate 68 (4·6 mg) and 2,3-dichloro-5,6-dicyano-1,4-benzoquinone (DDQ) [6·0 mg (1½ molar proportions)] were refluxed in benzene (1 ml) under nitrogen for 11 hours. The resultant yellow solution was filtered from a precipitate of the hydroquinone and evaporated to dryness, yielding a light brown residue which was dissolved in ether and examined by TLC (80% light petroleum-ethyl acetate). Three main spots were observed, two of which (R_f 0·32 and 0·52) corresponded to reaction products, and the third of which (R_f 0·47) represented unreacted starting material. Bands corresponding to these spots were separated by preparative TLC. As the α, β-unsaturated ketoacetate (69) was expected to be more polar than the ketoacetate (68), material from the band of lower R_f value (0·3 mg) was examined. GLC (3% OV-22) revealed two main components ($I^{197°}_{OV-22}$ 2150 and 2310), one of which (I 2310) gave M^+ 274 [GC-MS; $C_{17}H_{22}O_3$ (69) requires 274]. The crude material had λ^{EtOH}_{max} 232 nm (calculated for 69:239 nm). It was considered possible that the component of M^+ 274 had arisen by dehydrogenation with associated cleavage of the cyclobutanoid ring).

The reaction was repeated in the presence of excess DDQ, and then with excess DDQ in a sealed tube at 200° for 3 hours, but in neither case was the required product

obtained. Similarly, when the reaction was repeated in the presence of dioxan or chlorobenzene as solvent, no material of molecular weight 274 was produced.

It was concluded that the ketoacetate was almost completely resistant to DDQ treatment.

Acid Hydrolysis of Ketoacetate 68

A solution of ketoacetate 68 (1·7 mg) in absolute ethanol (0·43 ml) was prepared, and concentrated HCl (20 µl) was added to give a 0·6 N solution. The solution was shaken well. After 15 minutes, a portion (10 µl) of the reaction mixture was removed, diluted with ether (~1 ml), and small portions of sodium bicarbonate were added until a basic solution was obtained. The solution was dried over anhydrous magnesium sulphate, concentrated under nitrogen to about 20 µl, and examined by GLC on 1% OV-17 at 150°: the presence of only starting material (t_R 15·8 minutes) was evident. 1¼ Hours after the initial addition of HCl, further HCl (20 µl) was added to give a 1·2 N solution, and 10 µl of the reaction mixture was immediately removed and examined as before: two products of retention times (t_R 11·3 and 12·0 minutes) greater than that of the starting material were observed. After 3½ hours, the reaction was half-way to completion and, after 21 hours, no ketoacetate remained. The reaction mixture was neutralised with sodium bicarbonate, dried over anhydrous magnesium sulphate, and concentrated to dryness under nitrogen. The products (0·7 mg) were dissolved in ether (0·35 ml) and further chromatographic data were obtained. TLC (70% light petroleum-ethyl acetate): com-

ponents of R_f 0·18 and 0·26 (cf. ketoacetate 68, 0·32); $I^{160°}_{OV-1}$: two components at ~1790 (cf. ketoacetate 68, 1855); $I^{160°}_{XE-60}$: components at 2285 and 2295 (cf. ketoacetate 68, ~2295). UV (absolute ethanol): negligible absorption.

The reaction was repeated in aqueous ethanol to investigate the possibility that hemiketals (72, 73) had been produced in the last reaction. Ketoacetate 68 (0·3 mg) was dissolved in a mixture of absolute ethanol (35 µl) and water (35 µl). Concentrated HCl (7 µl) was added and, after 20 hours, the reaction mixture was worked-up as before. GLC examination of the products (1% OV-1; 0·5% XE-60) indicated the presence of components identical to those obtained from the last reaction.

A portion (0·2 mg) of the product mixture was dissolved in anhydrous pyridine (5 µl), anhydrous acetic anhydride (10 µl) was added, and the solution was warmed for 30 minutes. Solvents were evaporated under nitrogen, ether (0·1 ml) was added, and the reaction products were examined by TLC (70% light petroleum-ethyl acetate) and by GLC on OV-1 and XE-60. On TLC, a single spot was observed, corresponding in R_f value to ketoacetate 68; GLC also indicated one component: the peaks observed on OV-1 and XE-60 were of retention indices identical to those of

ketoacetate 68.

Base Hydrolysis of Ketoacetate 68

$^{N}/_{10}$ Sodium hydroxide (~0·2 ml) was added to a solution of ketoacetate 68 (0·5 mg) in absolute ethanol (0·5 ml), and the mixture was shaken and left for 20 minutes. The solution was then acidified to pH 6-7 by careful addition of dilute HCl (reaction of the keto-acetate in dilute mineral acid was known to be very slow), dried over anhydrous magnesium sulphate, filtered and examined by TLC and GLC (1% OV-1 and 0·5% XE-60), which indicated the presence of components identical to those obtained from mineral acid treatment of ketoacetate 68.

Preparation of Diastereomeric Ketols 76, 77

Treatment of ketoacetate 68 with mineral acid was repeated on a larger scale and, on this occasion, 19 mg of starting material afforded 13 mg of a mixture of products which were separated by careful preparative TLC (70% light petroleum-ethyl acetate). The following yields were recorded: component of lower polarity, 3·2 mg; component of higher polarity, 2·7 mg. The purities of both compounds were confirmed by GLC analyses (1% OV-1 and 0·5% XE-60), and chromatographic data were correlated: the component of lower mobility on silica gel corresponded

to the component of higher retention index on XE-60, and the component of higher mobility on silica gel to that of lower retention index on XE-60. $\nu_{max}(CCl_4)$: component of lower polarity, 3611(m), 2958(s), 2929(s), 2900(s), 2831(m), 1718 cm^{-1}(s); component of higher polarity, 3617(m), 2960(s), 2924(s), 2879(s), 2834(m), 1718 cm^{-1}(s); mass spectra (most abundant ions): component of lower polarity, m/e 234 [M^+; 6% ($C_{15}H_{22}O_2$ requires 234)], 216 (8%), 192 (19%), 177 (8%), 173 (13%), 166 (14%), 161 (19%), 159 (12%), 150 (27%), 145 (17%), 135 (39%), 132 (28%), 131 (26%), 119 (100%), 117 (61%), 108 (60%), 105 (55%), 91 (62%), 79 (57%), 77 (50%), 55 (56%), 43 (56%), 41 (95%); component of higher polarity - mass spectrum almost identical with above, but no peak at m/e 166.

Each isomer was separately re-acetylated on a micro scale with acetic anhydride/pyridine, and the respective ketoacetates were obtained. Mass spectra of these compounds were recorded (most abundant ions are indicated): derivative from ketol of lower polarity, m/e 276 [M^+; 2% ($C_{19}H_{20}O_4$ requires 276)], 233 (5%), 216 (19%), 201 (7%), 188 (4%), 174 (13%), 173 (21%), 166 (9%), 159 (15%), 146 (12%), 145 (20%), 132 (49%), 131 (36%), 117 (94%), 108 (22%), 105 (36%), 91 (47 %), 79 (18%), 77 (21%), 69 (17%), 67 (9%), 65 (11%), 55 (18%), 43 (100%), 41 (40%);

derivative from ketol of higher polarity-spectrum identical with that of ketoacetate 68, and almost identical with above but, as in the spectrum of the parent ketol, no peak was observed at m/e 166.

Epimerisation of the Brachylaenalones

A solution of Brachylaenalone B (1 mg) in absolute ethanol (1 ml) was prepared, and the purity of the solute was confirmed by GLC on 1% OV-1 and 0·5% XE-60. $^{N}/_{10}$ Sodium hydroxide (~0·2 ml) was added, and the solution rapidly assumed an intense pink coloration. After 30 minutes, 6 N HCl was added carefully to pH 7, when the solution again became colourless. The solution was dried over anhydrous magnesium sulphate, filtered and examined by GLC on OV-1 and XE-60: an epimeric mixture of Brachylaenalone A: Brachylaenalone B = 4:3 was observed.

Similarly, when a solution of Brachylaenalone A was treated with HCl, slow epimerisation to isomer B was recorded.

Preparation of Ketols 76, 77 from a Natural Source

Examination by GLC (1% OV-1 and 0·5% XE-60) of combined fractions 30-32 (an oil) from column chromatography of the heartwood of Brachylaena hutchinsii (Table 2) indicated that these comprised predominantly the ketol of lower

polarity. An IR spectrum (liquid film) of fractions 30-32 supported this postulate. An experiment in which a small quantity of the oil was treated with base, neutralised and then subjected to preparative TLC (70% light-petroleum-ethyl acetate) afforded poor yields of pure ketols, as the difficult TLC separation was further complicated by the presence of impurities in the starting material. It was necessary, then, initially to purify the isomer present in the above chromatographic fraction.

Preparative TLC (60% light petroleum-ethyl acetate) of a portion (430 mg) of fractions 30-32 afforded the less polar ketol (170 mg) in high purity, as estimated by GLC (OV-1 and XE-60). The apparently poor yield achieved in this separation was attributed to the presence in the starting material (an oil) of a consideration proportion of trapped solvent.

A solution of the pure ketol of lower polarity (170 mg) in absolute ethanol (10 ml) was prepared, and $^{N}/_{10}$ sodium hydroxide (0·5 ml) was added. The solution was left for 17 hours, acidified to pH 6-7, and solvent was removed in vacuo. Ether (40 ml) was added, and the ethereal solution was washed (x 5) with water (5 ml) until the washings were neutral to litmus. The solution was further washed (x 2) with brine (5 ml), dried over anhydrous magnesium sulphate, and examined by GLC (OV-1 and XE-60):

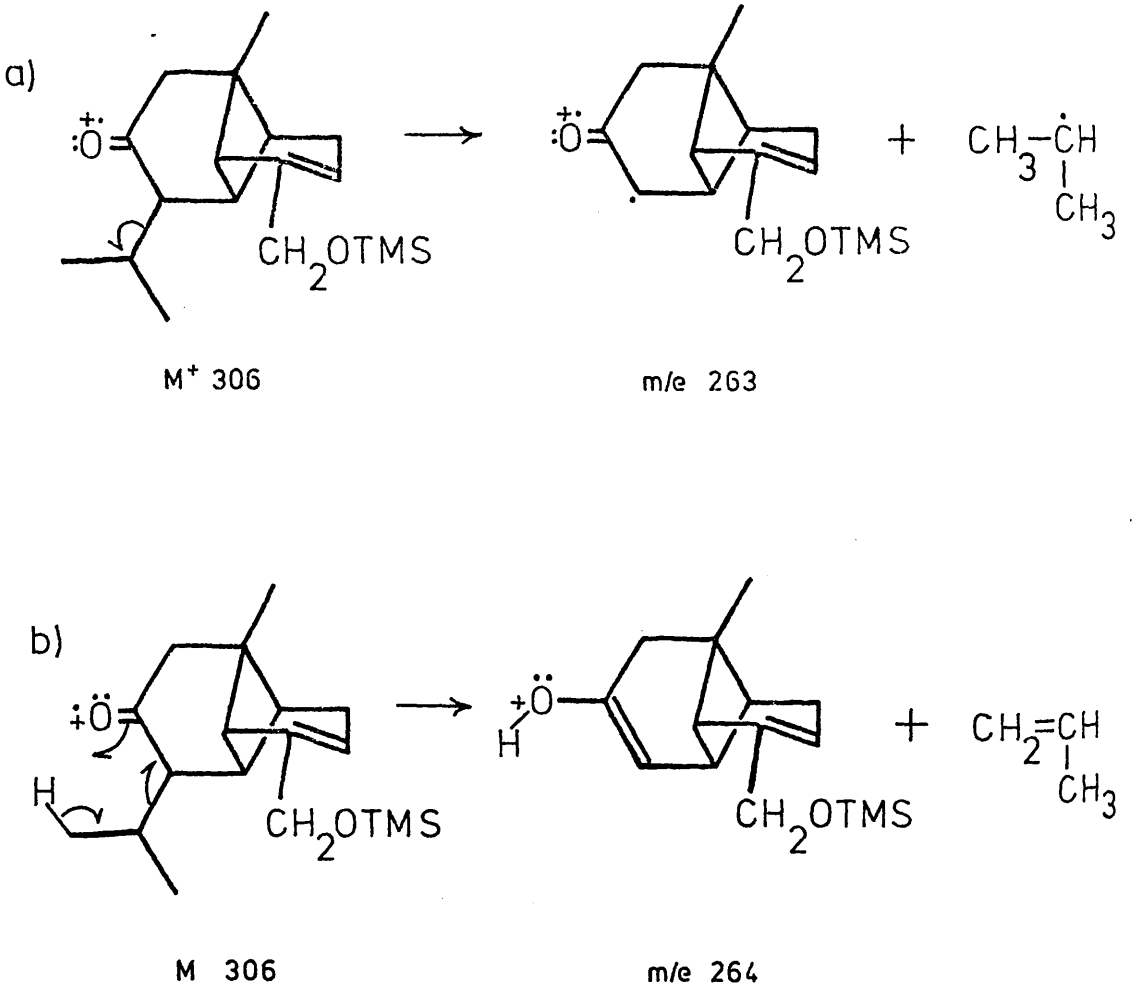
the presence of approximately equal proportions of both ketols was observed. Preparative TLC of this mixture (60% light petroleum-ethyl acetate) afforded pure samples of both isomers (GLC on XE-60): less polar ketol, 70 mg; more polar ketol, 30 mg. These compounds were sublimed for microanalyses. [Found: ketol of lower polarity, C, 76·81%; H, 9·59%; ketol of higher polarity, C, 77·04%; H, 9·41%. $C_{15}H_{22}O_2$ requires: C, 76·91%. H, 9·46%.] Cotton effect data were recorded: ketol of lower polarity, small negative Cotton effect: ΔE - 0·27 at 306 nm, a = -10·6; ketol of higher polarity, small positive Cotton effect: ΔE + 0·43 at 304 nm, a = +17·6.

Preparation of Trimethylsilyl Derivative of the Ketol of Lower Polarity

A solution of the ketol of lower polarity (2·6 mg) in anhydrous pyridine (30 µl) was treated with hexamethyldisilazane (40 µl) and a catalytic amount (5 µl) of trimethylchlorosilane. The solution was warmed for 30 minutes, solvents were removed under nitrogen, ether (1·0 ml) was added, and the mixture was well shaken and filtered through cotton-wool and cellulose powder. Examination of the product by GLC (1% OV-1 and 0·5% XE-60) indicated it to be of high purity. Mass spectrum (most abundant ions): m/e 306 [M^+ ; 7% ($C_{18}H_{30}O_2Si$ requires 306)], 291 (4%),

Figure 26

Mass Spectral Fragmentation of the Trimethylsilyl Derivative of the Less Polar Ketol: m/e 263 and 264



278 (2%), 263 (4%), 238 (6%), 223 (11%), 222 (10%), 216 (20%), 181 (12%), 173 (18%), 156 (13%), 145 (14%), 132 (28%), 117 (41%), 105 (31%), 103 (22%), 91 (42%), 75 (54 %), 73 (100%), 41 (38%). Of the peaks at m/e 263 (M^+ - 43) and m/e 264 (M^+ - 42), it is significant that the former is present in predominance. This observation suggests that in the structure of this compound, elimination of an isopropyl radical occurs more favourably than β-keto cleavage with associated γ-hydrogen rearrangement [Fig. 26, (a) and (b), respectively].

Preparation of Trimethylsilyl Derivative of Copaenol

A solution of copaenal [(80); 5 mg] in ether (~ 5 ml) was treated with excess lithium aluminium hydride and stirred for 25 minutes. Saturated sodium sulphate solution was then added dropwise until no further effervescence was observed. The ether solution was dried over anhydrous magnesium sulphate, filtered through cotton wool and cellulose powder, and concentrated to dryness in vacuo. GLC examination (1% OV-1 and 0·5% XE-60) of the product (5 mg) confirmed its purity.

A portion (1·6 mg) of this material, assumed (on account of its GLC behaviour) to be copaenol, was dissolved in anhydrous pyridine (40 µl), and hexamethyldisilazane (40 µl) was added, followed by a catalytic amount (5 µl)

of trimethylchlorosilane. The solution was warmed for 1 hour, and the trimethylsilyl derivative of copaenol was recovered in the usual manner and in high purity (GLC on OV-1 and XE-60).

Sarett[74] Oxidation of Ketols 76,77

A solution of the ketol of higher polarity (1 mg) in anhydrous pyridine (0·2 ml) was added to a suspension of chromium trioxide (13 mg) in pyridine (0·2 ml), and the mixture was shaken for 40 minutes. Water (1 ml) was added, and the reaction was worked-up in the usual manner to yield a product of chromatographic behaviour identical to those of Brachylaenalone B : R_f (ether) 0·70; $I^{156°}_{OV-1}$ 1780; $I^{156°}_{XE-60}$ 2395. Similar oxidation of the ketol of lower polarity afforded a product of R_f (ether) 0·70, $I^{156°}_{OV-1}$ 1765, $I^{156°}_{XE-60}$ 2370, corresponding to Brachylaenalone A.

Sodium Borohydride Reduction of Ketols 76, 77

The ketol of higher polarity (0·5 mg) was dissolved in methanol (1 ml), and excess sodium borohydride was added to the solution. The mixture was shaken for 45 minutes, water (3 ml) was added, and reaction products (0·4 mg) of chromatographic data identical to those of Brachylaena diols (b) and (c) were obtained by extraction with ether: R_f (ether) 0·50 and 0·30; $I^{156°}_{OV-1}$ 1810 and 1825; $I^{156°}_{XE-60}$ 2345 and 2400. Similarly, two products were obtained by reduction of the ketol of lower polarity. One of these corresponded in chromatographic behaviour to diol (a): R_f (ether) 0·40; $I^{156°}_{OV-1}$ 1810; $I^{156°}_{XE-60}$ 2390. The second component had R_f (ether) 0·40, $I^{156°}_{OV-1}$ 1810, $I^{156°}_{XE-60}$ 2335.

Both mass spectra showed M^+ 236 (see p. 44). These compounds were separated by careful preparative GLC on 1% OV 225, and their purities were estimated by GLC analyses on 0·5% XE-60. $[\alpha]_D^{25}$: former component, -52° (c, 1·0 in $CHCl_3$); second component, -31° (c, 1·0 in $CHCl_3$). IR spectra ($CHCl_3$; soluble with difficulty) were obtained: identical positions of absorption were recorded, with a prominent peak at 3620 cm^{-1} in both spectra; a close similarity of the spectra with those of diols (b) and (c) was observed.

It was concluded that in the reduction of the ketol of lower polarity, Brachylaena diols (a) and (d), of identical isopropyl configurations, had been produced. These pure compounds were not, however, obtained in a crystalline state.

Preparation of C-8 Deuterated Brachylaena Diols (a) and (d)

A solution of ketol A (130 mg) in sodium-dried ether (5 ml) was treated with excess lithium aluminium deuteride. The reaction mixture was stirred for 90 minutes. Saturated aqueous sodium sulphate was added dropwise until no more effervescence was observed, anhydrous magnesium sulphate was added, and the mixture was filtered through cotton wool and cellulose powder. Solvent was removed

from the filtrate *in vacuo*, yielding an oil (131 mg) which was examined by GLC on 1% OV-1 and 0·5% XE-60: no starting material remained, and two products of retention times similar to those of Brachylaena diols (a) and (d) were observed in approximately equal proportions. Attempted preparative GLC separation of these components on 1% OV-225 afforded only diol (d) in a pure state, due to an instrumental fault. A mass spectrum of C-8 deuterated diol (d) was recorded (see p. 45).

Preparation of Brachylaena Diol (b) Dibenzoate

Brachylaena diol (b) (51 mg) was dissolved in anhydrous pyridine (0·6 ml), and benzoyl chloride (150 µl: 6 molar proportions) was added: the solution immediately assumed a deep reddish brown coloration. The reaction mixture was refluxed for $2\frac{1}{2}$ hours, and then cooled. Water (4 ml) and saturated aqueous sodium bicarbonate (1 ml) were added to the mixture, and organic material was extracted with ether to yield an oil (150 mg), which was expected to contain some solvent. Examination by TLC (90% light petroleum-ethyl acetate) indicated a predominance of a product of R_f 0·41: no starting material was observed. Preparative TLC afforded the component of R_f 0·41 (75 mg) in a pure state, and its IR and mass spectra (pp. 48 and 49) respectively, were consistent with the formation of diol

(b) dibenzoate. Sublimation of the compound yielded a glass (Found: C, 78·16%; H, 7·15%; required for $C_{29}H_{32}O_4$: C, 78·34%; H, 7·26%). λ_{max}^{hexane} 229 nm (ε 20,400).

Preparation of Brachylaena Diol (b) Di-p-Chlorobenzoate

To a solution of Brachylaena diol (b) (37 mg) in anhydrous pyridine (0·4 ml), p-chlorobenzoyl chloride (70 µl: 3 molar proportions) of high purity, as estimated by GLC on 0·5% XE-60 at 55°* was added. The resultant deep yellow-brown solution was refluxed for 2 hours. The reaction mixture was cooled, water (5 ml) and saturated aqueous sodium bicarbonate (1 ml) were added, and organic material was extracted with ether to afford a semi-solid mass (70 mg ; containing crystalline p-chlorobenzoic anhydride), which was examined by TLC (90% light petroleum-ethyl acetate). A major product of R_f 0·50 was observed: no starting material remained. Preparative TLC afforded the major component (21 mg) in high purity, and its IR and mass spectral data were consistent with those expected for diol (b) di-p-chlorobenzoate. In particular, the presence of two chlorine atoms in the molecular structure was confirmed by the presence of isotope peaks in the mass spectrum. (Intensities

* It was expected that on XE-60 at this temperature, p-chlorobenzoyl chloride would be distinguishable from its corresponding o- and m- isomers, and from benzoyl chloride.

of molecular ions at m/e 514 and 516, relative to m/e 512: m/e 514, 70%; m/e 516, 16%; calculated for two chlorine atoms: m/e 514, 65%; m/e 516, 11%). A glass was obtained on purification of the product by sublimation. (Found: C, 67·71%; H, 5·95%; required for $C_{29}H_{30}O_4Cl_2$: C, 67·85%; H, 5·90%). λ_{max}^{hexane} 240·5 nm (ε 85, 100).

The corresponding pure di-p-chlorobenzoates of Brachylaena diols (a), (c) and (d), of R_f values (90% light petroleum-ethyl acetate) 0·48, 0·55 and 0·55, respectively, were obtained in a similar manner. (Found for diol (c) di-p-chlorobenzoate: C, 67·69%; H, 5·78%; required for $C_{29}H_{30}O_4Cl_2$: C, 67·85%; H, 5·90%. Insufficient quantities of the corresponding derivatives of diols (a) and (d) were available to allow microanalyses).

Wolff-Kishner Reduction of Brachylaenalone A

Anhydrous diethylene glycol (0.8 ml) was added to Brachylaenalone A (68 mg) in a Carius tube, together with pellets of potassium hydroxide (58 mg: 6 equivalents), dried first between filter papers. Anhydrous hydrazine was prepared by refluxing 100% hydrazine hydrate over sodium hydroxide for 12 hours, and distilling that fraction corresponding to pure hydrazine into a flask containing sodium hydroxide. A portion (1 ml) of this pure reagent was transferred to the tube, which was then cooled in liquid nitrogen, sealed, surrounded by a metal casing, and placed in an oven at 210° for 5 hours. The pale yellow solution of products was diluted with water and extracted with ether; the combined extracts were concentrated *in vacuo*, washed with small portions of water until the washings were neutral to litmus, dried over anhydrous magnesium sulphate and concentrated to dryness *in vacuo* at room temperature to yield a green-yellow gum (29 mg; expected to contain a small proportion of diethylene glycol). TLC (90% light petroleum-ethyl acetate): main products of R_f 0.12, 0.25, 0.44, 0.80; no starting material remained. GLC on 3% SE-30: components of retention indices 1310, 1390, 1410, 1490, 1500, 1900, 1920 (Fig. 21). Preparative TLC afforded the hydrocarbon

fraction of R_f 0.80, comprising components of I_{SE-30} 1310 to 1510 (Fig. 22). Mass spectra of these components were obtained (see p. 63). The area under peak 2 (Fig. 22), as a percentage of the total area of peaks in the chromatogram of this hydrocarbon fraction, was estimated by obtaining a Xerox copy of the chromatogram, cutting out the observed peaks, obtaining the total weight of these cuttings, and comparing this value with the weight of the cutting corresponding to peak 2. Retention times (minutes) of components of this fraction on 5% Bentone 34/5% dinonyl phthalate at 100°: 29.3, 31.3, 36.3 (ylangene), 37.6 (copaene), 41.6, 48.6, 52.3.

Preparation of Brachylaenalone A Bis-Ethylenethioacetal

A solution of Brachylaenalone A (65 mg) in boron trifluoride etherate (48% w/v BF_3; 250 μl) was treated with ethanedithiol (200 μl), added in small portions (50 μl) over a period of 10 minutes. The solution was shaken after each addition, and then shaken periodically for 2 hours. The reaction mixture was examined by TLC (80% light petroleum-ethyl acetate): main products of R_f values 0.59 and 0.78 were noted; components of R_f 0.53 and 0.73 were also observed. After 5 hours, water (5 ml) and dilute sodium carbonate (to pH ~ 7) were added to the mixture, which was extracted with ether to yield an oil

(170 mg; expected to contain some ethanedithiol). Examination by TLC again indicated the presence of main components of R_f values 0.59 and 0.78. These compounds were purified by preparative TLC, and the results of this separation were analysed by TLC comparison (60% chloroform-benzene) with the crude product: the components isolated appeared to be of high purity.

Brachylaenalone B was treated in a manner identical to the above, yielding corresponding products of R_f values (80% light petroleum-ethyl acetate) 0.53 and 0.78. From these results, it appeared that the crude product from Brachylaenalone A had contained a minor proportion of the main product of higher polarity from Brachylaenalone B (*cf.* R_f values). If, however, the less polar major product from each Brachylaenalone was considered to be the monoethylenethioacetal resulting from substitution at the ketone, then this observation was explainable in terms of epimerisation (to only a small extent) at the isopropyl centre in the presence of the Lewis acid, boron trifluoride.

IR and mass spectra of the major products derived from the Brachylaenalones were recorded (pp.66 and 67).

Reduction of Brachylaenalone A Bis-Ethylenethioacetal

To a solution of the bis-ethylenethioacetal of Brachylaenalone A (3.8 mg) in absolute ethanol (1.5 ml), excess Raney nickel of grade W2, prepared according to the method of R. Mozingo,[91] was added. The mixture was refluxed for 4½ hours, cooled and filtered through cotton wool and Celite 535, which retained the catalyst. The filtrate was examined by GLC (1% OV-1; 5% Bentone 34/5% dinonyl phthalate) and by GC-MS (1% OV-1), yielding the results described in the Discussion.

Reductions of Ylangene over Adams Catalyst

Preparative TLC (100% light petroleum) of a sample (33 mg) of ylangene (R_f 0.71) afforded ylangene of high purity (17 mg), as judged by GLC on 1% OV-1 and 0.5% XE-60, under conditions of programmed temperature.

Platinum oxide (0.8 mg) and glacial acetic acid (2 ml) were placed in the hydrogenation flask, and the catalyst was activated as described on page 69. A solution of ylangene (8.2 mg) in glacial acetic acid (200 µl) was injected into the flask, using a further small volume (50 µl) of acetic acid to transfer the last traces of the hydrocarbon. After a short induction period, rapid consumption of hydrogen was observed but, 20 minutes after the start of the reduction, a considerable decrease in the

rate of absorption of hydrogen was noted. $1\frac{1}{2}$ hours later, the reaction appeared to be complete. The level of mercury in the capillary tube of the valve was adjusted to its original position by addition of water (0.8 ml) from the burette. A further solution of ylangene (5 mg) in glacial acetic acid (100 μl) was injected into the reaction flask, and a small volume (50 μl) of acetic acid was used to transfer the last traces of ylangene, as before. After 2 hours, when no further absorption of hydrogen was apparent, water (0.5 ml) was again added from the burette to adjust the level of mercury in the valve to its position at the start of the reaction. Atmospheric temperature (23°) and pressure (745 mm mercury) were recorded, and the partial pressures of water (21 mm) and acetic acid (14 mm) under the conditions of the reduction were estimated graphically. Application of the equation given on p. 70 then afforded the following results.

First Reduction:

0.0392 mmoles ylangene reduced;

0.0404 mmoles hydrogen consumed.

Second Reduction:

0.0245 mmoles ylangene reduced;

0.0250 mmoles hydrogen consumed.

The acetic acid solution of the reaction product was

filtered through cotton wool, and examined on OV-1 and XE-60 at 88°: a peak of higher retention index (1392) than that of ylangene (1352) was observed, and the presence of a negligible amount of starting material was indicated.

The solution was adjusted to about pH 8 by addition of concentrated aqueous sodium bicarbonate, and saturated with sodium chloride. The aqueous mixture was extracted with ether, and the combined, dried extracts were concentrated in vacuo at room temperature. Traces of acetic acid were removed by azeotropic distillation with benzene in vacuo at room temperature, to yield an oil (6 mg), which was examined by GLC on OV-1 and XE-60: results identical to the above were obtained. Examination of the material on 5% Bentone 34/5% dinonyl phthalate at 100° indicated a homogeneous product. IR (liquid film), most prominent absorptions: 2984, 1475, 1393, 1381 cm^{-1} [negligible absorption at about 785 cm^{-1} (C-H out-of-plane deformation in $R_2C=CHR$)].

Reductions of Brachylaena Diol (c) over Adams Catalyst

Platinum oxide (2.1 mg) and glacial acetic acid (2 ml) were placed in the hydrogenation flask, and the catalyst

was activated in an atmosphere of hydrogen, according to the method indicated on page 69. A solution of Brachylaena diol (c) (27.2 mg) in glacial acetic acid (200 μl) was then injected into the stirred suspension of catalyst, using further small portions of solvent (2 x 50 μl) to transfer the last traces of diol. The reaction mixture was stirred for 2½ hours, by which time absorption of hydrogen appeared to be complete. Water (2.4 ml) was added carefully from the burette to replace the hydrogen consumed, and a calculation showed that 0.102 mmoles of hydrogen had been used to reduce 0.115 mmoles of reactant.

A further solution of Brachylaena diol (c) (12.2 mg) in acetic acid (200 μl) was added to the hydrogenation flask, and the above procedure was repeated. Absorption of hydrogen in this experiment, however, appeared to be very slow and when, after 2½ hours, the pressure in the apparatus was still falling, it was decided to terminate the reaction. Water (0.7 ml) was added from the burette to increase the pressure in the apparatus to its original value, and the contents of the hydrogenation flask were then filtered through cotton wool. The filtrate was examined by GLC on 0.5% XE-60 under conditions of programmed temperature: five main components were evident.

Water (5 ml) was added to this solution, followed by dilute aqueous hydroxide to pH 7-8. The solution was saturated with sodium chloride, and extracted with ether. Traces of acetic acid were removed from the combined, dried, concentrated extracts by azeotropic distillation with benzene *in vacuo* at about 35°, to yield a partially crystalline oil (~30 mg). Re-examination of the product mixture by GLC on XE-60 yielded results identical to those recorded before the extraction procedure (see p. 74). Mass spectra of the main components of the mixture were obtained (see Discussion).

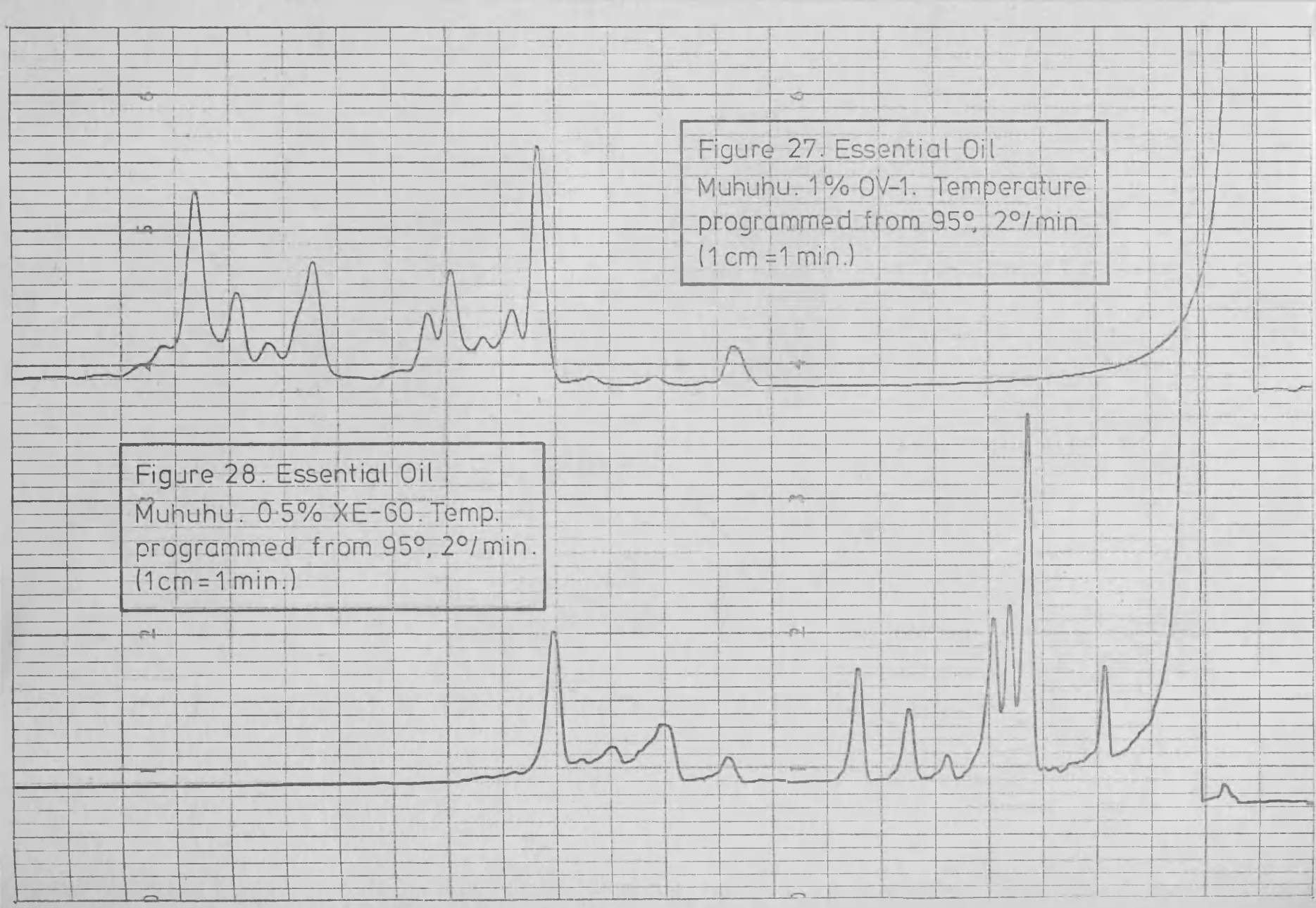


Figure 27. Essential Oil Muhuhu. 1% OV-1. Temperature programmed from 95°, 2°/min (1 cm = 1 min.)

Figure 28. Essential Oil Muhuhu. 0·5% XE-60. Temp. programmed from 95°, 2°/min. (1cm = 1 min.)

Part 2: Chemical Constituents of the Steam - Volatile Oil from Brachylaena hutchinsii (Essential Oil Muhuhu‡)

2.1. Discussion

A brief investigation into the chemical constituents of Essential Oil Muhuhu is described. Separations of the oil into fractions by GLC (analytical and preparative), together with combined GC-MS analyses※ of individual fractions, supplemented occasionally by IR spectrometry, has allowed the documentation of molecular weights and functional types present in the oil.

Preliminary Examination of the Oil

The oil was first examined by GLC on 1% OV-1 and on the highly polar phase, 0.5% XE-60, under conditions of programmed temperature, affording the results shown in Figures 27 and 28, respectively. On the phase OV-1, components of retention indices approximately 1350-1750

‡ R.C. Treatt and Co., 19 Watling Street, London, E.C.4. (see p. 14)

※ All mass spectra are reproduced in order in Appendix 2.

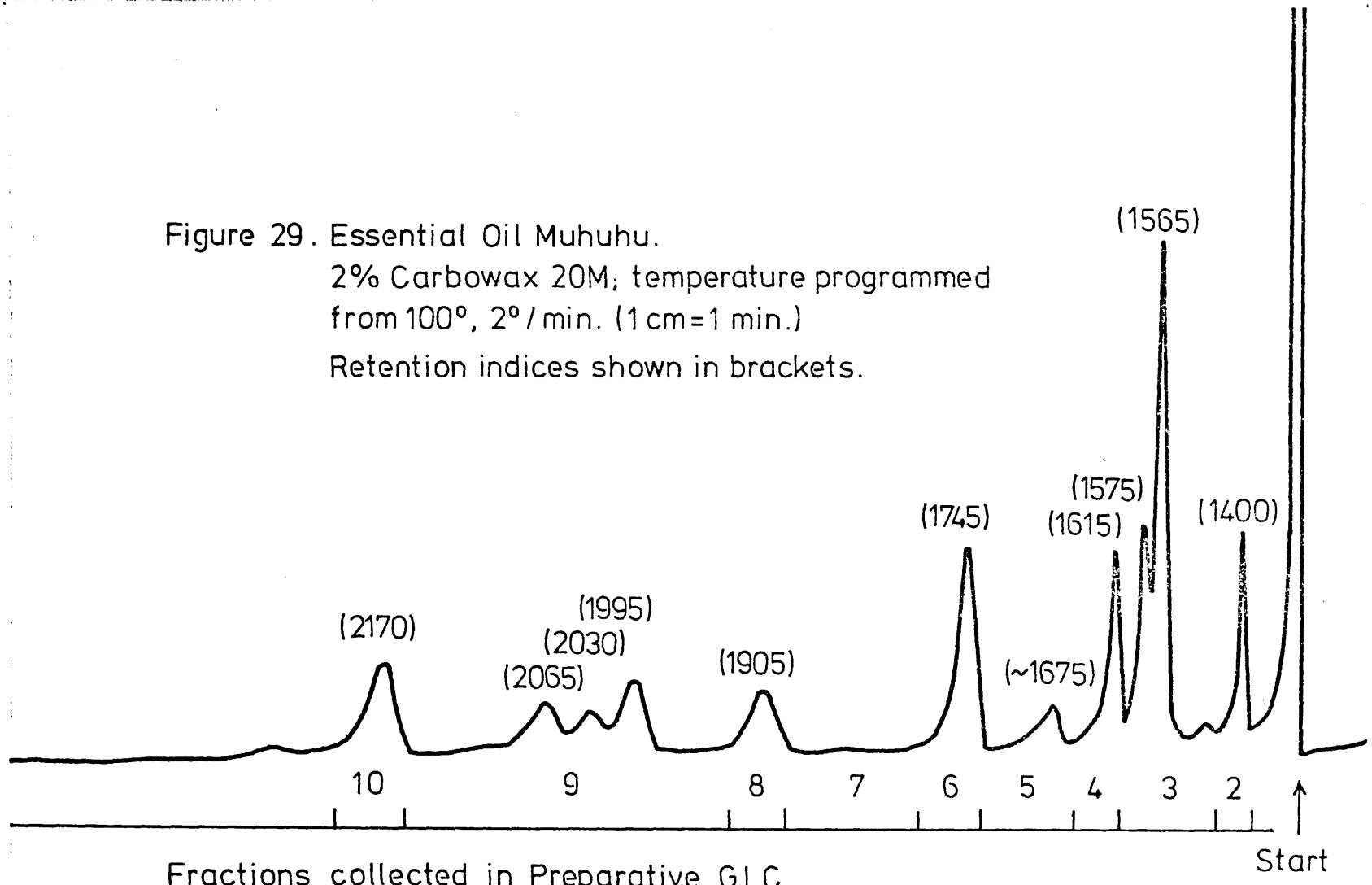
were observed: a superior separation of these components on XE-60, however, was apparent, and prominent peaks of retention indices approximately 1400-1900 were recorded.

Attempted formation of trimethylsilyl derivatives from the total oil afforded results which were difficult to interpret, due to the complexity of the oil: it appeared, however, that derivatives had been formed from several components of retention indices higher than 1600 on XE-60. Similarly, attempted formation of O-Me oximes from the total oil yielded ambiguous results, but the possible aldehydic/ketonic character of certain components of retention indices above 1700 on XE-60 was inferred.

Preparative GLC Separation of the Oil

Attention was directed towards a preparative GLC separation of the oil into fractions suitable for further analyses, and chromatograms of the mixture on QF-1 (of polarity slightly lower than that of XE-60, and of particular use in the separations of alcohols and ketones) and on Carbowax 20M (widely used to achieve separations of alcohols, aldehydes/ketones and hydrocarbons with varying degrees of unsaturation) were compared with the results achieved on XE-60, with a view

Figure 29. Essential Oil Muhuhu.
2% Carbowax 20M; temperature programmed from 100°, 2°/min. (1 cm = 1 min.)
Retention indices shown in brackets.



Fractions collected in Preparative GLC

Table 18

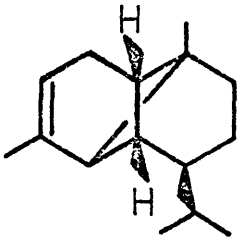
A) Main Fractions Collected from 2% Carbowax 20M (Weights recovered from 150 g Essential Oil Muhuhu‡ are indicated in brackets)	Retention Indices of Main Peaks		
	B) Carbowax 20M (1%)	C) XE-60 (0.5%)	D) QF-1 [(5%); where relevant: see text]
2(2 mg)	1400	1425	—
3(11 mg)	1565	1490	—
	1575	1515	—
4(5 mg)	1615	1540	—
5 (3 mg)	1670	1570	1615
	1685	1600	1660
6 (7 mg)	1745	1600	—
		1645	—
8 (6 mg)	1905	1740	1738
		1775	1780
			1800
			1940
9 (17 mg)	1995	Complex; ~1800	—
	2030		
	2065		
10 (8 mg)	2170	1835	1835
		1855	1855

‡ Due to an instrumental fault, only a 50% yield was obtained.

to selecting a phase which would afford an optimum resolution of the components present. The most satisfactory separation was recorded on 2% Carbowax 20M, and this phase was therefore employed to collect the fractions indicated in Figure 29. Fractions 2-6 and 8-10 were then examined on 1% Carbowax 20M and 0.5% XE-60: the success of the separation was confirmed (Carbowax 20M), and retention indices of the observed peaks were noted (Table 18, columns A-C).

Examination of Individual Fractions

Of the two apparently single-component fractions collected, fraction 2 had retention data identical to those of copaene and ylangene on 1% Carbowax 20M and 0.5% XE-60. Analysis of this fraction at 100° on the phase 5% Bentone 34/5% dinonyl phthalate, known to separate these hydrocarbons, revealed the presence of two components of retention times characteristic of copaene and ylangene: measurements of peak areas suggested the composition copaene:ylangene = 1:6. Mass spectra of this fraction were recorded by GC-MS on the phase 1% OV-225 (equivalent to XE-60): spectra corresponding to mixtures of copaene (3) and ylangene (4) were obtained. Mass spectra of the individual hydrocarbons are shown in Appendix 2.



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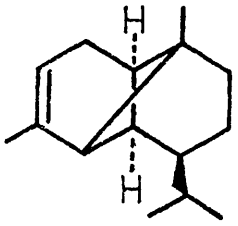

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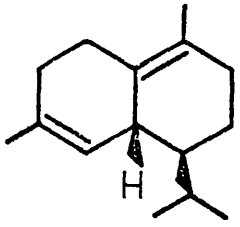

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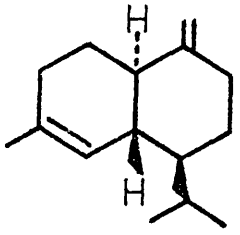


93

Table 19

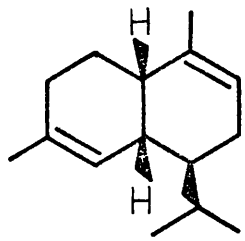
Major Mass Spectral Fragmentations of the Constituent of Fraction 4

m/e	Relative Abundance (%)	Proposed Nature of Fragment ‡	Metastable Peak (where necessary to support proposed transition)
204	45	M	
189	18	M - $^{\bullet}CH_3$	
161	100	M - $(CH_3)_2{}^{\bullet}CH$	m* 127.1
134	57	M - C_5H_{10}	m* 88.0
119	50	m/e 134 - $^{\bullet}CH_3$	m* 105.7
105	51	methyltropylium ion	
91	34	tropylium ion	
81	33	$(C_6H_9)^+$	
41	35	$(C_3H_5)^+$	

‡ For convenience, the molecular ion ($M^{+\bullet}$) is denoted by 'M'.

The homogeneity of fraction 4‡ was demonstrated by GLC analysis on 1% Carbowax 20M, 0.5% XE-60 and 5% QF-1, and a mass spectrum of this material was recorded by GC-MS on 1% OV-225. The spectrum indicated the presence of a hydrocarbon of molecular weight 204. Prominent fragments of the spectrum are indicated in Table 19. The retention indices of this fraction on Carbowax 20M and XE-60 were consistent with those of a bicyclic sesquiterpene, which would possess two double bonds, according to the presumed molecular formula $C_{15}H_{24}$. δ-Cadinene (92)[96] was found to possess a mass spectrum[97] which was almost identical to that of fraction 4. That the fragments at m/e 134 in these spectra were not a general feature of mass spectra of the cadinenes was evident from the spectrum[98] of γ-cadinene (93)[99], in which this ion was not observed.

‡ This fraction was found to decompose rapidly in air, at room temperature, to a mixture of compounds of higher molecular weight. A second batch of the material was isolated by further preparative GLC separation of the oil, and stored under nitrogen at a low temperature.



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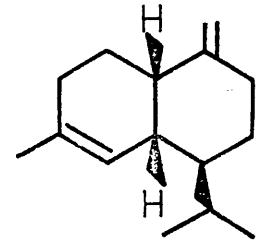

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δ- Cadinene (92) was thus tentatively proposed as the constituent of fraction 4.

Fraction 3 appeared to consist of two components, incompletely resolved on 1% Carbowax 20M and 0.5% XE-60. The phases 5% QF-1 and 5% Bentone 34/5% dinonyl phthalate failed to achieve complete separation of these constituents. The fraction was examined by GC-MS on 1% OV-225, and mass spectra were recorded at various positions on both peaks. The spectra obtained exhibited identical patterns of fragmentation, and the presence of diastereomeric hydrocarbons of molecular weight 204 was indicated. A mass spectrum recorded at top of the first peak (where the concentration of the second component would be expected to be small) is shown in Appendix 2, and its main features are summarised in Table 20. Comparison of this spectrum with the corresponding spectrum[100] of α-muurolene (46)[63,64], a bicyclic analogue of α-copaene (p. 12), indicated that these compounds were identical, a postulate further supported by a consideration of the retention indices of fractions 2, 3 and 4 on Carbowax 20M. Assuming fraction 2 to comprise α-copaene and α-ylangene, and fraction 4 to comprise δ-cadinene, then the retention index of the first component of fraction 3 was in close agreement with that required for α-muurolene on the same phase.[93]

Table 20

Major Mass Spectral Fragmentations of $3^{(1)}$ ‡

m/e	Relative Abundance (%)	Proposed Nature of Fragment	Metastable Peak (where necessary to support proposed transition)
204	30	M	
189	9	M - $^{\bullet}CH_3$	
161	49	m/e 189 - C_2H_4	m* 137.1
105	100	methyltropylium ion	
94	64	m/e 161 - $^{\bullet}C_5H_7$	m* 54.9?
41	37	$(C_3H_5)^+$	

‡ '$A^{(b)}$' = Component b of fraction A.

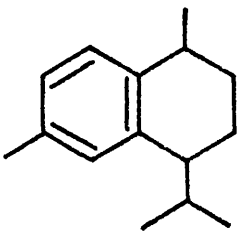
Table 21

Major Mass Spectral Fragmentations of $5^{(1)}$

m/e	Relative Abundance (%)	Proposed Nature of Fragment	Metastable Peak (where necessary to support proposed transition)
202	30	M	
173	16	M - $^{\bullet}C_2H_5$	
159	100	m/e 173 - $^{\bullet}CH_2$	m* 146.1

Due to the poor resolution of the components of fraction 3 on OV-225, no mass spectrum corresponding to a pure sample of the second component was obtained. Spectra corresponding to mixtures of the components in which the second component was predominant possessed base peaks at m/e 161 (contrast m/e 105 in the spectrum of the first component), and the relative abundances of the fragments at m/e 94 in these spectra were much lower than in the spectrum of the first component. The mass spectrum[101] of γ-muurolene (59)[63,64] appeared to be closely similar to that of the second component of fraction 3: on Carbowax 20M, however, γ-muurolene would have been expected to occur at a lower retention index than the corresponding α-isomer.[93] If, therefore, the presence of α-muurolene in fraction 3 was assumed, it was not possible to postulate the γ-isomer as a major constituent of the fraction.

Fraction 5 constituted a minor proportion of the total oil, and was observed as two poorly resolved peaks on 1% Carbowax 20M and 0.5% XE-60. On 5% QF-1, however, a superior separation of these peaks was achieved (Table 18). Mass spectra were recorded on 1% OV-210 (equivalent to QF-1). Spectra corresponding to the peak of lower retention index on OV-210 (the predominant peak) were indicative of a hydrocarbon of molecular



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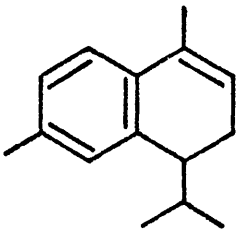

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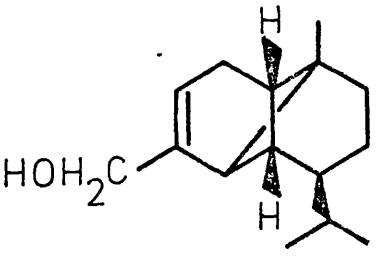

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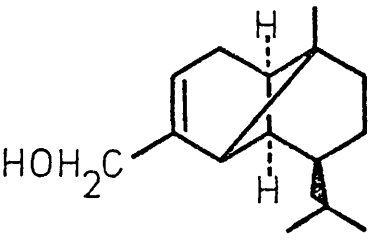

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Table 22

Major Mass Spectral Fragmentations of 6(1)

m/e	Relative Abundance (%)	Proposed Nature of Fragment	Metastable Peak (where necessary to support proposed transition)
200	17	M	
157	100	M - $(CH_3)_2{}^{\bullet}CH$	
142	35	m/e 157 - $^{\bullet}CH_3$	m* 128.4

Table 23

Major Mass Spectral Fragmentations of 6(2)

m/e	Relative Abundance (%)	Proposed Nature of Fragment	Metastable Peak (where necessary to support proposed transition)
220	65	M	
205	3	M - $^{\bullet}CH_3$	
187	3	m/e 205 - H_2O	
121	100	$(C_9H_{13})^+$	
108	38	m/e 187 - $^{\bullet}C_6H_7$	m* 62.4
81	85	$(C_6H_9)^+$	
41	36	$(C_3H_5)^+$	

weight 202 (Table 21), which necessitated the molecular formula $C_{15}H_{22}$, as in calamenene (94), for example. The mass spectrum[102] of calamenene, however, showed very little similarity with the corresponding spectrum of this component. No further mass spectra of hydrocarbons of this molecular formula were available. Spectra corresponding to the second peak of fraction 5 revealed that it was not homogeneous and, as this peak represented only a minor proportion of the total oil, it was not investigated further.

Each of fractions 6, 8 and 10, collected as single peaks from 2% Carbowax 20M, was separated into two components on 0.5% XE-60 and, in respect of fraction 6, the resolution achieved on the latter phase was considered adequate to allow GC-MS examinations of the individual peaks. Mass spectra of the constituents of fraction 6 (present in equal proportions) were recorded by GC-MS on 1% OV-225. Prominent fragments in the spectra are indicated in Tables 22 and 23. The peak of lower retention index on OV-225 was found to represent a hydrocarbon of molecular weight 200, requiring the molecular formula $C_{15}H_{20}$. The aromatic nature of this highly unsaturated compound was suggested by its mass spectrum, in which only three prominent fragments were observed. A molecular structure such as that of calacorene (95) was

thus indicated, and the retention indices of the first component of fraction 6 on Carbowax 20M and on XE-60 were consistent with those expected for a structure of this type. The mass spectrum of calacorene itself, however, showed little similarity with the spectrum of the component under investigation and, unfortunately, no spectra of compounds structurally similar to calacorene could be found in the literature.

The mass spectrum of the more polar constituent of fraction 6 was indicative of an alcohol of molecular weight 220: an ion at m/e 187, corresponding to dehydration of the fragment at m/e 205,[103] was observed in the spectrum. From the molecular weight, the molecular formula $C_{15}H_{24}O$ was inferred, corresponding either to a tricyclic structure with one double bond or to a bicyclic structure with two double bonds; in respect of the former system, it was noted that the retention times of copaenol (96) and ylangenol (97) on Carbowax 20M and XE-60 were higher than the retention indices of the alcohol present in fraction 6 on these phases (cf. p.134).

Examination of fraction 8 by GC-MS on 1% OV-225 indicated that the peaks of retention indices 1740 and 1775 on XE-60 were not homogeneous. On the phase 5% QF-1, this fraction was separated into four major components of retention indices 1738, 1780, 1800 and 1940

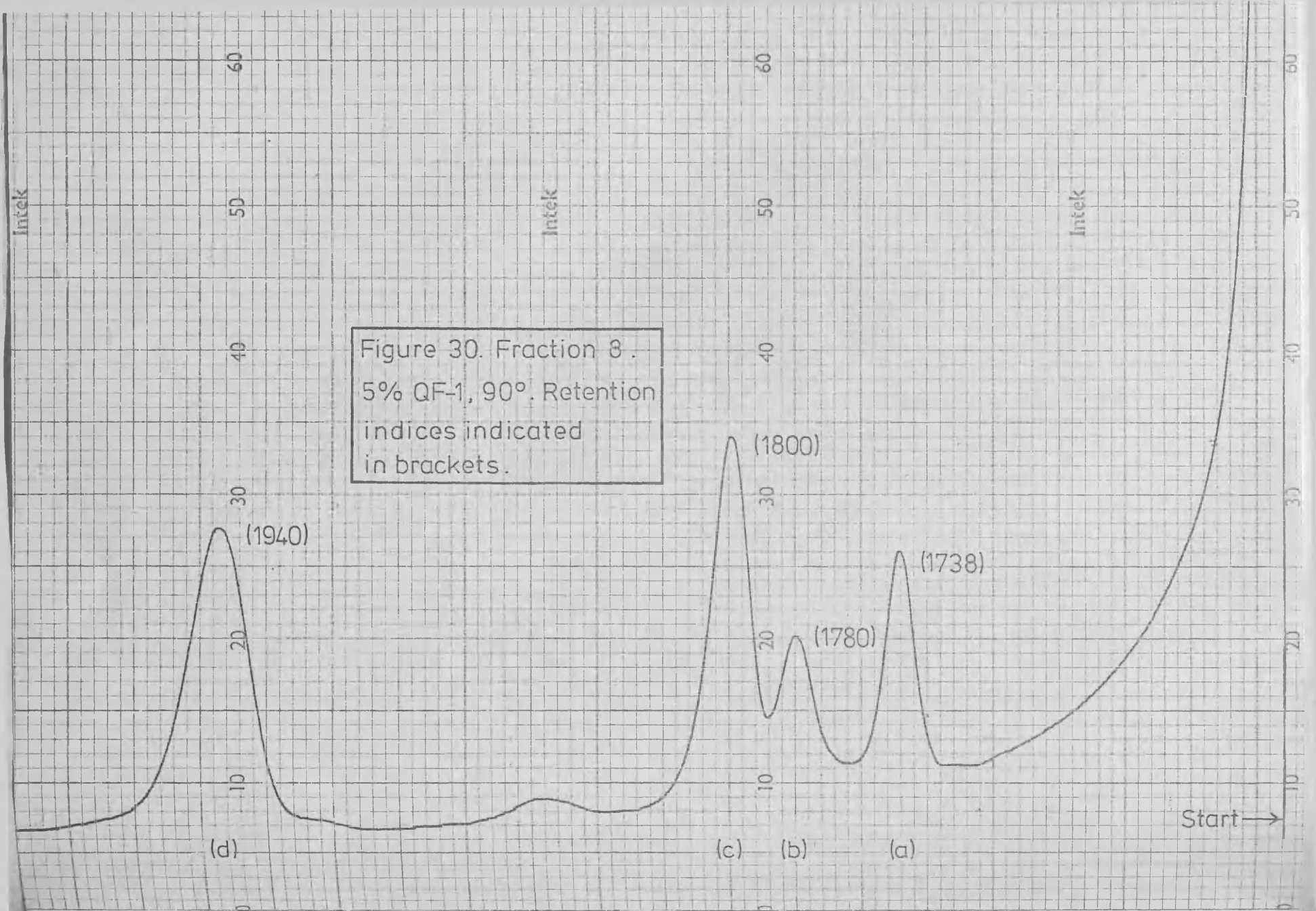


Figure 30. Fraction 8. 5% QF-1, 90°. Retention indices indicated in brackets.

Table 24

Major Mass Spectral Fragmentations of 8[4]

m/e	Relative Abundance (%)	Proposed Nature of Fragment	Metastable Peak (where necessary to support proposed transition)
220	16	M	
205	4	M - $^{\bullet}CH_3$	
202	2	M - H_2O	
187	3	m/e 205 - H_2O	m* 170.6
177	100	M - $(CH_3)_2{}^{\bullet}CH$ <u>or</u> M - CH_2=CH-O$^{\bullet}$	m* 142.4
107	40	$(C_8H_9)^+$	
41	39	$(C_3H_5)^+$	

[designated as peaks (a) - (d), respectively, in Figure 30]. Mass spectra corresponding to these peaks were obtained by GC-MS on 1% OV-210. Peak (d) was found to represent an alcohol or aldehyde of molecular weight 220 ($C_{15}H_{24}O$): prominent fragments in the mass spectrum of this compound are indicated in Table 24. The relative abundance of the molecular ion was higher than usual for an alcohol, for which a fragment of higher intensity than that at m/e 220 ($M^{+\cdot}$), corresponding to loss of water from the molecular ion (m/e 202), would have been expected. On treatment of fraction 8 with methoxylamine hydrochloride/pyridine, this component was completely converted to its O-methyl oxime, as judged by GLC on 5% QF-1, further supporting the proposed presence of an aldehyde; the other major constituents of this fraction did not form corresponding derivatives. If the aldehydic nature of the component of retention index 1940 on QF-1 was thus assumed, the fragment at m/e 177 in its mass spectrum could be interpreted as loss of either $(CH_3)_2{}^{\cdot}CH$ or $CH_2{=}CH{-}O{\cdot}$ from the molecular ion cf. 104. The latter fragmentation would require the presence of a methylene group adjacent to the aldehyde.

Mass spectra corresponding to peaks (a) - (c) were examined: it was evident from the spectra that these peaks were not homogeneous; indeed, on 1% OV-210, a

Figure 31. AVA examination of fraction 8, peaks (a)-(c) [cf. Fig. 30]

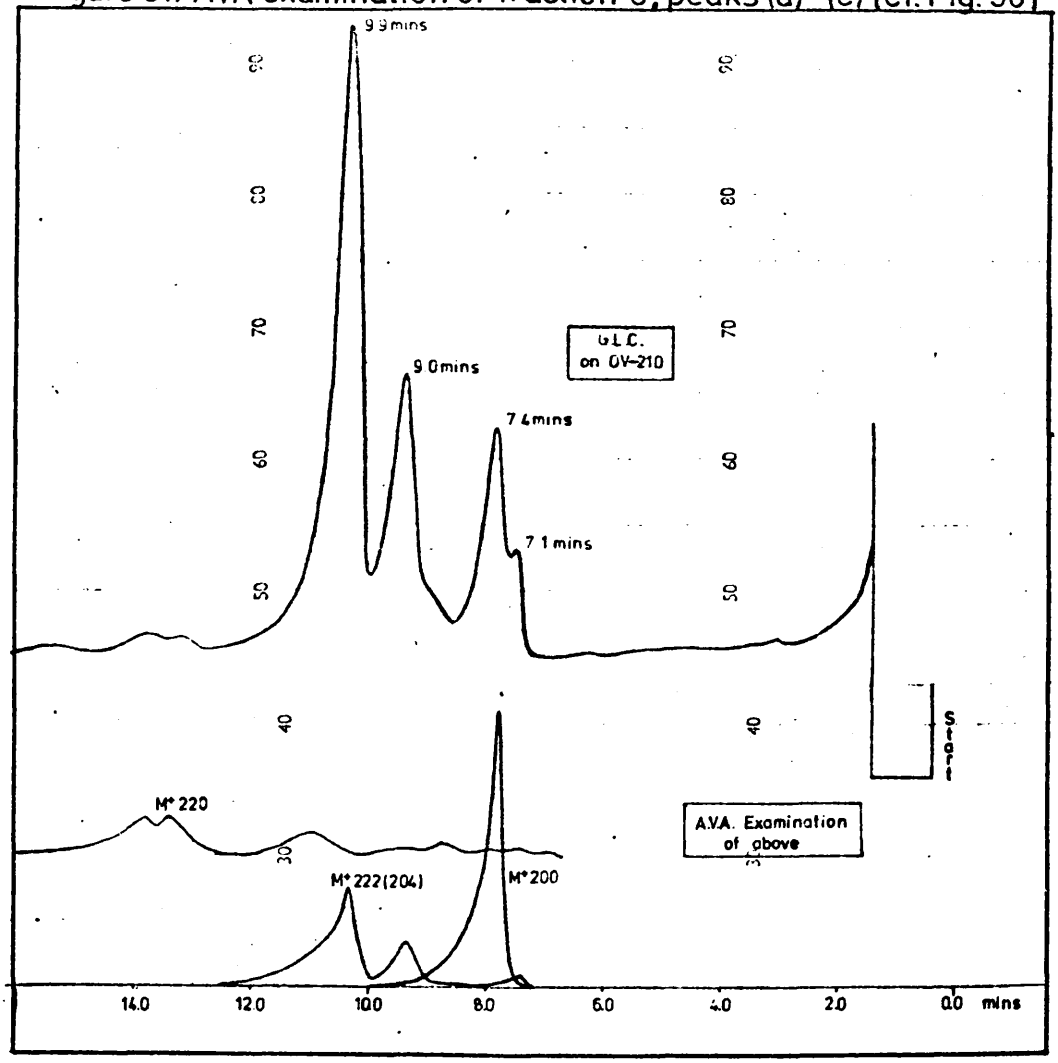


Table 25

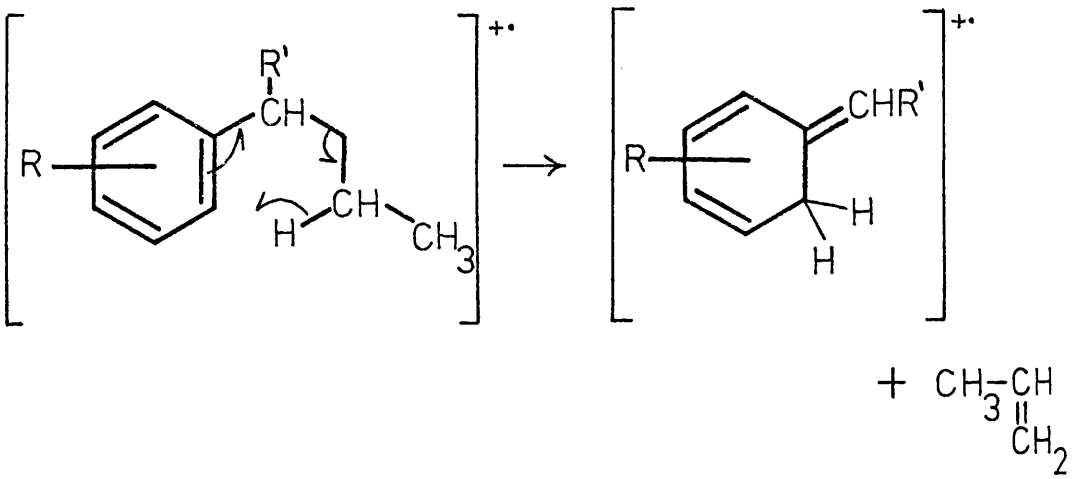
Major Mass Spectral Fragmentations of 8[1]

m/e	Relative Abundance (%)	Proposed Nature of Fragment	Metastable Peak (where necessary to support proposed transition)
200	69	M	
185	100	M - $^{\bullet}CH_3$	
157	30	M - $(CH_3)_2{}^{\bullet}CH$	
143	30	m/e 185 - $CH_2{=}CH{-}CH_3$	m* 110.5
43	23	$(C_3H_7)^+$	

'shoulder' was resolved on peak (a) which had not been visible on 5% QF-1. This peak appeared to comprise predominantly a hydrocarbon of molecular weight 200; similarly, peaks (b) and (c) essentially comprised diastereomeric alcohols of molecular weights 222. Small concentrations of hydrocarbons of molecular weights 220, however, were also evident throughout the range of retention indices represented by peaks (a) - (c). Variations in the intensities of ions at m/e 200, 204 (for an alcohol of molecular weight 222) and 220 were recorded throughout this region of the chromatogram by means of an accelerating voltage alternator (Fig. 31), to determine the positions on peaks (a) - (c) at which mass spectra of the major constituents of fraction 8 of retention indices approximately 1700-1800 on 1% OV-210, with a minimum level of impurity, might be obtained. These spectra are shown in Appendix 2, and major fragmentation patterns are indicated in Tables 25-27.

Consideration of the mass spectrum corresponding to the predominant constituent of peak (a) indicated the presence of a hydrocarbon of molecular formula $C_{15}H_{20}$. This formula suggested either a bicyclic sesquiterpene with four double bonds or a monocyclic sesquiterpene with five double bonds. The mass spectrum of this

Figure 32



m/e 185 ($C_{14}H_{17}$; corresponding to M - $^{\bullet}CH_3$)

m/e 143 ($C_{11}H_{11}$)

(R, R' = H or alkyl)

Table 26

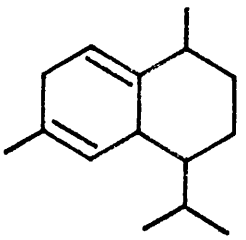
Major Mass Spectral Fragmentations of 8[2]

m/e	Relative Abundance (%)	Proposed Nature of Fragment	Metastable Peak (where necessary to support proposed transition)
222	2	M	
204	40	M - H_2O	
179	64	M - $(CH_3)_2{}^{\bullet}CH$	
161	100	m/e 204 - $(CH_3)_2{}^{\bullet}CH$	m* 127.1
119	62	m/e 161 - C_3H_6	m* 88.0
105	45	methyltropylium ion	
81	37	$(C_6H_9)^+$	
43	56	$(C_3H_7)^+$	

Table 27

Major Mass Spectral Fragmentations of 8(3)

m/e	Relative Abundance (%)	Proposed Nature of Fragment	Metastable Peak (where necessary to support proposed transition)
222	2	M	
204	30	M - H_2O	
179	22	M - $(CH_3)_2{}^{\bullet}CH$	
161	61	m/e 204 - $(CH_3)_2{}^{\bullet}CH$	m* 127.1
119	100	m/e 161 - C_3H_6	m* 88.0
105	41	methyltropylium ion	
82	32	$(C_6H_{10})^+$	



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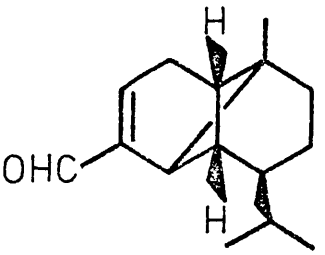
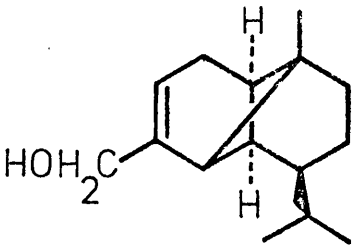


80



97

compound resembled that of the first component of fraction 6 on XE-60: in the former spectrum, however, an intense ion was observed at m/e 143, almost entirely absent in the latter spectrum. This ion was shown to have arisen by elimination of C_3H_6 from the fragment at m/e 185 (m* 110.5), for which transition a possible mechanism is outlined in Figure 32.

Peaks (b) and (c) appeared essentially to represent diastereomeric sesquiterpenoid alcohols of molecular formula $C_{15}H_{26}O$, corresponding to bicyclic structures with one double bond, for example. The mass spectra of these compounds exhibited almost identical patterns of fragmentation (Tables 26 and 27). A significant ion at m/e 82 in the spectrum corresponding to peak (c), however, was present in very low relative abundance in the spectrum corresponding to peak (b): the constitution of the fragment represented by this ion is uncertain, and no metastable peak was observed to account for its formation. A marked resemblance was noted between the mass spectra of these diastereomeric alcohols and that[105] of 4,10-dimethyl-7-isopropyl-bicyclo (4.4.0)-1,4-decadiene (98), indicating that the products of dehydration of the alcohols in the mass spectrometer[103] possessed structural features similar to this hydrocarbon. The fragments at m/e 179 in the

spectra of the alcohols did not appear in the spectrum of the hydrocarbon: these fragments represented losses of $(CH_3)_2{}^{\bullet}CH$ from the molecular ions, and correspond to a fragment at m/e 161 in the spectrum of the hydrocarbon (also observed in the spectra of the alcohols).

Fraction 10 was examined on the phase 5% QF-1: two components were observed, of retention indices (1835 and 1855) identical to those of fraction 10 on 0.5% XE-60. The component of lower retention index constituted approximately 10% of the total fraction. An IR spectrum (carbon tetrachloride solution) of the mixture was recorded: absorptions (weak) were observed at 3607 and 1687 cm^{-1}, suggesting the presence of an alcohol and of an olefinic double bond [cf. $\nu_{C=C}$ in the corresponding spectra of Brachylaena ketols A and B: 1688 cm^{-1} (w)]. Both components readily formed trimethylsilyl derivatives, as judged by GLC on 0.5% XE-60 and 5% QF-1; on treatment of the fraction with methoxylamine hydrochloride in dry pyridine, however, no O-methyl oxime formation was observed. Under identical conditions a sample of copaenal (80), obtained from a separate batch of Essential Oil Muhuhu by Dr. E. Klein of Dragoco (Holzminden)‡, and of

‡ Private communication: see also pages 40 and 135

Table 28

Major Mass Spectral Fragmentations of 10[1]

m/e	Relative Abundance (%)	Proposed Nature of Fragment	Metastable Peak (where necessary to support proposed transition)
218	6	M	
200	6	M - H_2O	
187	14	M - $^{\bullet}CH_2OH$	
175	57	M - $(CH_3)_2{}^{\bullet}CH$	
157	76	m/e 175 - H_2O	
145	63	m/e 187 - CH_2=CH-CH_3	m* 112.4
131	100	m/e 145 - $^{\bullet}CH_2$	
119	53	$(C_9H_{11})^+$	
105	83	methyltropylium ion	
91	63	tropylium ion	
41	49	$(C_3H_5)^+$	

Table 29

Major Mass Spectral Fragmentations of 10[2]

m/e	Relative Abundance (%)	Proposed Nature of Fragment	Metastable Peak (where necessary to support proposed transition)
220	18	M	
202	10	M - H_2O	
189	8	M - $^{\bullet}CH_2OH$	
177	74	M - $(CH_3)_2{}^{\bullet}CH$	
159	36	m/e 177 - H_2O	m* 142.8
135	84	m/e 202 - $^{\bullet}C_5H_7$	m* 90.2?
105	76	methyltropylium ion	
93	77	$(C_7H_9)^+$	
91	100	tropylium ion	
41	66	$(C_3H_5)^+$	

retention index on 0.5% XE-60 identical to that of the major component of fraction 10, readily formed an O-methyl oxime (as indicated by GLC). It was concluded, therefore, that the major constituent of fraction 10 was not copaenal and, indeed, that neither constituent of this fraction possessed an aldehydic/ketonic functionality.

Mass spectra of the components of fraction 10 were obtained by GC-MS on 1% OV-210. The peak of lower retention index on this phase was found to represent a primary alcohol of molecular weight 218, requiring a molecular formula $C_{15}H_{22}O$, which corresponded to a bicyclic alcohol with three double bonds, for example. Prominent fragments in the mass spectrum of this compound are indicated in Table 28.

The mass spectrum of the major constituent of fraction 10 corresponded, similarly, to that of a primary alcohol of molecular weight 220: significant ions of the spectrum are indicated in Table 29. This spectrum was identical with that of an alcohol isolated in the early fractions (fractions 10 and 11 in Table 3) collected during column chromatography of the products of reduction of a naturally-occurring mixture of Brachylaenalones A and B (fractions 19-27 in Table 2). This compound was believed to be ylangenol (97), on the basis of the close similarity between the

Table 30

Physico-Chemical Data of Copaenal (80) and Compound postulated as Ylangenal (p.135)

a) Chromatographic Data

R_f values (80% light petroleum-ethyl acetate):
0.77 (identical)

Retention times on 1% OV-1 and 0.5% XE-60 at 126°:
OV-1, 14.0 minutes (identical); XE-60, 5.6 minutes (identical).

b) IR Spectra (carbon tetrachloride solutions).

ν_{max} (cm^{-1}):

Copaenal	Compound Postulated as Ylangenal
2950 (s)	2958 (s)
2867 (m)	2872 (m)
2809 (w)	2807 (w)
2706 (w)	2719 (w)
1685 (s)	1688 (s)
1626 (w)	1630 (w)

cont'd.

Table 30 cont'd.

c) Mass Spectra (prominent fragments are indicated):

Copaenal		Compound postulated as Ylangenal	
m/e	% Relative Abundance	m/e	% Relative Abundance
218	20	218	21
203	6	203	13
189	8	189	8
185	25	(185	2)
175	100	175	96
(157	9)	157	37
147	26	147	28
133	57	133	57
119	32	119	40
105	64	105	73
91	90	91	100
81	27	81	37
79	31	79	36
77	32	77	39
69	23	69	29
55	38	55	47
43	33	43	65
(41	4)	41	66

physico-chemical data of its product of mild oxidation (Sarett[74]) and those of copaenal (80), as indicated in Table 30. The major constituent in fraction 10 of Essential Oil Muhuhu was therefore tentatively designated as ylangenol.

Finally, fraction 9 was investigated. Only poor resolutions of the components of this mixture were achieved on the GLC phases 1% OV-1, 1% Carbowax 20M, 0.5% XE-60 and 5% QF-1. Superior separations of the constituents were observed on TLC (80% light petroleum-ethyl acetate): major components of R_f values 0.23, 0.34 and 0.58 were noted; in addition, minor components of R_f values 0.28, 0.39, 0.67 and 0.75 were recorded. It was concluded, however, that fraction 9 was too complex to merit further investigation within the scope of this brief survey.

The above findings are summarised in Table 31, and may be compared with the results of an investigation by Dr. E. Klein of Dragoco (Holzminden) into the composition of a separate sample of Essential Oil Muhuhu (Table 32).

Table 31

Classification of Constituents of Essential Oil Muhuhu

		Mol. Weight	Presumed Mol. Formula	Proposed Identities	Origin‡
a)	Hydrocarbons	200	$C_{15}H_{20}$	-	$6^{(1)}$
		200	$C_{15}H_{20}$	-	$8^{(1)}$
		202	$C_{15}H_{22}$	-	$5^{(1)}$
		204	$C_{15}H_{24}$	ylangene (4)	$2^{(1)}$
		204	$C_{15}H_{24}$	copaene (3)	$2^{(2)}$
		204	$C_{15}H_{24}$	α-muurolene (46)	$3^{(1)}$
		204	$C_{15}H_{24}$	-	$3^{(2)}$
		204	$C_{15}H_{24}$	δ-cadinene (92)	4
b)	Alcohols	218	$C_{15}H_{22}O$	-	$10^{(1)}$
		220	$C_{15}H_{24}O$	-	$6^{(2)}$
		220	$C_{15}H_{24}O$	ylangenol (97)	$10^{(2)}$
		222	$C_{15}H_{26}O$	-	$8^{(2)}$
		222	$C_{15}H_{26}O$	-	$8^{(3)}$
c)	Aldehyde	220	$C_{15}H_{24}O$	-	$8^{(4)}$

‡ 'A^b' = Component b of fraction A.

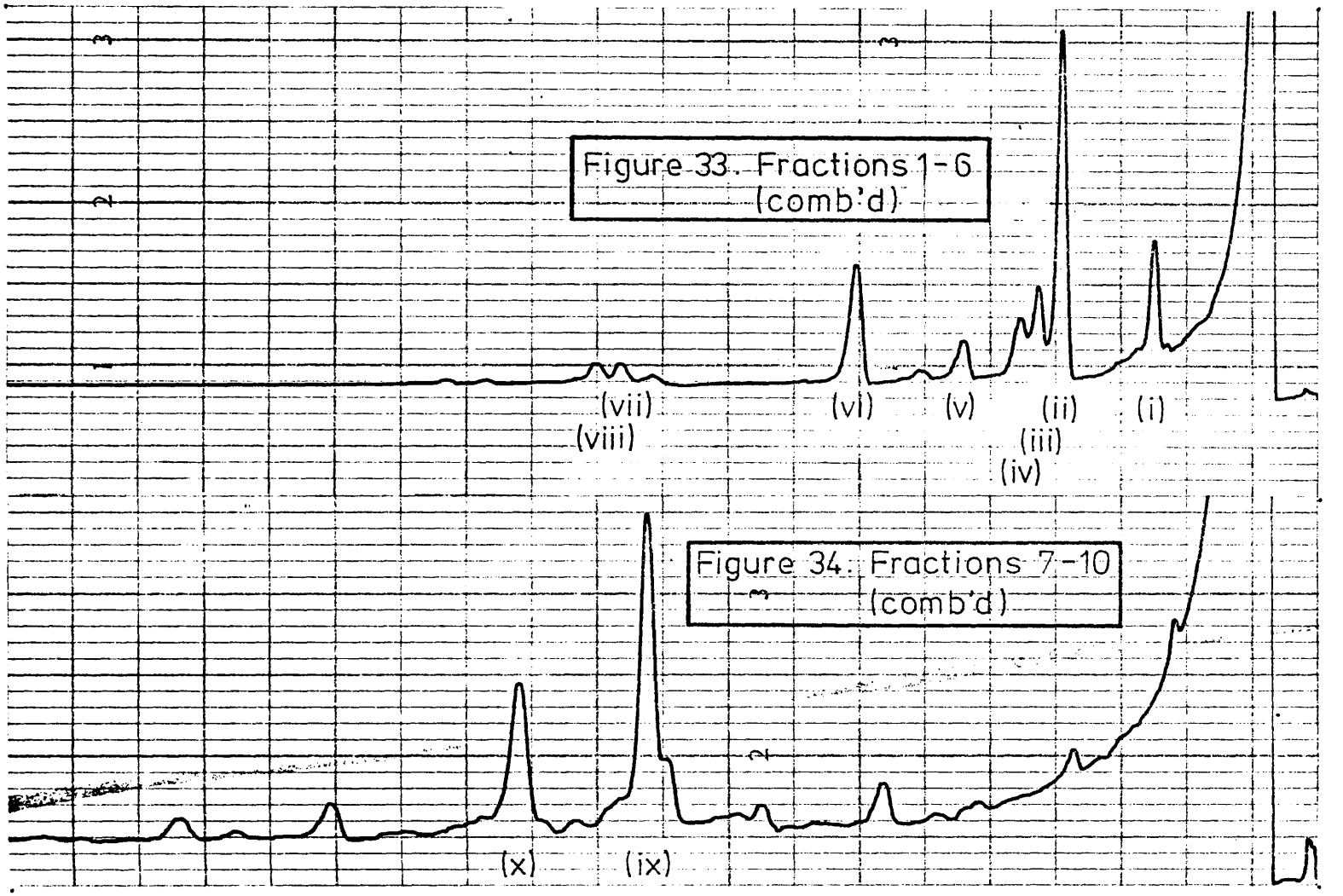
Table 32

Constituents of Essential Oil Muhuhu reported by Dr. E. Klein, Dragoco (Holzminden)‡

	Molecular Weight	Molecular Formula	Identity
a) Hydrocarbons	198	$C_{15}H_{18}$	cadalene (99)
	200	$C_{15}H_{20}$	1,6-dimethyl-4-isopropyl-7,8-dihydronaphthalene
	200	$C_{15}H_{20}$	α-calacorene (95)
	202	$C_{15}H_{22}$	calamenene (94)
	204	$C_{15}H_{24}$	ylangene (4)
	204	$C_{15}H_{24}$	copaene (3)
	204	$C_{15}H_{24}$	δ-cadinene (92)
	-	-	'zizanene'
b) Alcohols	220	$C_{15}H_{24}O$	ylangenol (97)
	220	$C_{15}H_{24}O$	copaenol (96)
c) Aldehydes	218	$C_{15}H_{22}O$	ylangenal
	218	$C_{15}H_{22}O$	copaenal (80)
d) Ether	-	-	'a sesquiterpenoid ether'

‡ Private communication.

Fractions from Column Chromatography of Heartwood Extract (Table 2, opp. p. 17). 0·5% XE-60. Temperature programmed from 95°, 2°/min. (1 cm = 1 min).



Comparison of the Constitution of Essential Oil Muhuhu with that of the Heartwood Extract of Brachylaena hutchinsii

The composition of the steam-volatile oil from Brachylaena hutchinsii was compared with the constitutions of early fractions from column chromatography of the heartwood extract. Fractions 1-6 and 7-10 Table 2) comprised components of retention indices 1400-1900 and 1400-2300, respectively, on the phase 0.5% XE-60 (Figs. 33 and 34). Fractions 11-16 (not included in Table 2) were composed predominantly of components of retention indices higher than 1900 on XE-60: no correspondence, therefore, between the constituents of fractions 11-16 and the constituents of Essential Oil Muhuhu was possible. The retention indices of individual main peaks of fractions 1-6 on 0.5% XE-60 [peaks (i) - (viii) in Figure 33], together with mass spectra corresponding to these peaks, recorded by GC-MS on 1% OV-225, were compared with data obtained from the constituents of Essential Oil Muhuhu, and similarities in the compositions of these mixtures were inferred (Table 33). Peak (viii) was found to represent a hydrocarbon of molecular weight 198, requiring the molecular formula $C_{15}H_{18}$ ($C_{14}H_{30}$ unlikely), as in cadalene (99), for example. While the retention

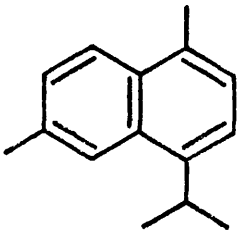


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Table 33

Main Peaks in Chromatogram (XE-60 of Fractions 1-6 (Fig. 33) from Column Chromatography of the Heartwood Extract of Brachylaena hutchinsii	Correspondence with Constituents of Fractions of Essential Oil Muhuhu‡ (cf. Table 31), according to Retention Indices and Mass Spectra
Peak (i)	$2^{(1)}$; $2^{(2)}$
Peak (ii)	$3^{(1)}$
Peak (iii)	$3^{(2)}$
Peak (iv)	4
Peak (v)	$5^{(1)}$
Peak (vi)	$6^{(1)}$
Peak (vii)	$8^{(4)}$
Peak (viii)	Hydrocarbon of molecular weight 198 [mass spectral base peak, m/e 183 $(M^+ - {}^{\cdot}CH_3)$]

[‡ 'A^b' = Component b of fraction A.]

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index of this peak on XE-60 was not inconsistent with the presence of cadalene, it was hoped to make direct comparisons of the mass spectra of these compounds, but no description of the mass spectrum of the latter hydrocarbon could be found in the literature.

The presence of both copaene and ylangene in

fractions 1-6 from column chromatography of the heartwood extract was indicated by a chromatogram of the combined fraction on 5% Bentone 34/5% dinonyl phthalate: peak (i) was separated into two peaks, of retention indices characteristic of copaene and ylangene, in the approximate ratio copaene:ylangene = 1:7 [cf. ratio of these hydrocarbons in Essential Oil Muhuhu (p. 124)].

The main peaks on 0.5% XE-60 [peaks (ix) and (x) in Figure 34] of fractions 7-10 (Table 2) were examined in a manner identical to the above, yielding the results shown in Table 34. It was not possible to obtain satisfactory mass spectra of minor constituents of the total combined fraction without serious overloading of the instrument.

Table 34

Main Peaks in Chromatogram (XE-60) of Fractions 7-10 (Fig. 34) from Column Chromatography of Heartwood Extract of Brachylaena hutchinsii	Correspondence with Constituents of Fractions of Essential Oil Muhuhu, according to Retention Indices and Mass Spectra
Peak (ix)	$8^{(4)}$ [corresponding to peak (vii) in Table 33]
Peak (x)	Hydrocarbon of molecular weight 198 (mass spectral base peak m/e 91 (tropylium ion)].

2.2. Conclusions

The sample of Essential Oil Muhuhu examined was found to comprise mainly hydrocarbons of molecular weights 200-204, alcohols of molecular weights 218-222, and an aldehyde of molecular weight 220 (Table 31). One complex fraction (fraction 9) collected during preparative GLC of the oil remains to be examined. The Brachylaenalones were not present in the oil.

A high degree of correspondence was observed between the composition of the steam-volatile oil and the constitutions of early fractions from column chromatography of the heartwood extract of Brachylaena hutchinsii.

2.3. Experimental

Formation of Oximes and Trimethylsilyl Derivatives : Examples

Attempted Formation of Oxime Derivatives from Fraction 8 of Essential Oil Muhuhu

A solution of fraction 8 (3.2 mg) in dry pyridine (0.15 ml) was treated with methoxylamine hydrochloride (2.3 mg), and warmed in a metal block ($\sim 45^{\circ}$) for 18 hours. Solvent was removed under nitrogen at room temperature, and ethyl acetate (0.5 ml) was added to give a solution of concentration ~ 1 mg/$\frac{1}{4}$ml. Analysis by GLC on 5% QF-1 at 90° indicated complete conversion of component (iv) to its O-methyl oxime.

Retention times (minutes)

Starting material : 16.0, 19.5, 22.0, 41.5.

Products : 16.0, 17.3, 19.5, 22.0.

Attempted Formation of Trimethylsilyl Derivatives from Fraction 10 of Essential Oil Muhuhu

A solution of fraction 10 (0.5 mg) in anhydrous pyridine (40 μl) was treated with hexamethyldisilazane (20 μl), followed by trimethylchlorosilane (3 μl). The reaction mixture was warmed in a metal block ($\sim 45^{\circ}$)

for 45 minutes, and then concentrated to dryness under nitrogen. Ethyl acetate (0.1 ml) was added, and this solution was examined by GLC on 0.5% XE-60 at 123°, and on 5% QF-1 at 100°. Formation of the trimethylsilyl derivatives of both components was evident.

Retention times (minutes)

Starting material

0.5% XE-60 : 3.0, 3.3.

5% QF-1 : 21.0, 23.0.

Products

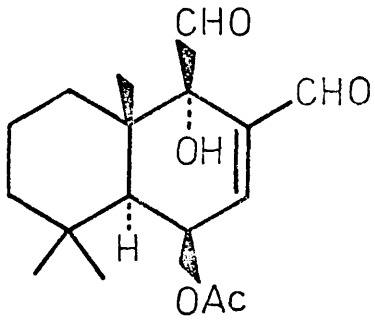
0.5% XE-60 : (in solvent front)

5% QF-1 : 14.5, 15.5.

Product of Oxidation of Combined Fractions 10 and 11 (Table 3)

IR and mass spectra were recorded [Table 27, parts (b) and (c), respectively]; λ_{max}^{EtOH} 242 nm [(ϵ 4,625); cf. Brachylaenalones A and B, λ_{max}^{EtOH} 244 nm (ϵ 9,000)].

Appendix 1



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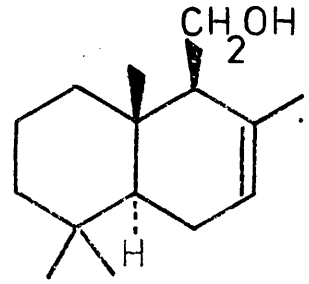

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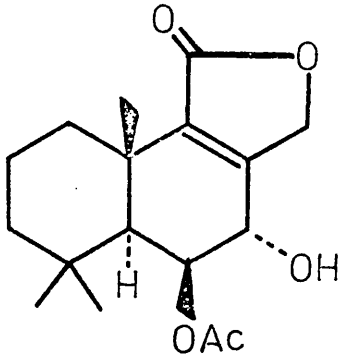


102

Cinnamodial (100) in Heartwood Extracts of Cinnamodendron Corticosum and Warburgia Stuhlmannii

The small family Canellaceae is a member of the ancient group of woody plants of the order Magnoliales, and is scattered in distribution from Florida, east tropical South America and the West Indies, including the Bahamas, to tropical and sub-tropical East Africa and Madagascar.[106] Early botanical studies[107] suggested a close relationship between the Canellaceae and the family Winteraceae, which occurs within the same order; more recently, however, it has been claimed[106] that the gap between these families is considerable, both from a morphological standpoint and also geographically, as the latter family is entirely absent from Africa and Madagascar. In view of the fact that drimenol (101) and related bicyclofarnesane sesquiterpenoids have been found almost exclusively in the Winteraceae, the recent discoveries of the drimanic sesquiterpenoid, cinnamodial (100), in two genera of the Canellaceae, Warburgia[108] and Cinnamosma,[109] and of its congener, ugandensolide (102), in Warburgia[108] have established an interesting chemotaxonomic link between the Canellaceae and Winteraceae. In the present work, the occurrence of cinnamodial in a third genus of the Canellaceae,

Cinnamodendron, and in another Warburgia species has been demonstrated.

Cinnamodendron Corticosum

It appears that no previous chemical investigation within the genus Cinnamodendron has been undertaken.

A supply of C. corticosum Miers (Red Canella or Mountain Cinnamon) was obtained from Jamaica.* Extraction of the dried, powdered heartwood with n-hexane afforded a light brown oil, constituting 0.5% by weight of the wood. GLC analyses of fractions obtained from the oil by preparative TLC revealed that the apparent major components of R_f values 0.18, 0.32 (corresponding in mobility to cinnomodial), 0.47 (present in highest concentration), 0.67, 0.86 and 0.94 (according to preliminary analytical TLC in 70% light petroleum-ethyl acetate) were themselves complex mixtures. From a concentrated hexane solution of the oil, however, crystalline material separated and crystallised from ether as colourless needles of m.p. 136-139°. GLC examination on 1% SE-30, under conditions of programmed temperature, indicated a single component of retention index 1625. An IR spectrum (carbon tetrachloride

* By courtesy of Mr. A. G. Kenyon, Tropical Products Institute, London.

Figure 35. IR spectrum (CCl_4) of Cinnamodial isolated from Cinnamodendron corticosum

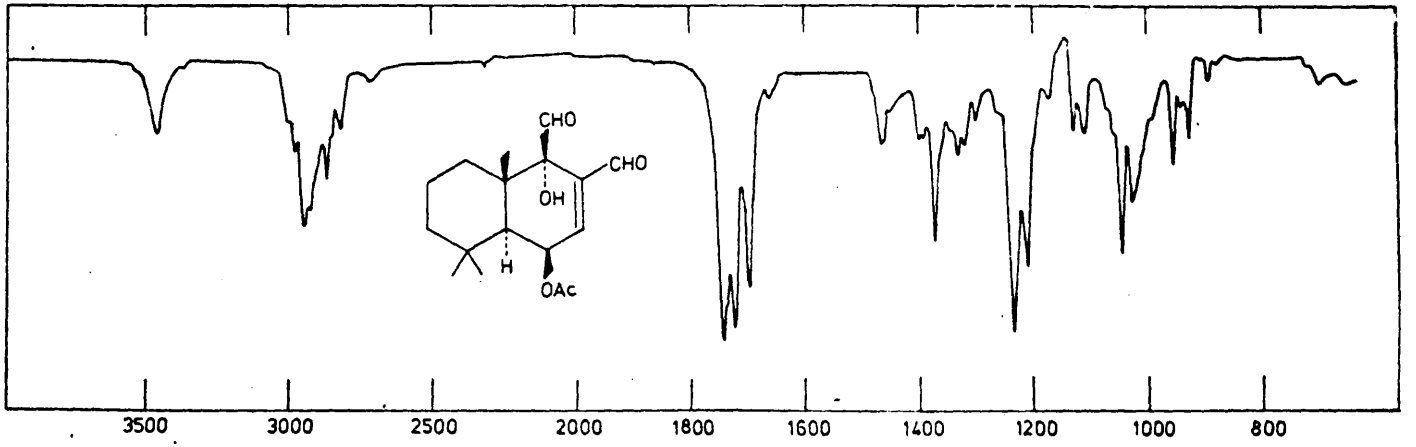
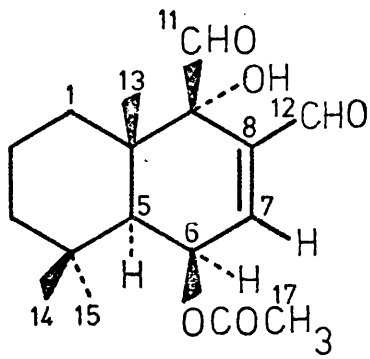


Table 35

Significant Peaks in NMR Spectrum of Cinnamodial (100) in carbon tetrachloride. Chemical Shift Data (60 MHz) and Observed Coupling Constants after deuterium exchange of the hydroxylic proton are indicated*.

* Prior to D-exchange, the hydroxylic proton appeared at 6.12 τ (m): $J_{11\text{-}OH}$ = 1.5 Hz

‡ s = singlet; d = doublet; t = triplet; m = multiplet



100

Proton	Chemical Shift (τ) and observed Splitting Pattern‡	Coupling Constants (J)
H_5	7.98 (d)	$J_{5\text{-}6}$ = 5 Hz
H_6	4.15 (t)	$J_{6\text{-}5}$ = 5 Hz
		$J_{6\text{-}7}$ = 5 Hz
H_7	3.12 (d)	
H_{11}	0.33 (s)	
H_{12}	0.53 (s)	
$3H_{13}$	8.71 (s)	
$3H_{14}$	8.85 (s)	
$3H_{15}$	8.97 (s)	
$3H_{17}$	7.93 (s)	

(ν_{O-H} ; unaffected by dilution)

solution; Fig. 35) showed prominent absorptions at 3462, 2869 and 2818 (ν_{C-H} of aldehyde(s)), 1744 ($\nu_{C=O}$ of ester), 1724 ($\nu_{C=O}$ of saturated aldehyde), 1697 ($\nu_{C=O}$ of α,β-unsaturated aldehyde), 1233 and 1209 cm^{-1} (ν_{C-O} of ester), suggesting the presence of a dialdehyde ester in which one aldehyde was conjugated. In the UV region, absorption in ethanol at 223 nm (ε 15, 500) was observed. These data exhibited a close similarity with those of cinnamodial (100)[108,109]. An NMR spectrum of the material (Table 35) was almost superimposable with that recorded by C.J.W. Brooks and G.H. Draffan[108] for cinnamodial. In the mass spectrum, the molecular ion expected for cinnamodial (at m/e 308) was not observed: cf.[108] a fragment of low relative abundance at m/e 290, however, corresponded to elimination of water from the molecular ion; the base peak of the spectrum, at m/e 43, supported the presence of an acetate.

On the basis of this evidence, the identity of this compound with cinnamodial was proposed. Column chromatography of the heartwood extract on alumina afforded fractions containing cinnamodial in admixture with other compounds, as judged by GLC on 1% SE-30. Estimations of peak areas in chromatograms of these fractions indicated that cinnamodial constituted less than 0.5% by weight of the total extract: all fractions collected by column chromatography, however, were shown by GLC to be very com-

plex, precluding further investigation within the scope of this preliminary survey.

Warburgia Stuhlmannii

No report of previous chemical investigations on Warburgia stuhlmannii Engl. ("Karambusi") has appeared in the literature, except for an early description[110] of the essential oil derived from its bark.

In the present work, a supply of W. stuhlmannii was obtained from Tanganyika.* Extraction of the dried, powdered heartwood with n-hexane yielded a dark brown, partially crystalline oil, comprising 1.5% by weight of the wood. TLC analysis of the oil indicated principal components of R_f values (80% light petroleum-ethyl acetate) 0.24 (corresponding in mobility to cinnamodial), 0.32 (most prominent constituent), 0.48, 0.58, 0.63, 0.75 and 0.91.

The oil was separated into fractions by extraction first with light petroleum (A) and then with light petroleum/ether, 1:1 (B): these fractions were shown by TLC to be of considerable complexity. The residue (C), however, appeared to be of high purity (TLC; GLC on 1% SE-30), and crystallised from ether as colourless needles with physical data identical to those of cinnamodial

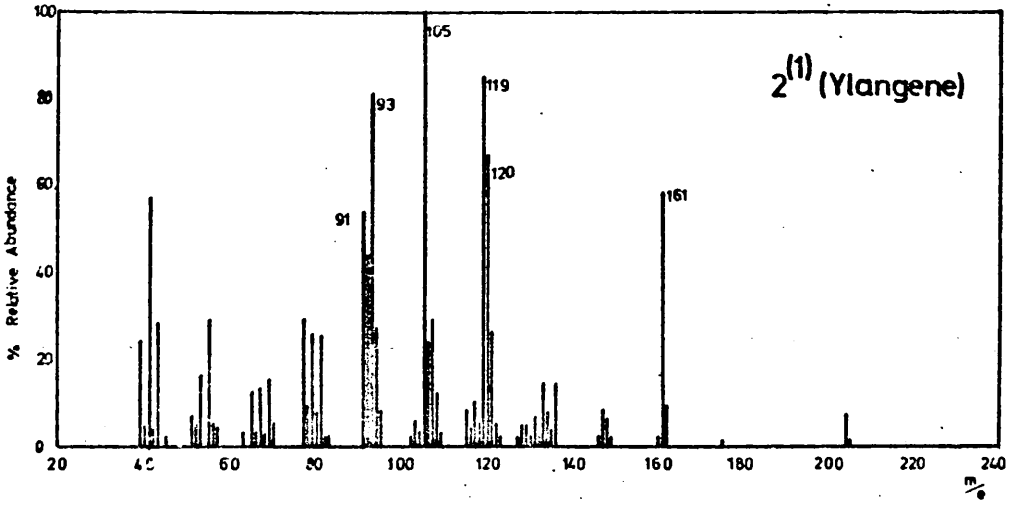
* By courtesy of Mr. A.G. Kenyon, Tropical Products Institute, London.

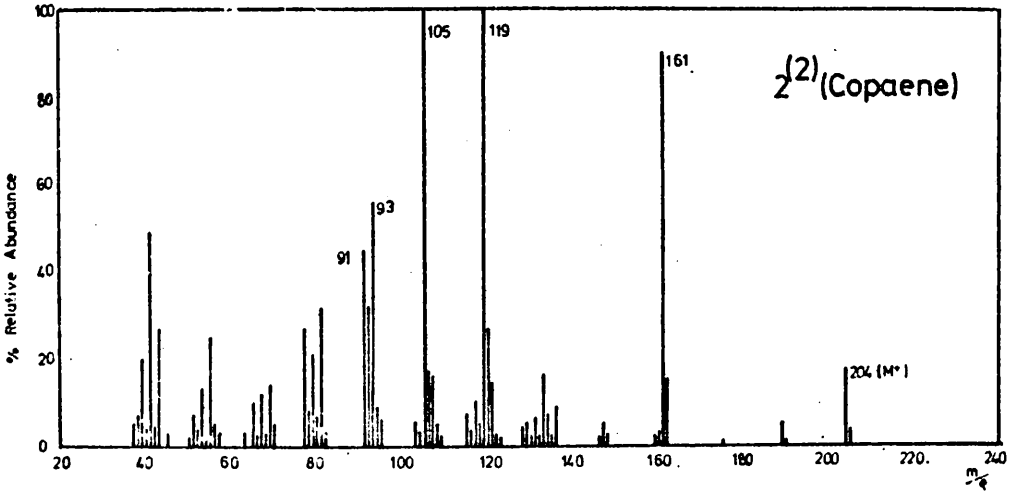
(cf.p.144). As cinnamodial could not be detected in fractions A and B by TLC, its concentration in the total oil was estimated directly from the weight of crystalline material afforded by portion C. Cinnamodial was thus found to represent 3% by weight of the total heartwood extract.

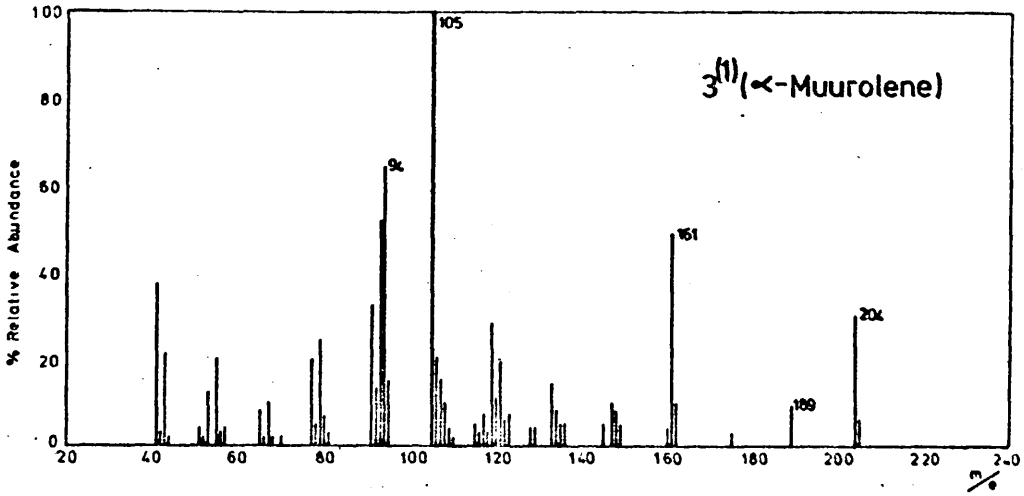
Appendix 2

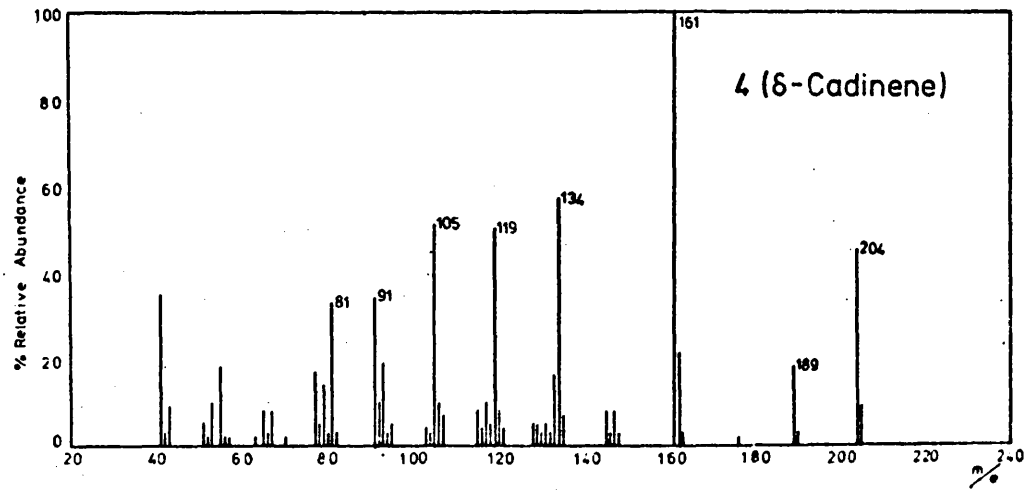
Mass Spectra of Constituents* of Essential Oil Muhuhu (see Part 2 of thesis). Proposed Identities of Compounds are indicated where possible, according to Table 31.

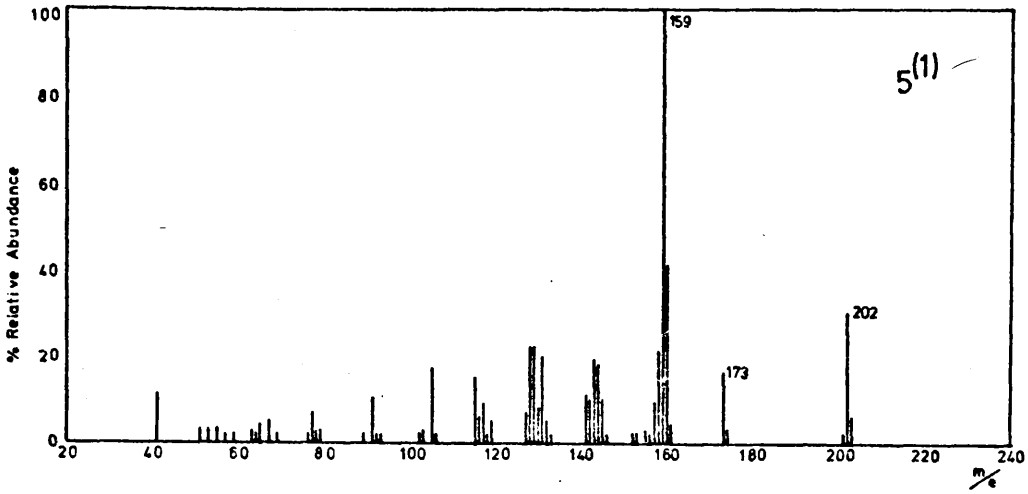
* $'A^{(b)}'$ = Component b of fraction A.

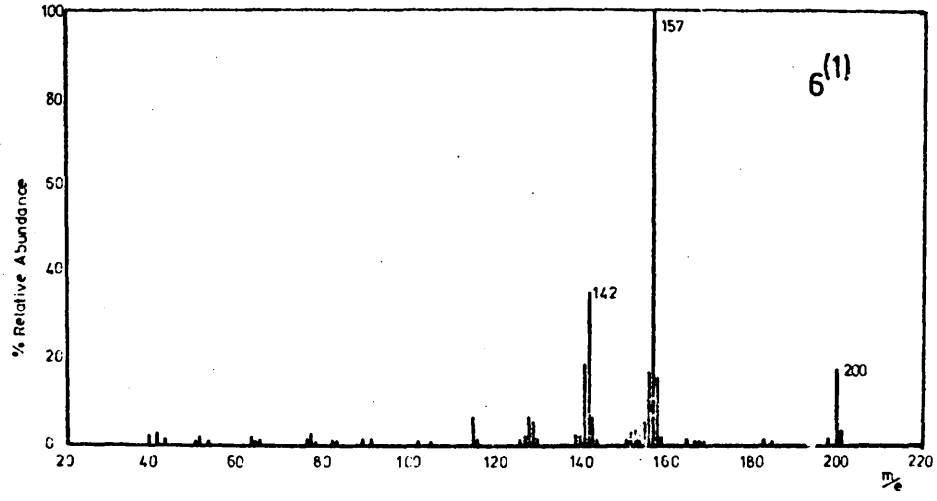

% Relative Abundance
100
80
60
40
20
0
20
40
60
80
100
120
140
160
180
200
220
240
m/e
105
93
119
120
91
161
2(1) (Ylangene)

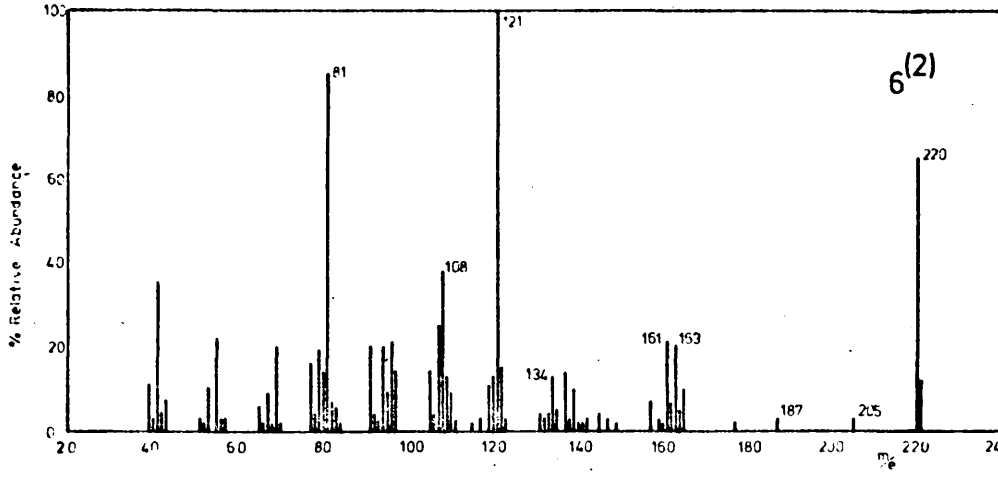

% Relative Abundance
100
80
60
40
20
0
20
40
60
80
100
120
140
160
180
200
220.
240
m/e
105
119
161
93
91
204 (M+)
2(2) (Copaene)

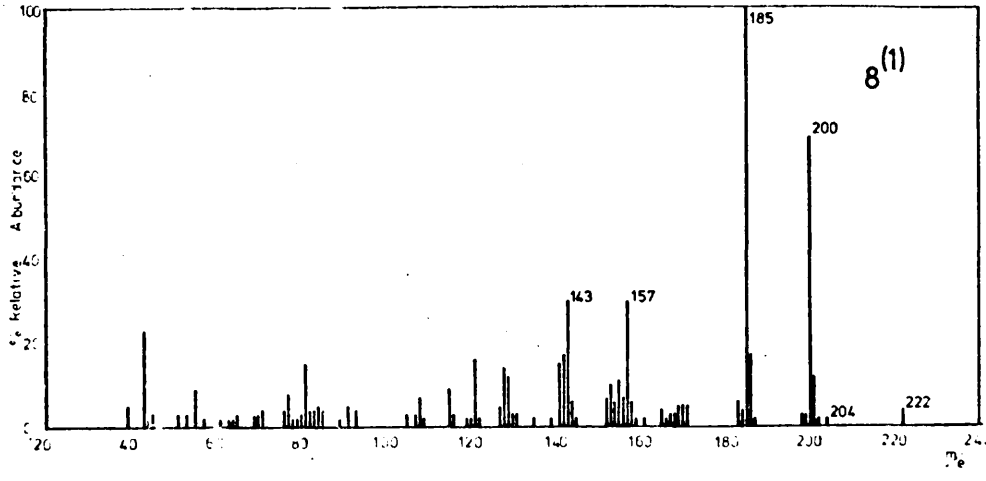

3(1)(∝-Muurolene)
% Relative Abundance
105
94
161
204
189
m/e

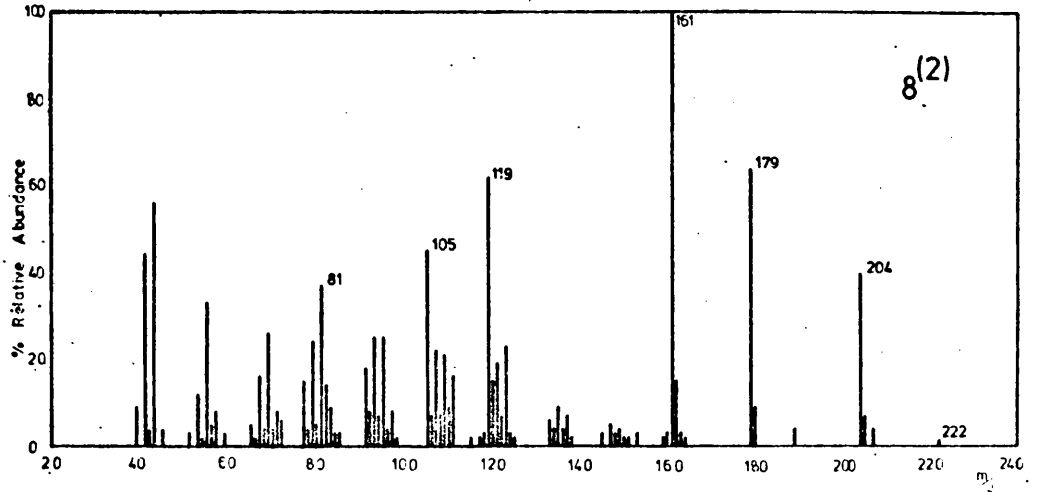

4 (δ-Cadinene)
% Relative Abundance
161
134
105
119
81
91
204
189
m/e

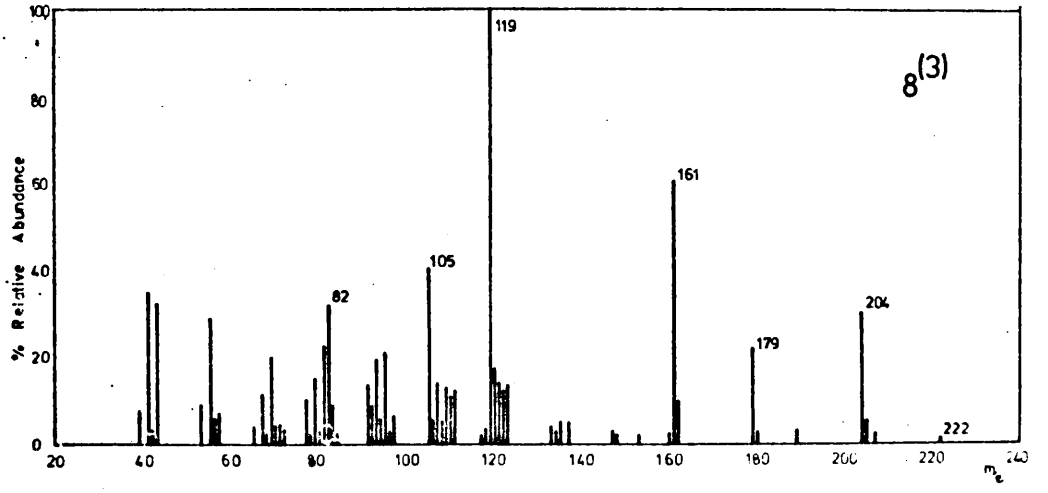

100
80
60
40
20
0
% Relative Abundance
159
202
173
20
40
60
80
100
120
140
160
180
200
220
240
m/e
5(1)

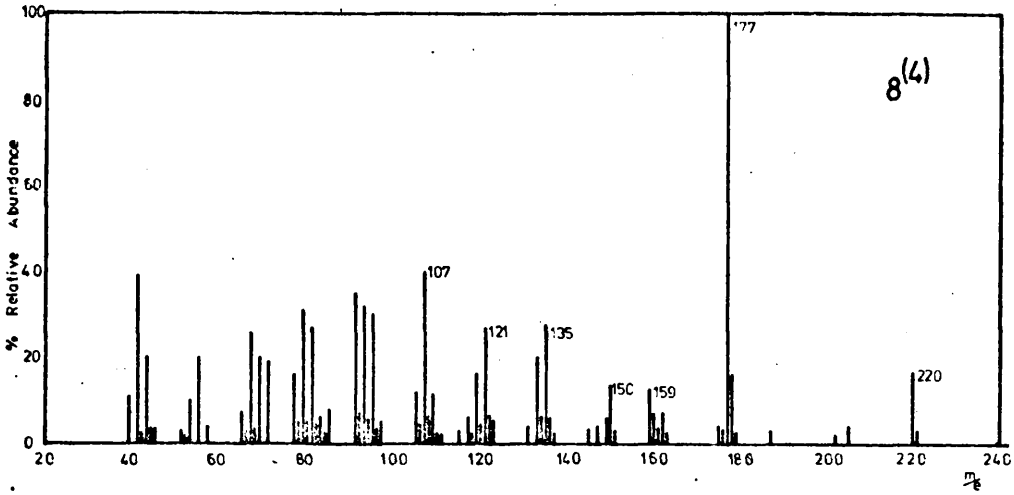

100
80
60
40
20
0
% Relative Abundance
157
142
200
20
40
60
80
100
120
140
160
180
200
220
m/e
6(1)

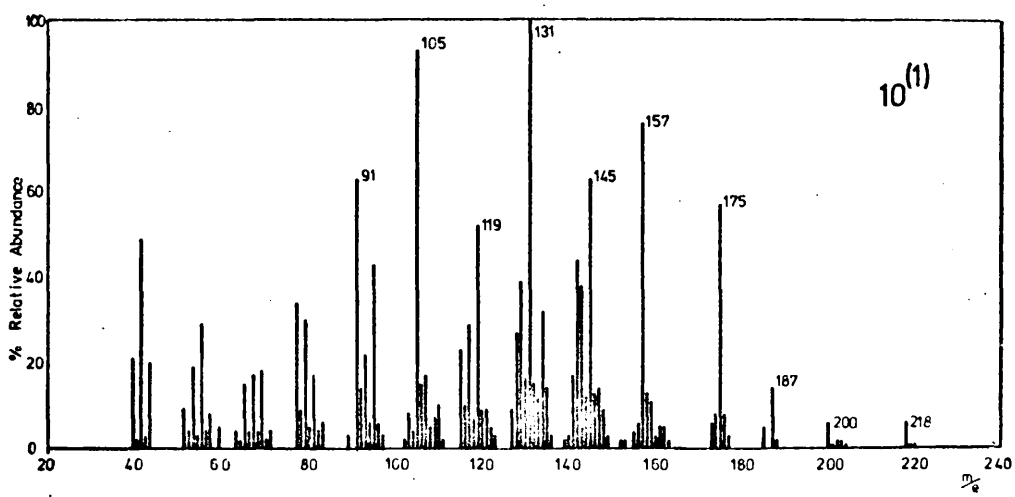

6(2)
% Relative Abundance
121
81
220
108
161
163
134
187
205
m/e

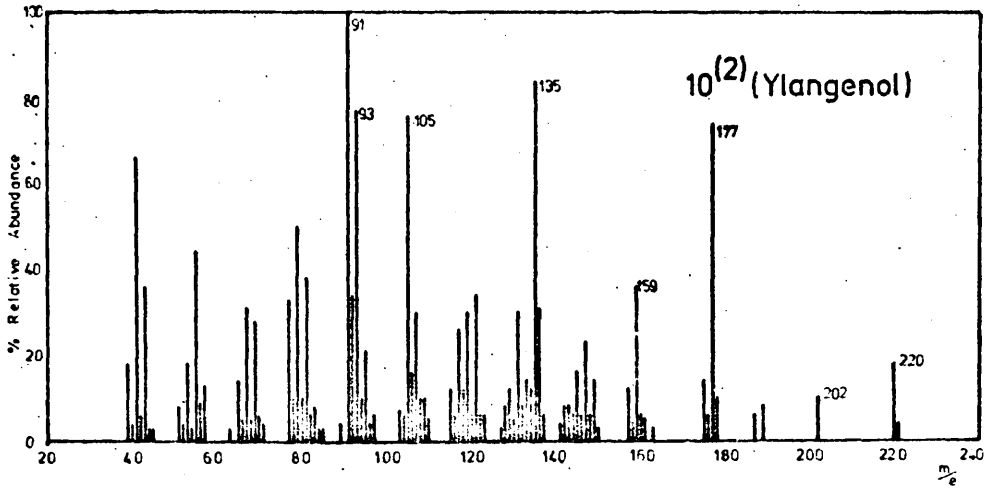

8(1)
185
200
143
157
204
222
m/e


8(2)
% Relative Abundance
100
80
60
40
20
0
20
40
60
80
100
120
140
160
180
200
220
240
m/e
161
179
119
105
204
81
222


8(3)
% Relative Abundance
100
80
60
40
20
0
20
40
60
80
100
120
140
160
180
200
220
240
m/e
119
161
105
204
82
179
222


8(4)
% Relative Abundance
177
107
121
135
150
159
220
m/e

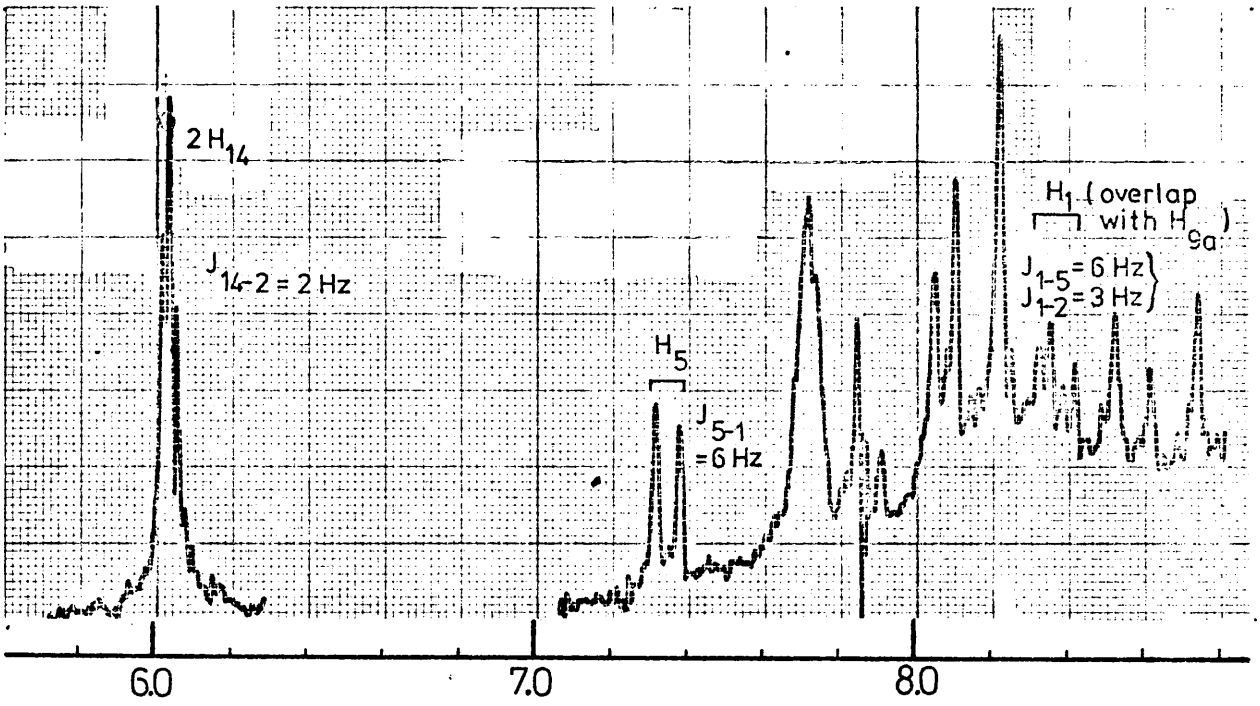

10(1)
% Relative Abundance
105
131
91
157
145
119
175
187
200
218
m/e


10(2) (Ylangenol)
% Relative Abundance
m/e
91
93
105
135
159
177
202
220
0
20
40
60
80
100
120
140
160
180
200
220
240

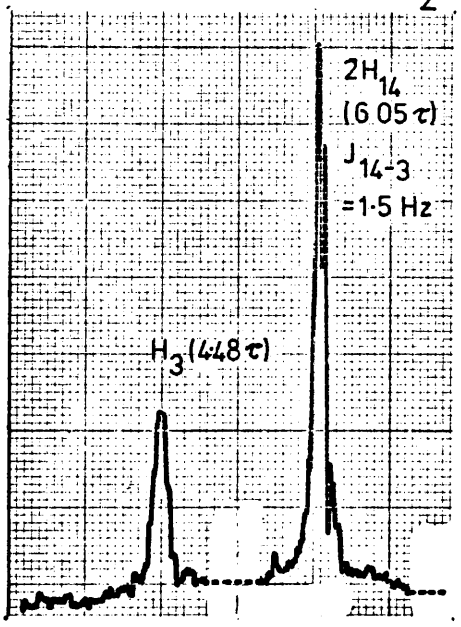
Appendix 3

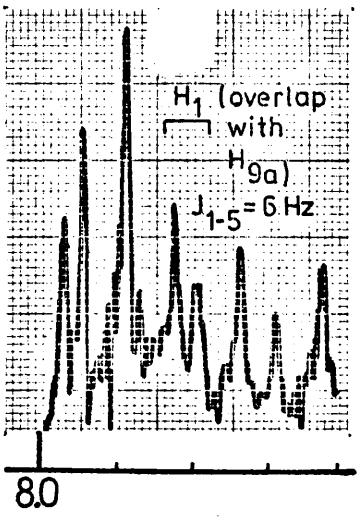
100 MHz NMR Spin Decoupling Experiments on C-8 Deuterated Brachylaena Diol (d): Examples (cf. pp. 55-58).

1. Irradiation on H_3 (c.f. Fig. 17).



2. Irradiation on H_2 (c.f. Fig. 17).





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