PHYSICO-CHEMICAL TECHNIQUES APPLIED TO ORGANIC NATURAL PRODUCTS

Studies of Sesquiterpenoids from *Warburgia ugandensis* (Sprague)

A thesis submitted to the University of Glasgow

for the degree of Ph.D.

by

George Harry Draffan

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Thanks are also due to my many colleagues for useful discussions and in particular to Dr. J.A. Zabkiewicz who has been associated with me in the work described in the final section. I gratefully acknowledge the assistance of Dr. L. Novotný of the Czechoslovak Academy of Sciences, who has provided several invaluable samples. Finally, I thank the technical staff of this Department for excellent service.

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

Department of Chemistry, University of Glasgow, August 1967.

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NOMENCLATURE

In drawings of formulae, bonds unless thickened or broken do not denote stereochemistry. "Wavy" lines indicate epimeric mixtures. Identical compounds may be referred to as e.g. 16 and later as 16A. This implies either that stereochemistry has been subsequently deduced, or that it is convenient to distinguish between the same compound derived from different sources.

GENERAL INTRODUCTION

Traditional interest in the extraction of plant essential oils came from their application in medicine, perfumery and colouring, properties which of course remain important.[1] As techniques developed very extensive studies of individual constituents were pursued leading to an immense body of chemical knowledge of natural products (exemplified in the terpenoid field by Simonsen's books[2]) and culminating in the unifying principles accepted today.[3,4] Trends in the study of natural products are now towards rapid structural elucidation with smaller quantities of material, and also to the clarification of more general biological and botanical problems.[5,6]

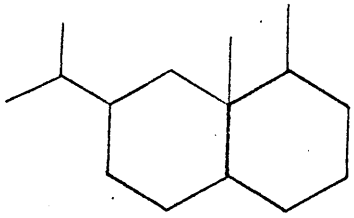
Our principal interests are in the isolation and identification of new plant products, notably those of the terpenoid group, and here modern physical methods have greatly altered the classical approach. Progress particularly in chromatographic separation techniques has enabled the purification of quantities on the gram or sub-milligram scale. At the same time methods of structural determination have developed from the classical oxidative fragmentations to the use of more specific reagents, and now to

the increasingly refined application of spectroscopic data. In many cases isolation of individual components is no longer a necessary first step in identification. The use of thin-layer and gas-liquid chromatography (TLC and GLC) and more recently the direct linking of GLC with mass spectrometry (GC-MS) allow rapid screening of a total oil. Thus, for example, a flavour extract can be examined by capillary GC-MS. In principle, with a suitable bank of mass spectra of known compounds, many constituents can be identified at once and structures proposed for other new compounds, or separations directed specifically at their isolation. Screening of this type also permits analysis of patterns of compounds in plants supposedly related botanically, thus simplifying once laborious chemotaxonomic studies.

The aim at the outset of the work described in this thesis was to select plant materials likely to contain sesqui- or diterpenoids and to examine these by TLC and GLC[‡] before attempting to isolate and identify new compounds. We also hoped to apply new techniques to comparative examination of related plants and eventually to study biosynthesis.

The species Warburgia ugandensis (Sprague) Canellaceae, native to East Africa, and a member of a group of aromatic trees, was selected. Interest was first aroused by a report[7] that it afforded "a fragrant wood, also a resin used locally for fixing

‡ GC-MS later became available but has chiefly been employed in the analysis of products from small scale reactions.



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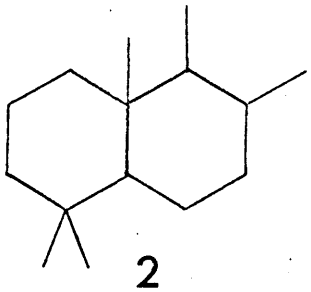

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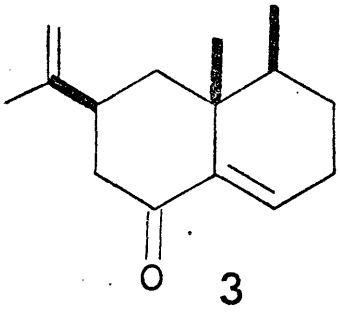

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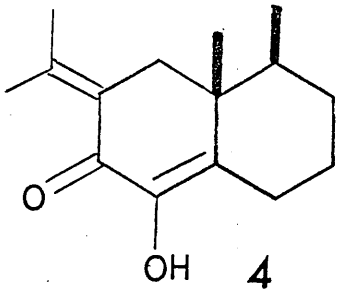

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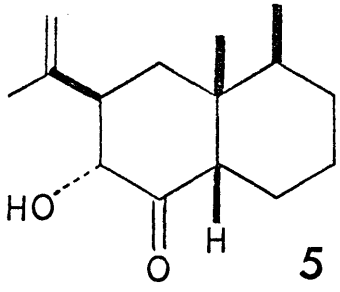

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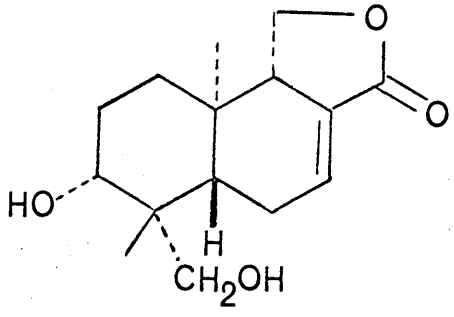

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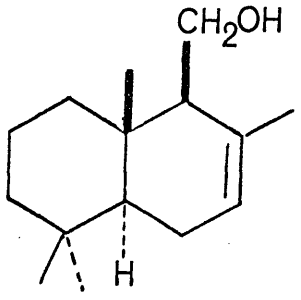

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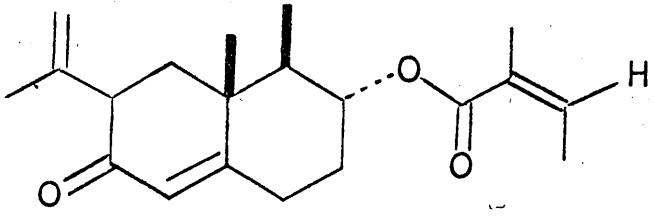

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tools in handles". There appeared to be no report in the literature of chemical studies on any species of Warburgia except W. stühlmannii, the bark of which gave an essential oil examined by W. Lenz in 1910.[8] It was our original intention to undertake comparisons of Warburgias with plants of related genera but this has had to be deferred because of difficulties in obtaining supplies of material.

The heartwood of Warburgia ugandensis proved to be a rich source of crystalline sesquiterpenoids. A consequence was that the scope of the work has been more classically oriented than was originally intended. The major part of the discussion deals with the identification of four new crystalline compounds. They belong to groups based on the eremophilane (1) and bicyclofarnesane (2) carbon skeletons. The eremophilanes have been of interest to terpene chemists since it was first observed that their biogenesis could not directly follow the classical isoprene rule.[9] The first members identified, and for almost twenty years the only known examples of the class, were eremophilone (3), hydroxyeremophilone (4) and hydroxydihydroeremophilone (5).[9] The bicyclofarnesanes too are of interest. They may be subdivided into the iresin (6)[10] and drimenol (7)[11] types which have antipodal configurations. Iresin and its analogues are oxygenated in ring A as in many of the higher terpenoids,

while compounds assumed to be elaborated from drimenol have so far been found oxygenated in ring B only.

An interest in the biogenesis of the eremophilane carbon skeleton (1) has led us to undertake a long-term project involving the feeding of ^{14}C-labelled precursors to the plant Petasites hybridus (L) Compositae. The sesquiterpenoid constituents of the Scottish variety of this species are of the petasin (8) type. The final section of the discussion describes the practical difficulties involved in attempting to confirm the proposed biogenetic pathway. The necessary groundwork has involved examination of the various plant parts by TLC and GLC and the development of small scale extraction and purification procedures. Preliminary incorporation results and proposed degradation schemes are also discussed. This work is currently being undertaken in collaboration with Dr. J.A. Zabkiewicz of this Department and Dr. A.M.M. Berrie of the Botany Department (Glasgow University).

Preliminary Examination of Warburgia ugandensis Heartwood: Isolation of Drimenol, Warburgin and Warburgiadione.

Supplies of Warburgia ugandensis were obtained from Uganda through the agency of the Tropical Products Institute of London. Two consignments of heartwood have been examined and found to differ quite markedly in physical appearance and in the chemical composition of their oils. It was the resinous nature and pleasant fragrance associated with the heartwood of the first batch received which caused us to make a preliminary small scale solvent extraction of the sawdust for GLC examination. The oil was extremely complex; however from the range of retention times on the phase SE-30[‡] the components were inferred to be chiefly of molecular weight consistent with sesquiterpenoid character, and we were encouraged to proceed with large scale separations.

The heartwood was finely ground and air-dried. Extraction of 2.1 kg with petroleum ether at room temperature gave 39 g of yellow oil (A). Despite careful removal of solvent, the odour associated with the untreated wood was greatly reduced and its chemical origin has not been determined. Chromatography of this first extract on neutral alumina allowed the isolation of

‡ The nature of the GLC phases, referred to by the customary abbreviations, is set out on p.34.

three crystalline components, shown to comprise the known sesquiterpene alcohol drimenol (7)[11] and two new compounds which we have named warburgin and warburgiadione. Fractions eluted later from the column consisted mainly of two non-crystalline compounds, W.H./V and W.H./XIV which have not been identified. Presently available physical data concerning these are noted in the experimental section (p. 38) and in table (13). Further extraction of the wood with ethanol, evaporation of the solvent, and partitioning of the resulting mixture of oily and solid material with chloroform gave 41 g of a dark viscous oil (B) from the chloroform soluble fraction. This extract also contained warburgin and warburgiadione: chromatography of (B) together with the mother liquors from (A) allowed the isolation of further quantities of these two most interesting compounds.

It was fortuitous that the original structural determination on drimenol should have been completed in Glasgow.[11] A supply of authentic material was therefore available and identity was proved by mixed melting point determination, comparison of optical rotation data, infrared, mass spectra and chromatographic behaviour.

Drimenol and related bicyclofarnesanes had previously been obtained from Drimys species in the family Winteraceae.* Our finding of drimenol in Warburgia ugandensis provided an

* Polygodial has also been obtained from Polygonum hydropiper (L).[12]

interesting chemotaxonomic link between the Winteraceae and Canellaceae. This supports the view that the families are allied despite the considerable morphological and geographical gap between them.[13]

GLC (SE-30 and QF-1) appeared to show warburgin to be the major component of the oil from this first heartwood yet a total of only 2.8g, before final purification, was obtained. This relatively low recovery was due in part to the complexity of the oil but chiefly to the remarkable instability of the compound to air and light. On silica gel TLC plates, spots exposed to air for a few minutes became purple. Preparative layer purification of mother liquors was therefore a wasteful procedure. Unless chromatographic columns were wrapped in dark paper the alumina became discoloured as the warburgin band was eluted. Solutions of the compound also darkened on standing. Crystallisation (from methanol) had to be carried out rapidly and was best followed by vacuum sublimation yielding pale yellow prisms, which showed only slight decomposition during storage for more than a year.

60 Mc./sec. NMR Spectrum of Warburgin ($CDCl_3$)

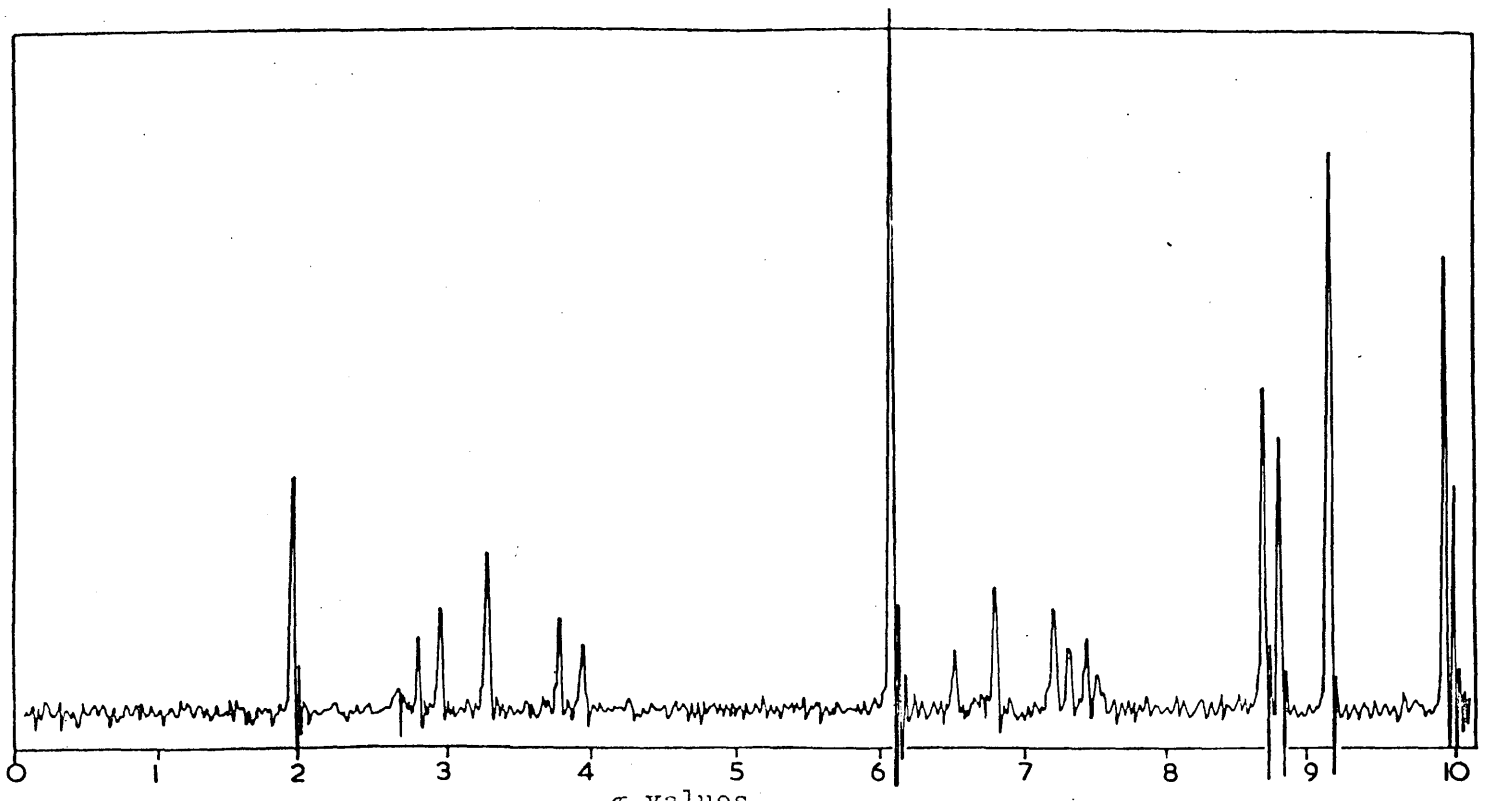


TABLE (1)

Warburgin: Physical Data

$C_{16}H_{16}O_4$ (analysis and mass spectrum).

Pale yellow prisms; m.p. 159-161°; $[\alpha]_D$ + 120° ($CHCl_3$)

λ_{max} (ethanol) 370 mμ (ε, 20,000): 285 mμ (inflection), 260 mμ (inflection), 252 mμ (ε, 3,800), 232 mμ (inflection), 220 mμ (ε, 5,000): unaltered in 0.05 N sodium hydroxide.

ν_{max} (CCl_4) 3156 w., 1734, 1679 and 1566cm^{-1}.

Mass spectrum (Appendix); molecular ion m/e 272 (base peak)

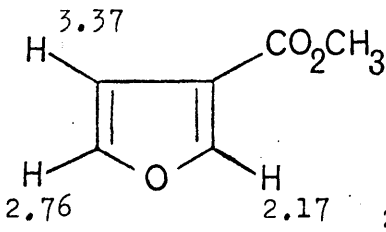
60 Mc./sec. NMR ($CDCl_3$) reproduced on facing page.

The Constitution of Warburgin

Proposed Structure

Table (1) gives the physical data recorded for warburgin. Elemental analysis indicated the formula $C_{16}H_{16}O_4$. The mass spectrum, beyond confirming the molecular ion, m/e 272, gave no obvious structural clues. An infrared band at 1679cm^{-1} indicated a conjugated ketone. This was supported by the preparation of a scarlet 2:4-dinitrophenylhydrazone. The absorption at the higher carbonyl frequency remained unchanged in the derivative. This band (1734cm^{-1} for carbon tetrachloride solution) could be accounted for by a simple ester, δ-lactone or α-pyrone.[14] A methoxyl group, evident from a peak at 6.10τ in the NMR spectrum was not at first associated with the possible ester function. However, alkaline hydrolysis followed by methylation regenerated warburgin as the major product and disclosed the presence of a methyl ester, rather than a lactone or pyrone with a methyl ether elsewhere in the molecule.

Evidence that the remaining oxygen should be accommodated in a furan ring came from infrared bands at 3156 and 1566cm^{-1}. These bands can be associated with furan ν_{CH} and ν_{ring} modes.[15,16] Confirmation of this structural feature, and further important evidence of constitution, was derived from nuclear magnetic resonance (NMR) spectrometric data as follows. In an NMR study of variously 2- and 3-mono-substituted furan derivatives, Gronowitz



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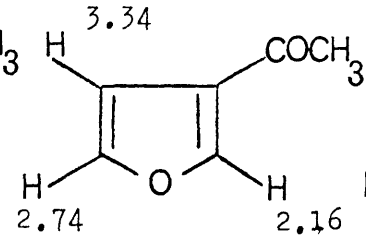

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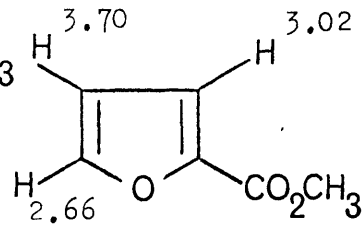

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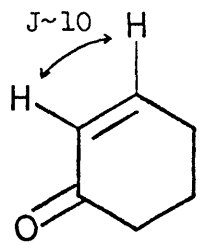

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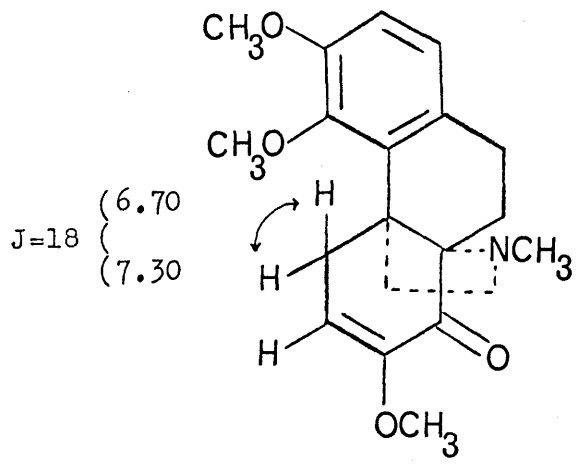

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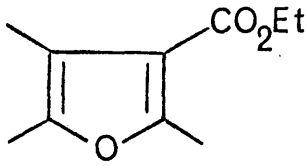
Formulae 9-13: chemical shifts as τ values; J values in c./sec.



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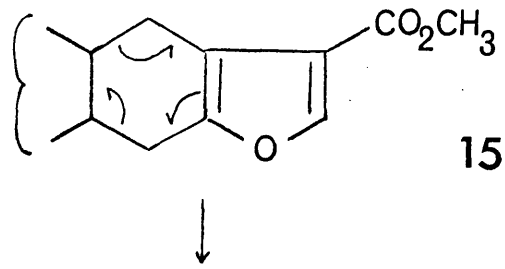
λ_{max} 259 mμ (ε,3,300)



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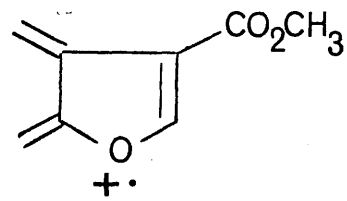


m/e 152

et al.[17] report ring proton τ values in the range 2.17 to 5.04 and coupling constants 0.7 to 3.55 c./sec. Electron donating substituents tend to shield the ring protons while electron withdrawing groups have a deshielding effect. Some examples are quoted: formulae (9)-(11). Of the four low-field protons in the NMR spectrum of warburgin, two were strongly coupled (doublets at 3.86 and 2.91τ, J= 9 c./sec.) and could not be associated with the furan ring. They best fitted the ethylenic protons of an enone of type (12).[18] The remaining protons were sharp singlets at 3.29 and 1.96 τ. By analogy with (9) and (10) the low-field proton was tentatively assigned to a suitably deshielded furan α-position. The peak at 3.29 τ could then be due to the proton of a tri-substituted double bond.

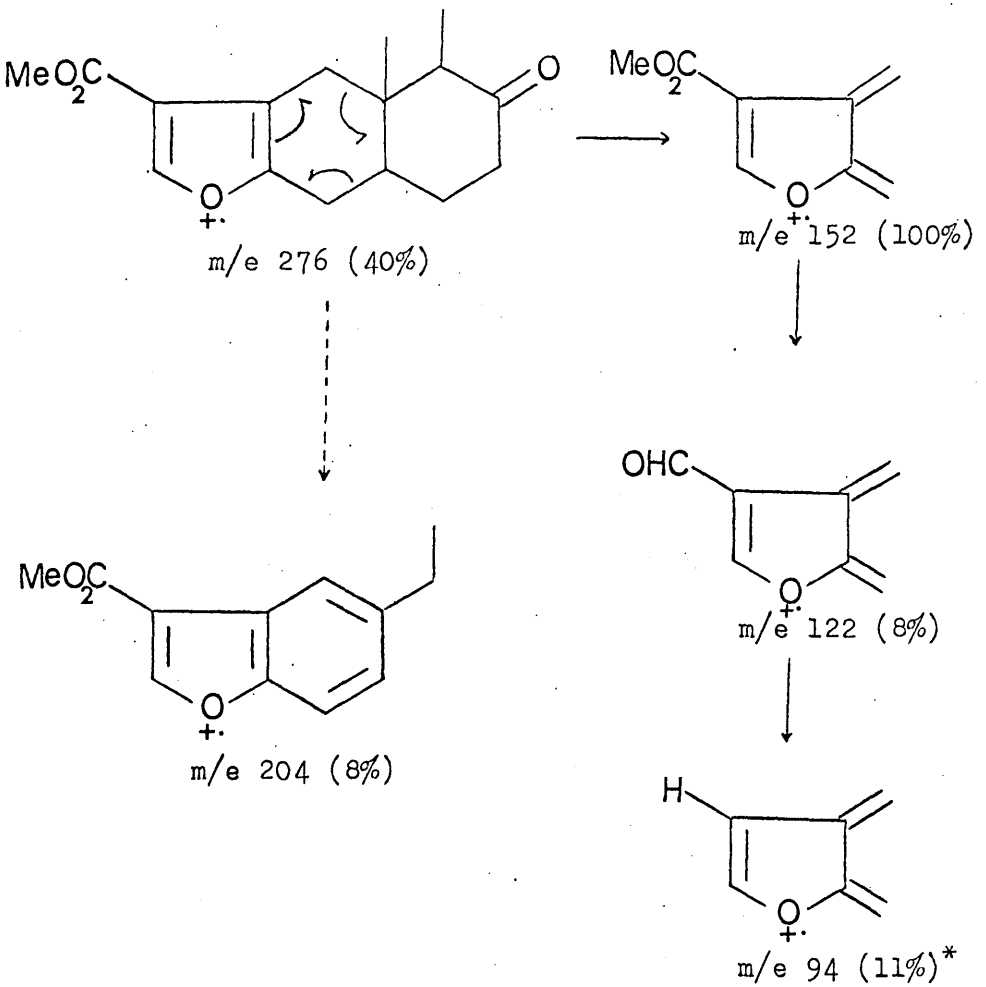
The NMR spectrum also indicated a tertiary methyl group (9.17 τ) and a secondary methyl (8.76 τ, doublet J=7 c./sec.) coupling with a deshielded methine (7.39, quartet J= 7 c./sec.). Finally a coupling of 17 c./sec. between protons at 7.31 and 6.72 τ strongly suggested a deshielded methylene in an environment that was both strained (implied by the large geminal coupling, J_{AB}) and dissymmetric (implied by the marked difference in chemical shift between A and B). A situation of this type must exist, for example, in compound (13).[19]

Before proposing a structure we considered the following

further evidence. Catalytic hydrogenation of warburgin in ethyl acetate with 10% palladium on charcoal as catalyst gave a single colourless crystalline product, tetrahydrowarburgin. Elemental analysis indicated the formula $C_{16}H_{20}O_4$ and the uptake of two moles of hydrogen. The infrared spectrum in carbon tetrachloride showed the expected weak furan ν_{CH} frequency at 3155cm^{-1} and carbonyl bands at 1730 (ester) and 1721cm^{-1} (saturated ketone). In the NMR spectrum the only low-field proton was that assigned to the furan which appeared as a sharp singlet at 2.07 τ. The spectrum also showed the expected peaks indicative of angular methyl (9.33 τ), secondary methyl (8.91 τ, J= 7 c./sec.) and methoxyl (6.16 τ) groups. The ultraviolet spectrum which had λ_{max} 255 mμ (ε, 2650) was as expected for a furan 3-carboxylic ester [compare (14)].[20] Evidence supporting the placing of the carbomethoxyl function on the furan ring also came from the mass spectrum which had the base peak, m/e 152, which we considered to arise by "retro-Diels-Alder"[21] cleavage promoted by the furan ring as in (15).

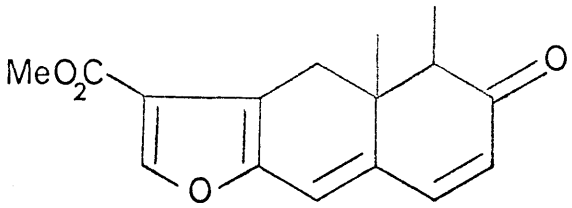
The above physical and chemical data allowed few structural possibilities for warburgin. Structure (16) (excluding stereochemistry) was preferred since it fitted the known sesquiterpene carbon skeleton (1), that of the eremophilane class. The

Fig.(1) Suggested Mass Spectral Fragmentation for Compound (17)‡



‡ Solid arrows denote transitions for which metastable ions were recorded. Other modes of transition are not ruled out.

* Alternatively m/e 94 may arise by loss of CO from the furan nucleus.



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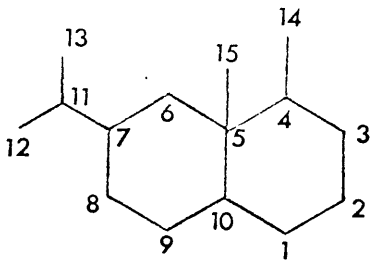

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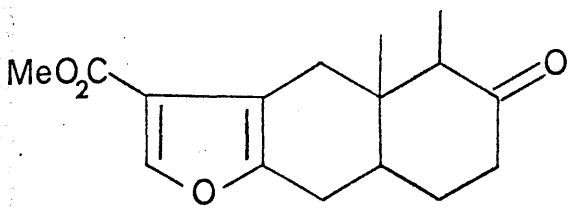

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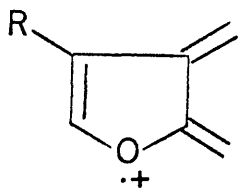

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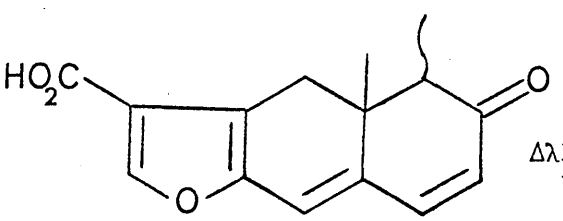
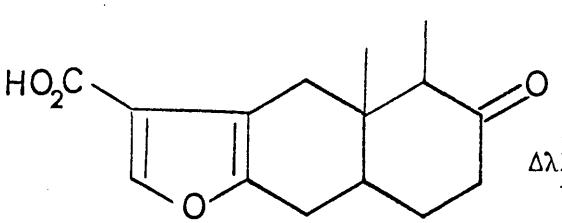
16 ⟶



$\Delta\lambda^{EtOH}_{EtOH\text{-}OH^-}$ + 13 mμ

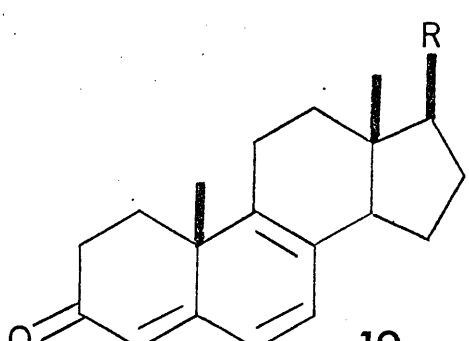
20

17 ⟶



$\Delta\lambda^{EtOH}_{EtOH\text{-}OH^-}$ -11 mμ

22



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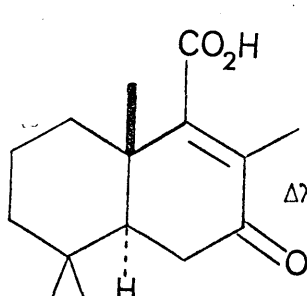


$\Delta\lambda^{EtOH}_{EtOH\text{-}OH^-}$ + 13 mμ

21

hydrogenation product, tetrahydrowarburgin, then has structure (17). An analysis of the mass spectrum is given in fig.(1). "Retro-Diels-Alder" cleavage, where ions corresponding to the furan-derived fragments (18) were the most abundant in the spectra, was later found to be characteristic in a series of degradation products from tetrahydrowarburgin.

No direct analogy for the ultraviolet spectrum of warburgin could be found. However the steroidal trienone (19) has a comparably extended chromophoric system and shows absorption at 388 mμ (ε, 12,300).[22]

Hydrolysis of warburgin in refluxing ethanolic potassium hydroxide gave two products as judged by TLC using the solvent system benzene-dioxan-acetic acid (90:25:4 by volume) (R_f values 0.45, 0.48; warburgin 0.70). Both gave red stains with 2:4-dinitrophenylhydrazine reagent: they were inferred to be ketoacids (20) from their TLC behaviour and from the infrared spectrum of the total product in chloroform, which had a broad hydroxyl band (2400-3500cm^{-1}), and carbonyl absorptions at 1740cm^{-1} (acid "monomer": weak), 1698cm^{-1} ("dimer") and 1675cm^{-1} (enone). The ultraviolet spectrum had λ_{max} (mμ) 372 (ethanol), 389 (ethanol-OH^-) and 375 (reacidified with HCl). Similar bathochromic shifts with alkali have been observed in the ultraviolet spectrum of (21)[11] and other γ-oxo αβ-unsaturated

acids where the shift probably depends on the ability of the ketone to enolise.[23] It is significant that the tetrahydro-ketoacid (22) obtained by hydrolysis of tetrahydrowarburgin (17) (see below) shows a distinct hypsochromic shift in alkali ($\Delta\lambda$= -11 mμ). The opposite shift observed with (20) must then also be due to enolisation of the ketone as in the γ-oxo series.

No attempt was made to purify the acidic products (20). The mixture was treated with ethereal diazomethane. Warburgin accounted for approximately 70% of the product and was identified by TLC through its characteristic fluorescence, decomposition and staining, and also by GLC (1% SE-30). A second product not fully resolved but estimated as 30% by GLC was slightly more polar and highly unstable in air. It was isolated by preparative TLC as a discoloured oil and had ν_{max} 1734 and 1676cm^{-1} in carbon tetrachloride and λ_{max} 372 mμ (ε, ca. 20,000) in ethanol. Difficulties in isolation and purification have prevented complete identification. However, the mass spectrum had the molecular ion, m/e 272, corresponding to $C_{16}H_{16}O_4$. The fragmentation pattern was similar to that for warburgin. It is reasonable to assume that this compound is the C-4 epimer of warburgin.

Alkaline hydrolysis of tetrahydrowarburgin gave a product which appeared homogeneous by TLC in the solvent system benzene-dioxan-acetic acid (90:25:4 by volume). The crude product had λ_{max} (mμ) 253 (ethanol), 242 (ethanol-OH^-) and 253 (reacidified

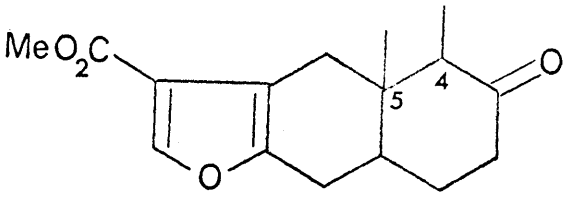
with HCl). Methylation with diazomethane regenerated (17). There was no evidence of the presence of possible C-4 epimers in the total methylation product by TLC or GLC under conditions which resolved the warburgin epimers.

Several other reactions of warburgin were briefly investigated in the preliminary stages of the study. Reduction with lithium aluminium hydride or with sodium borohydride gave complex mixtures of unstable products. Attempted dehydrogenation with 5% palladium on charcoal not surprisingly also gave numerous products. When these reactions were found to be complicated they were not pursued further in view of the limited availability of starting material.

Structural Proof

We considered that the evidence detailed above strongly supported structure (16) (excluding stereochemistry) for warburgin. It was decided to attempt a proof of the gross structure by a stepwise degradation to a furanohydrocarbon for comparison with the naturally occurring furanoeremophilane (23) of known absolute configuration[24] (see also p. 21).

The position of the ketone function in warburgin would have remained uncertain. We originally hoped to prove this point by making the enol acetate in which the C-4 methyl would give rise to a singlet at about 8 τ in the NMR spectrum. This was not



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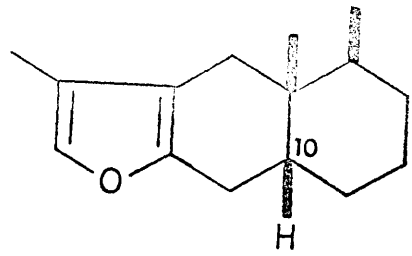

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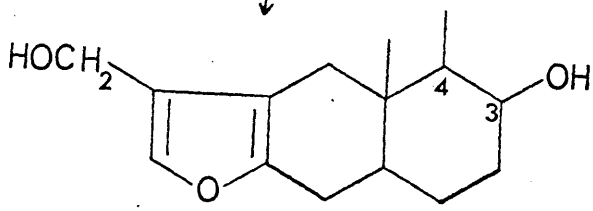

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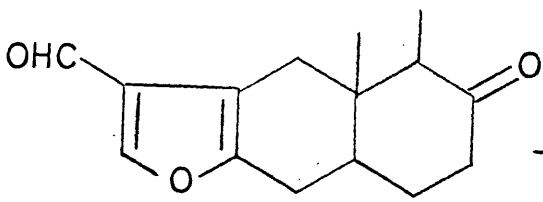

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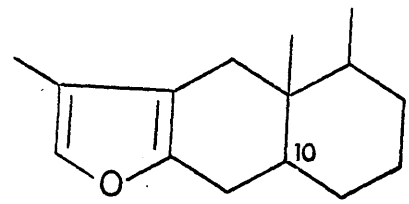

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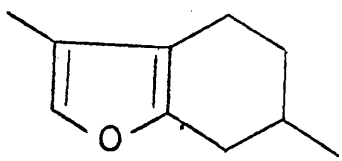

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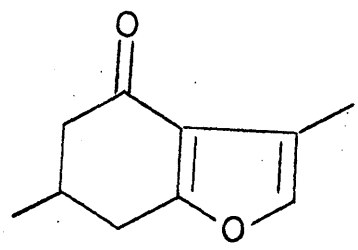
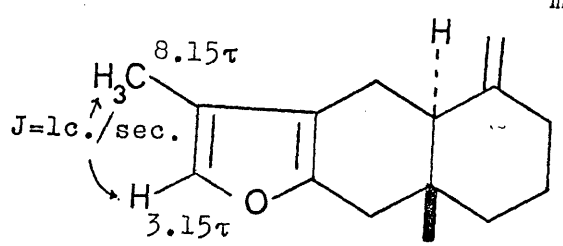
λ_{max} 222 mμ (ε,6,020)



28

λ_{max} 265 mμ (ε,3,700)



29

successful. Enol acetylations using a variety of conditions gave unstable products which were not adequately characterised. However, subsequent work by Ourisson and co-workers, discussed fully below, made further pursuit of this theme unnecessary.

It was proposed to obtain the required furanohydrocarbon (26) from tetrahydrowarburgin (17) by lithium aluminium hydride reduction to diol (24), oxidation to keto-aldehyde (25) and Huang-Minlon reduction.[25]

Reduction of tetrahydrowarburgin with excess lithium aluminium hydride in anhydrous tetrahydrofuran at room temperature gave a rather unstable low melting product. The infrared spectrum showed no carbonyl absorption and at high dilution in carbon tetrachloride two hydroxyl bands of approximately equal intensity at 3624 and 3615cm^{-1}. Absorption in the ultra violet region at λ_{max} 221 mμ (ε, ca.5000) was also consistent with structure (24). [Compare menthofuran (27) which has λ_{max} 222 mμ (ε, 6020).[26]] A singlet in the NMR spectrum at 5.52 τ was assigned to the methylene protons of the primary furan alcohol and a multiplet at 6.15 τ to the methine proton of the secondary alcohol. The mass spectrum had the required molecular ion, m/e 250 and the expected "retro-Diels-Alder" ion as the base peak, m/e 124 [(18), R= CH_2OH]. The crude reaction product was homogeneous according to GLC on the phases SE-30 and Apiezon L.

Fig.(2) Suggested Mass Spectral Fragmentation for Ketoaldehyde (25) ǂ

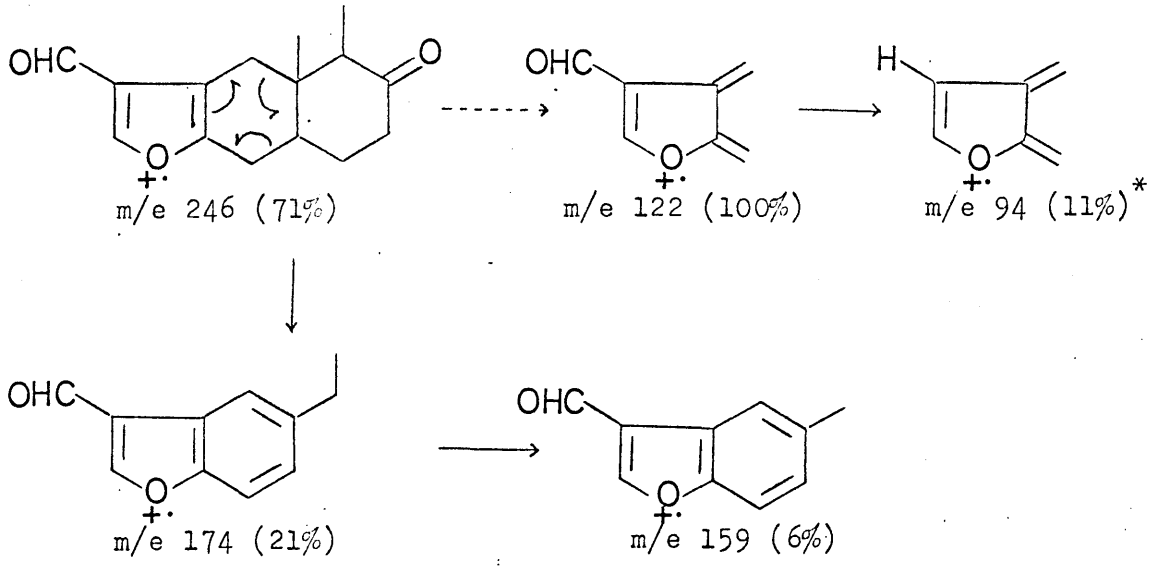
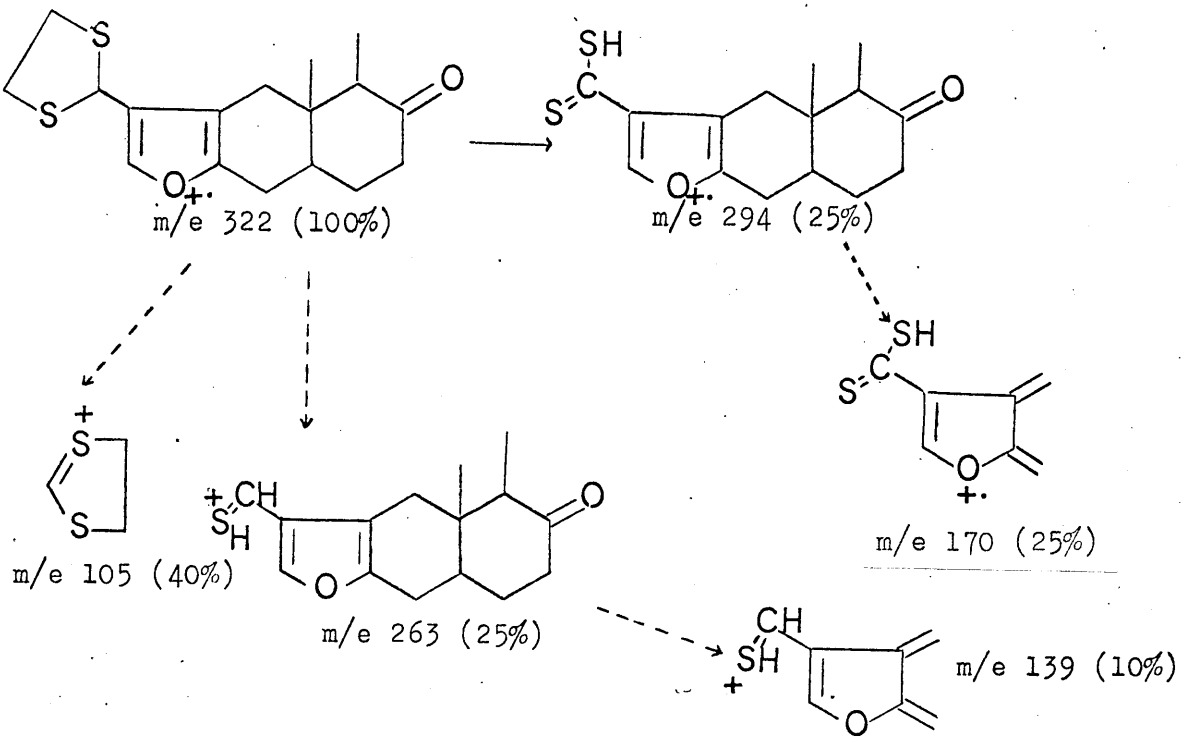


Fig.(3) Suggested Mass Spectral Fragmentation for Thioacetal (38) ǂ



ǂ Solid lines denote transitions for which metastable ions were recorded. Other modes of transition are not ruled out.

* m/e 94 may arise by loss of CO from the furan nucleus [see also fig.(1)].

TLC indicated a major product (85-90%) accompanied by minor polar components.

Difficulties arising from the instability of the diol and doubts as to its stereochemical homogeneity at C-3 and C-4 were resolved when oxidation with chromium trioxide in pyridine (Sarett)[27] gave the crystalline and therefore readily purifiable ketoaldehyde (25). The infrared spectrum had bands at 2724 (ν_{CH} of aldehyde), 1718 ($\nu_{C=O}$ of ketone) and 1690 ($\nu_{C=O}$ of aldehyde)cm^{-1}. The unsaturated aldehyde chromophore absorbed in the ultraviolet region at 271 mμ (ε, 2600). Evodone (28) with λ_{max} 265 mμ (ε, 3700) is a suitable analogy.[28] In the NMR spectrum the furan proton appeared at 2.03 τ and the aldehydic proton at 0.01 τ. The mass spectrum, fig.(2), was similar to that from tetrahydrowarburgin (17). An ion, m/e 174 (21%) apparently formed from the molecular ion may be due to a substituted benzofuran. A possible corresponding fragment is present in the spectrum of (17), [m/e 204 (8%)].

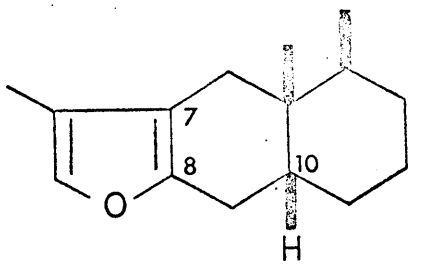
Huang-Minlon reduction of the ketoaldehyde (25) (in refluxing ethylene glycol) gave a product mixture containing approximately 60% furanohydrocarbon (26) as judged by TLC. This product resembled authentic furanoeremophilane‡(23) in polarity (R_f 0.70 in benzene) and staining characteristics. A

‡ A sample of cis-furanoeremophilane (23) was supplied by Dr. L. Novotný of the Czechoslovak Academy of Sciences, Prague.

second more polar product (R_f 0.40 in benzene) may have been incompletely reduced material but proved to be unstable and was not characterised. Compound (26) was isolated as an unstable fragrant oil by preparative TLC and was found to be clearly separable from furanoeremophilane (23) by GLC on the phase 10% Apiezon L. An unresolved peak at 3.05 τ in the NMR spectrum was assigned to the furan α-proton coupling with the furan methyl group at 8.13 τ (doublet J, ca. 1.5 c./sec.). Closely similar data are recorded for atractylon (29)[29] Weak bands in the liquid film infrared spectrum of furanoeremophilane (23) at 1576, 1660, 1776 and 1810cm^{-1} have been assigned to the furan ring.[30] We observed only two corresponding bands (at 1565 and 1648cm^{-1}) in the spectrum of (26). The spectra showed other significant differences. However the closely similar mass spectra (Appendix) proved the two compounds to be isomeric. Conversion of tetrahydrowarburgin into an isomer of cis-furanoeremophilane suggested a possible trans-ring fusion in (17), (24), (25) and (26). It is convenient to assume, for purposes of exposition, that the observed distinction between (23) and (26) is due to the 10α-H stereochemistry in (26) i.e. as (26A).

Hydrogenation of (26A) in acetic acid with platinum oxide catalyst gave two major products in a ratio of 3:2 as judged by GLC. They were separable from an authentic sample of tetrahydrofuranoeremophilane (30)[24 ≠] and were proved to be isomeric

≠ A sample of (30) was provided by Dr. L. Novotný.



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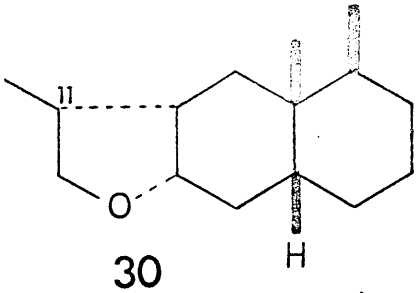

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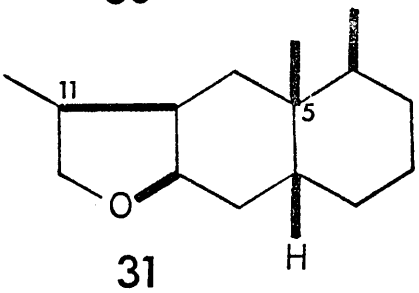

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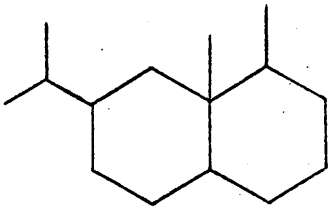

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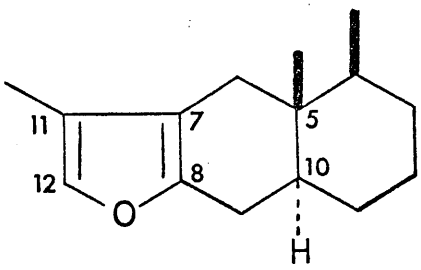
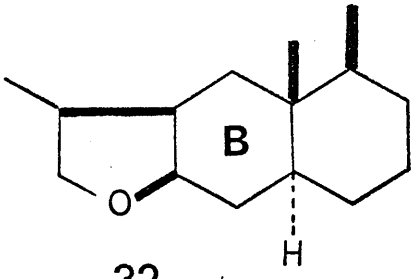


26A



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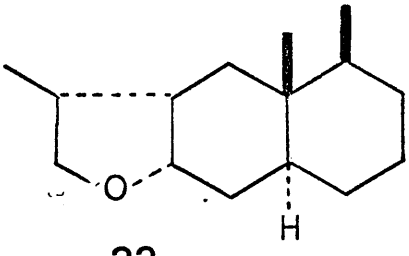


33

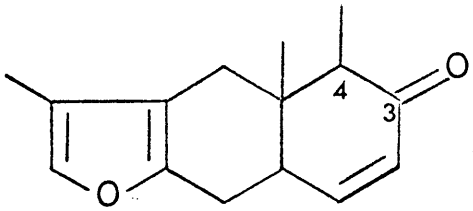
with (30) by GC-MS. A minor, poorly-resolved peak of markedly shorter retention had molecular ion 208 corresponding to $C_{15}H_{28}$ and was evidently a mixture of isomeric eremophilanes (1). Hydrogenation of Novotný's cis-furanoeremophilane (23) under the same conditions gave as the major product the known compound (30). Analysis by GLC disclosed a minor peak attributable to an eremophilane, results consistent with those reported.[24,30] We observed a further minor product (about 2%) which proved (from GC-MS evidence) to be another isomer of tetrahydrofuranoeremophilane. GLC and GC-MS data for the isomeric eremophilanes and tetrahydrofuranoeremophilanes are summarised in table (5) in the experimental section p.47.

Eight possible configurational isomers can be formally obtained from the catalytic hydrogenation of each isomer of furanoeremophilane. We have observed two from each reaction. Of these only (30) is known and even in this case a configurational assignment at C-11 has not been made. From examination of a model it would appear that hydrogenation of the 7-8 double bond of cis-furanoeremophilane (23) should take place preferentially from the β-face giving (30) as the major product. The minor isomeric product (2%) which we observed may then be (31) resulting from reaction from the α-face. Conformations in which potential steric interaction between the C-11 and C-5 methyls is relieved - regardless of the configuration at C-11 - can be

postulated for (31). We regard as fortuitous the apparent similarity of retention and mass spectral data for this compound and one of the isomers from trans-furanoeremophilane, [experimental section table (5)].

The 7-8 double bond of trans-furanoeremophilane (26A) is less hindered to hydrogenation from the α-face of the molecule. The product would then be (32). However with a β-oriented 11-CH_3, interaction with the 5-CH_3 would be extreme and could only be relieved by distortion of ring B. With an α-oriented 11-CH_3, the situation is more favourable, but this would require that hydrogenation of the 7-8 and 11-12 double bonds should take place from opposite sides of the molecule - a rather remote possibility. Hydrogenation of the 7-8 double bond from the β-face would give (33) which is relatively strain free. The possibility of a trans-7-8 ring fusion in the tetrahydrofurano-eremophilanes arising from an initial 1,4 reduction of the furan ring also exists. We are therefore not justified in attempting to predict the stereochemistry of the products of hydrogenation from trans-furanoeremophilane.

At this stage we were informed by Dr. K.H. Overton of this Department of the then unpublished work of Professor G. Ourisson and collaborators on the constitution as (34) (excluding stereochemistry) of furanoligularenone isolated from



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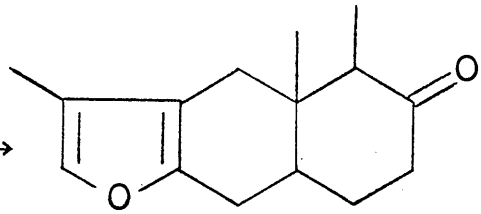

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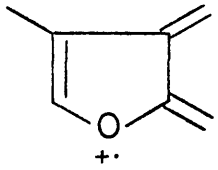
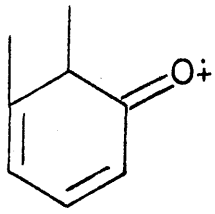


m/e 108

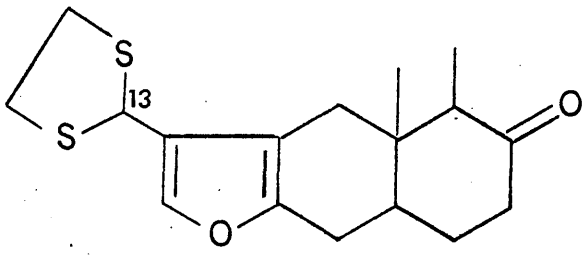
36



m/e 122

37

25 ⟶



38

⟶ 35A ≡ 35

Ligularia fischeri (Compositae).[31] A significant point in the structural proof was the base-catalysed exchange deuteration of furanoligularanone (35), when the C-4 doublet methyl resonance in the NMR spectrum became a broad singlet: (CH_3-CH-)--->(CH_3-CD-). This observation placed the carbonyl function unambiguously at C-3. The mass spectrum of (34) showed the expected cleavage to the base peak, m/e 108 (36). The complementary fragment, m/e 122 (37), was also recorded as a positive ion.

We achieved a correlation of the ketoaldehyde (25) with furanoligularanone (35) by making use of the relatively hindered nature of the ketone function in (25). Treatment with 1.1 moles of ethanedithiol in ether with boron trifluoride etherate as catalyst resulted in selective conversion to the thioacetal (38).‡ This compound was characterised by its infrared absorption ($\nu_{C=O}$ 1717cm^{-1} in carbon tetrachloride) and by its NMR and mass spectra. The NMR spectrum was especially characteristic. The four thioacetal methylene protons appeared as a sharp singlet at 6.72 τ. Singlets at 4.58 and 2.79 τ were assigned to the C-13 proton and furan proton respectively. The mass spectrum was also of interest in that it did not give an observable direct "retro-Diels-Alder" cleavage of the parent ion. A tentative rationalisation of the spectrum is given in fig. (3), [facing p.15].

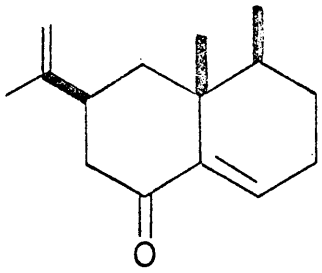
Difficulties were encountered in the Raney nickel reduction of (38). It was found that mild conditions (refluxing in acetone) left starting material unchanged while forcing

‡ We thank Dr. K.H. Overton for suggesting this experiment.

conditions (refluxing in dioxan) caused decomposition. After a number of trial reactions a 40% yield of the required furanoketone was obtained by brief treatment in refluxing dioxan using freshly prepared Raney nickel.[32] The product was purified by preparative layer chromatography as an unstable oil and proved to be inseparable from an authentic sample of furanoligularanone (35)‡ on five GLC phases. The infrared, NMR and mass spectra were identical. It was necessary to "seed" our product with Ourisson's sample in order to obtain it in crystalline form. Sublimation gave material of m.p. 86-88° not depressed on mixing with furanoligularanone. Final proof of identical stereochemistry came from a comparison of optical rotatory dispersion data, to be discussed fully below.

At this stage we had satisfactorily proved the gross structure of warburgin as (16). The stereochemistry of the C-4 and C-5 methyl groups and the nature of the ring fusion (tentatively assumed to be trans) in tetrahydrowarburgin (17) and its congeners remained in doubt.

‡ A sample of furanoligularanone was supplied by Professor G. Ourisson, Institut de Chimie, Strasbourg. Furanoligularanone is designated as (35) and our degradation product [proved to be identical with (35)] is denoted (35A).



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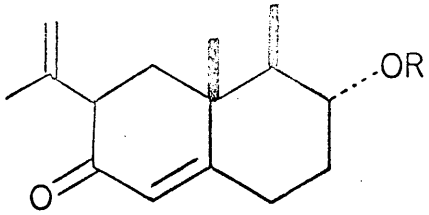
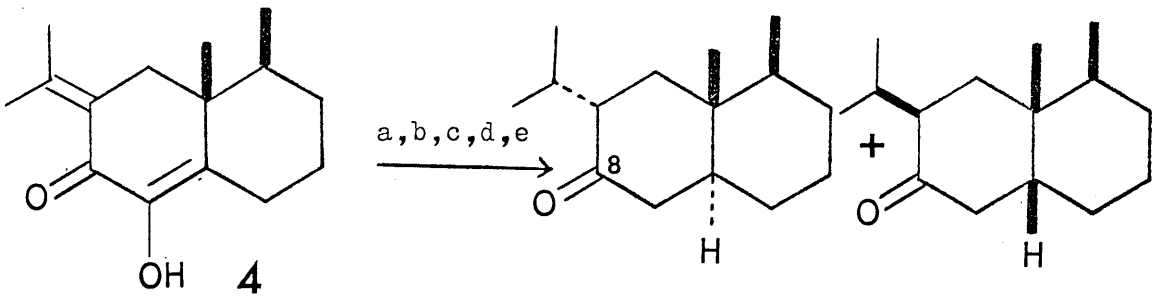


8 R = CO.CH(CH_3)=CH(CH_3) cis-

Fig.(4) Transformations of Hydroxyeremophilone (4)



4

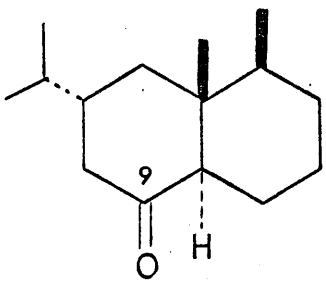
a,b,c,d,e

39

40

a,e (ketone-alcohol exchange)

b,c,d,e



41

a) H_2/Pd-C; b) Ac_2O/pyridine;

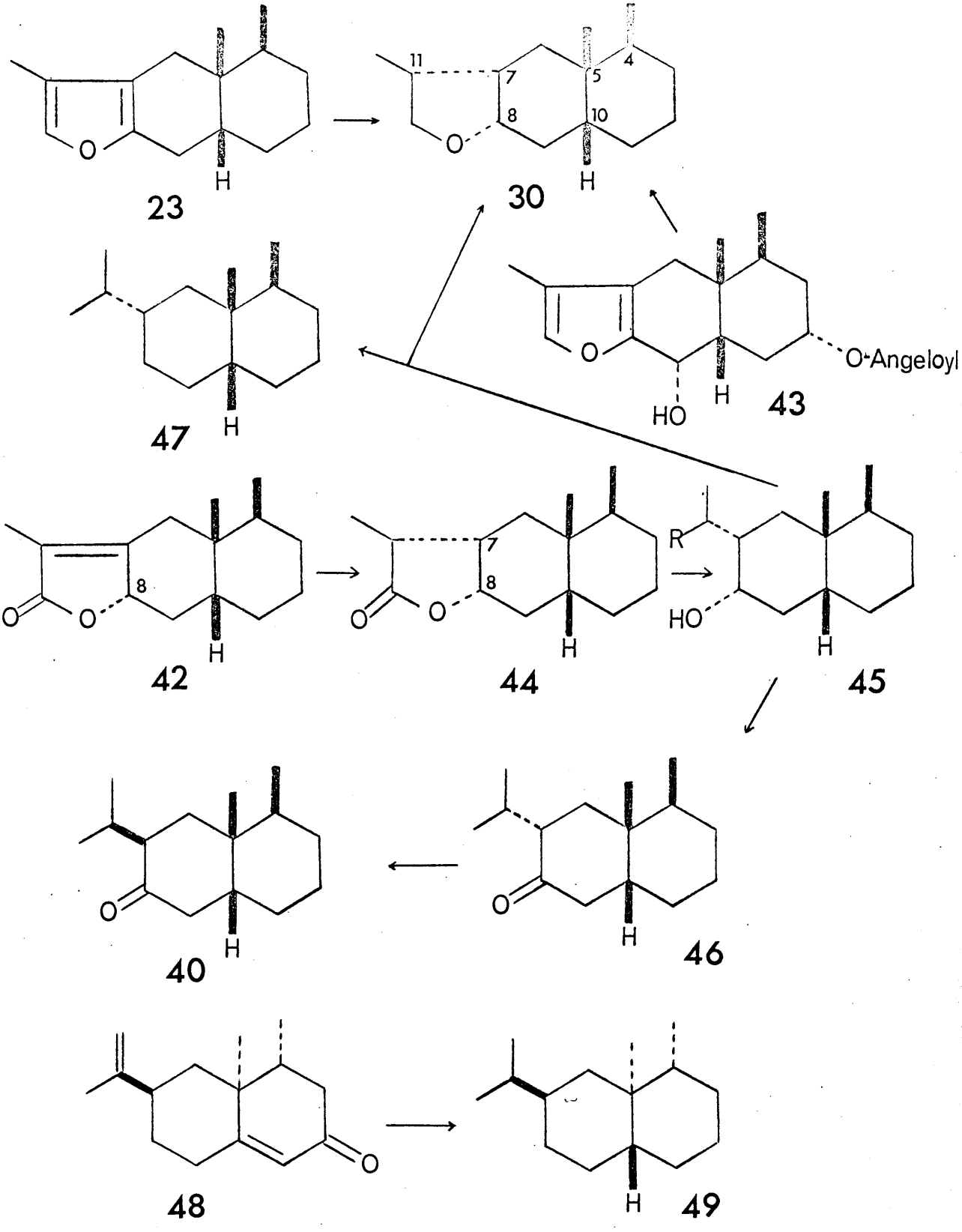
c) Ca/NH_3; d) CrO_3; e) OH^-

Absolute Stereochemistry of the Eremophilane Sesquiterpenes

Some comment on the absolute configurational assignments in the eremophilane series is necessary at this point in the discussion. Until recently all the members of the group proved to have the stereochemistry implied in formula (3) for eremophilone.[33] A key compound is hydroxyeremophilone (4) to which all the eremophilanes, with the exception of petasin (8) (where the absolute configuration was independently proved[34,35]), have been directly or indirectly related‡. The absolute configuration of hydroxyeremophilone was determined by the unambiguous synthesis of a degradation product, the C-8 ketone (39).[33] Two other stable ketones (40) and (41) have also been obtained from (4) by the steps noted in fig.(4).[36]

Of particular relevance to our work are furanoeremophilane (23)[24,30], eremophilenolide (42)[24] and furanopetasin (43).[37] They are typical of a family of eremophilane sesquiterpenoids, isolated from Petasites species by the Prague group of Šorm, which are based on a cis-fused decalin system. The absolute configuration of eremophilenolide derives from a correlation with hydroxyeremophilone.[24] Controlled lithium aluminium hydride reduction of dihydroeremophilenolide (44) gave the hydroxy-aldehyde [(45), R=CHO]. Huang-Minlon reduction followed by reoxidation gave the ketone (46) which was isomerised with base to the known stable isomer (40) also obtained from hydroxy-

‡ In many cases other evidence e.g. optical rotatory dispersion data, confirms the configurational assignments.


23
30
47
43
O-Angeloyl
42
44
45
40
46
48
49

eremophilone [fig.(4)]. Treatment of the ditosylate of diol [(45), R=CH_2OH]with lithium aluminium hydride gave a mixture of the hydrocarbon (47) and tetrahydrofuranoeremophilane (30). Both furanoeremophilane (23) and furanopetasin (43) have been reduced to (30) thus proving their configuration at C-4, C-5 and C-10. Assignment of the α-configuration at C-7 in compounds (30), (44), (45) and (47) is secured by the isolation of the base labile ketone (46). The C-8 α-configuration in eremophilenolide and its congeners depends on the application of the Klyne-Hudson rule to dihydro-eremophilenolide and tetrahydrofuranoeremophilane. Rotation values of -12° for (44) and $+42^{\circ}$ for (30) give a negative molecular rotation difference and permit an α-assignment at the carbon atom (C-8) bearing the potential hydroxyl group in (42). The absolute configuration of tetrahydrofuranoeremophilane (30) has thus been determined at all positions except C-11, as noted above.

Recently the structure and absolute stereochemistry as (48) has been established for nootkatone[38], a member of a smaller group having the opposite stereochemistry at C-4 and C-5 to the previously reported eremophilanes. Again establishment of absolute configuration came from a correlation with hydroxy-eremophilone. The hydrocarbon (49) obtained from nootkatone proved to be the antipode of that obtained by Huang-Minlon reduction of the C-9 ketone (41) from hydroxyeremophilone [fig.(4)].

Fig. (5)

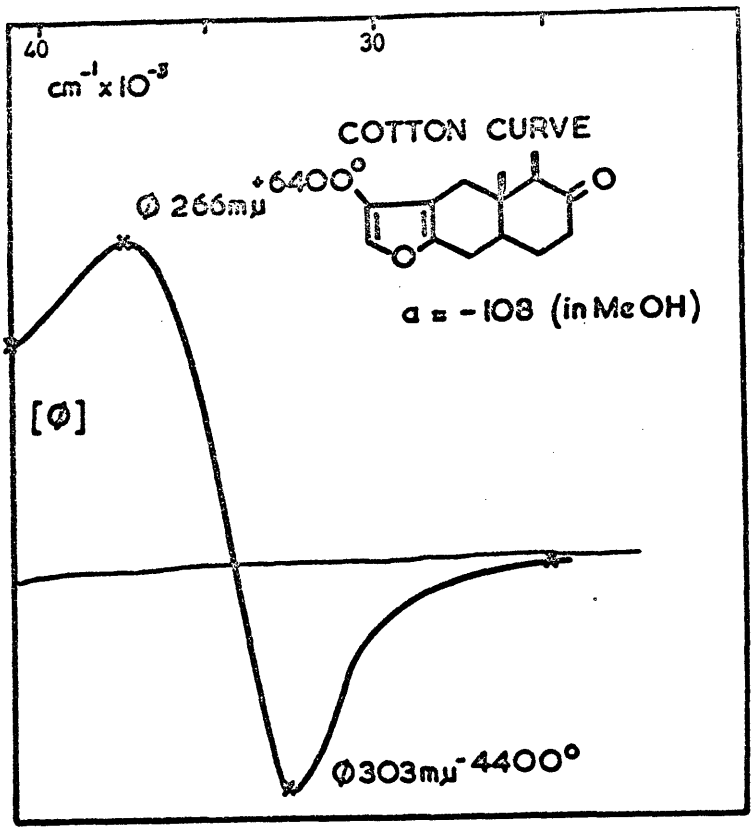
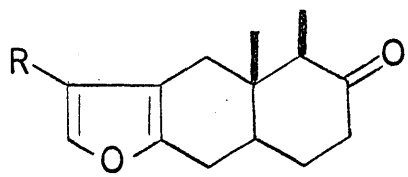


Table (2)

Cotton Effect Extrema (Methanol) for:



Compound	Molecular Rotation $[\phi]$ mμ	Molecular Amplitude (a)
(17) R = COOMe	$[\phi]_{304}$ -4180; $[\phi]_{279}$ + 5300	-95
(25) R = CHO	$[\phi]_{313} \pm 0$; $[\phi]_{275}$ + 7620	-76
(35A) R = CH_3	$[\phi]_{303}$ -4400; $[\phi]_{266}$ + 6400	-108

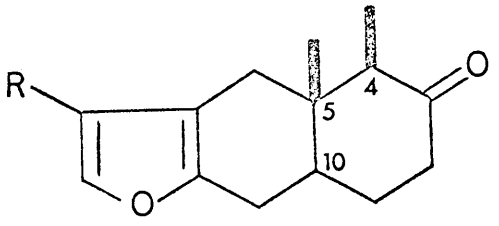
Stereochemistry at C-4 and C-5

(a) Optical Rotatory Dispersion (O.R.D.) Data ‡

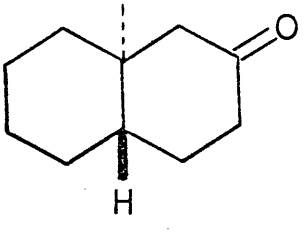
The Cotton effect curves due to the carbonyl chromophore in compounds (17), (25) and (35A) had amplitudes in the range -76 to -108 [table (2) and fig.(5)]. Comparison of the data for our furanoketone (35A): $[\phi]_{303\ m\mu}$ -4400°, $[\phi]_{260\ m\mu}$ + 6400°; a = -108 with that for authentic furanoligularanone (35): $[\phi]_{304\ m\mu}$ -4770°, $[\phi]_{267\ m\mu}$ + 6680°; a = -114.5 proved the stereochemical identity of these compounds. [It should be noted that the curve obtained from the ketoaldehyde (25) was rather anomalous. The profile and amplitude altered with dilution (changing from a 1 cm to a 1 mm cell) although the sign of the effect remained negative. The $n-\pi^x$ transition of the aldehyde chromophore may well contribute to the observed dispersion curve so that a firm interpretation of the result in this case is not justified].

Assuming a cyclohexanone "chair", application of the octant rule[39] indicates the absolute stereochemistry as represented in (50) with 4β-CH_3 (equatorial), 5β-CH_3 (axial) rather than the antipodal stereochemistry of the nootkatone (48) series. Useful analogies are the bicyclic ketones (51)[40] and (52)[41] and the 17-keto D-homo steroids (53) and (54).[42] In case (54) where the

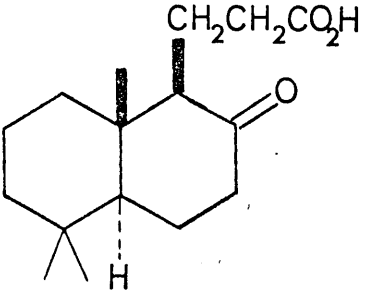
‡ Cotton curves were recorded by Professor W. Klyne, Westfield College, London.



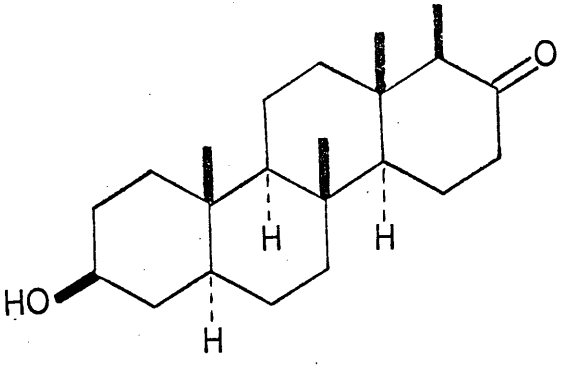
50 a = -76 to -108



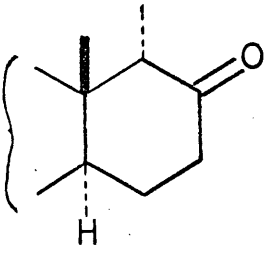
51 a = + 71.2



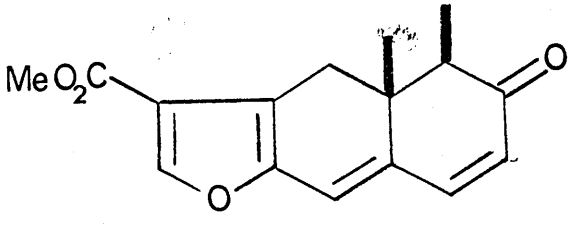
52 a = -123



53 a = -91



54 a = -22



16A

α-CH_3 is axial and in a positive octant the amplitude is greatly reduced. The results do not permit assignment of stereochemistry at C-10 in the degradation products from warburgin but prove the absolute stereochemistry as (16A) for warburgin itself.

(b) Benzene - Induced Shifts in NMR Spectra

Evidence supporting the above conclusions from the O.R.D. data came from the benzene solvent shifts in the NMR spectra of the ketones (17), (25) and (35A).

The chemical shift difference for a proton resonance measured in two solvents may be defined[43] as $\Delta = \delta_{\text{inert solvent}} - \delta_{\text{interacting solvent}}$ where the "inert" solvent is normally carbon tetrachloride, chloroform or cyclohexane. Although numerous papers have been published on this effect, using a variety of solutes and solvents, the subject has not yet been reviewed. However an assessment of the mechanism of benzene-induced shifts has been made by Ronayne and Williams.[43] They suggest that benzene molecules form transient non-planar 1:1 collision complexes at each electron-deficient site in the solute molecule and that the orientation of the benzene molecules is governed by local dipole (rather than molecular dipole) - induced dipole interactions. Regardless of the actual mechanism a useful empirical generalisation in the case of alicyclic carbonyl compounds is the so-called "plane rule":[44,45] "if a reference

Fig. (6)

Δ Values for Methyl Resonances in Ketoandrostane Derivatives[44]

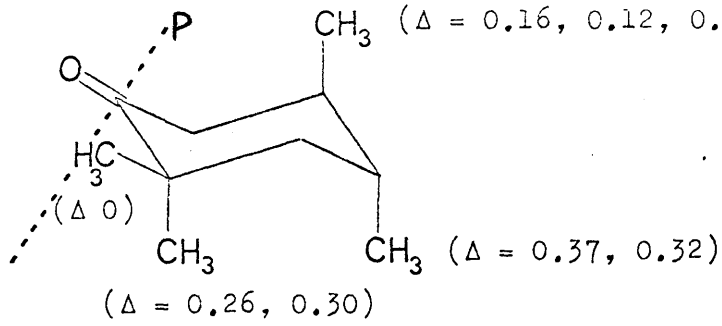
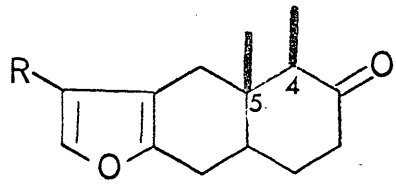


TABLE (3)

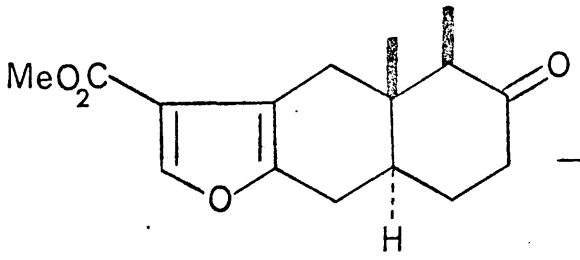
Δ values (δ_{CHCl_3} - $\delta_{benzene}$) for:



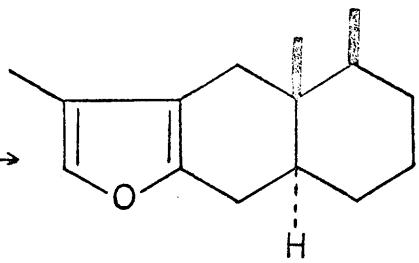
Compound	4-CH_3	5-CH_3	Furan substituent
(17) R = CO_2Me	+ 0.05	+ 0.27	+ 0.37 (OCH_3)
(25) R = CHO	+ 0.10	+ 0.34	+ 0.37 (CHO)
(35A)ǂ R = CH_3	- 0.01	+ 0.20	+ 0.11 (CH_3)

ǂ measured in CCl_4 and benzene

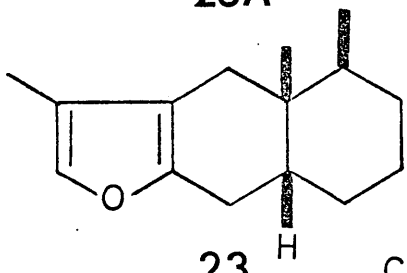
plane (P) is drawn through the carbon atom of the carbonyl group at right angles to the C=O bond then protons close to (P) show very small shifts (in benzene); protons in front of (P), i.e. on the same side as the oxygen of the carbonyl, are deshielded, while protons behind are shielded". [Quoted from ref. (45]. The results of observations made by Williams and Bhacca chiefly on the shifts of the C-18 and C-19 methyl groups attached to cyclohexanone "chairs" in a number of mono-keto androstane derivatives are summarised in fig. (6).(44 The most important point is the clear distinction between an axial and equatorial methyl group α- to a ketone function. Our results for compounds (17), (25) and (35A) are summarised in table (3) and are consistent with the stereochemistry implied in formula (50). However, since a second benzene molecule may be associated with the substituted furan ring in each case, the overall effect may not be a simple one and the results must be interpreted with some caution.



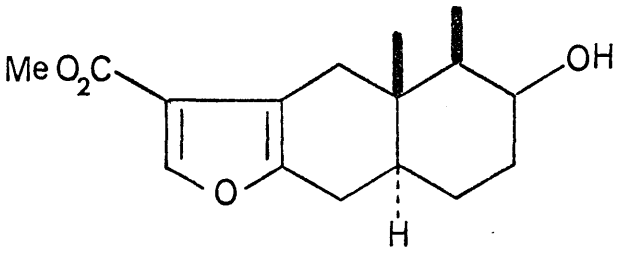
17A



26A



23



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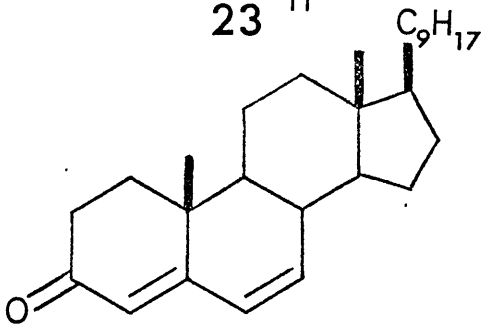

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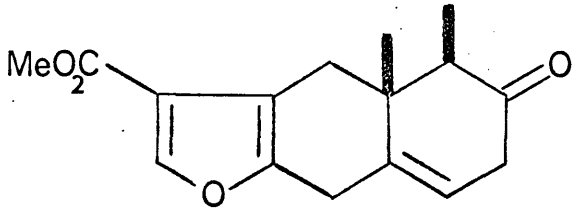

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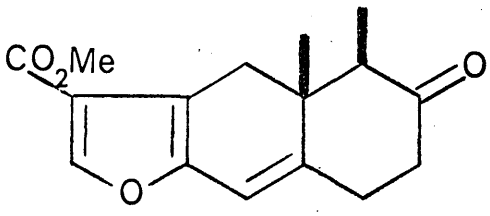

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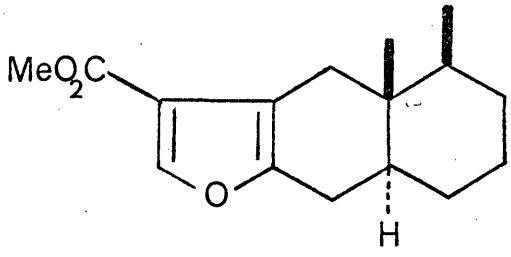


59

Stereochemistry at C-10 in Tetrahydrowarburgin and its Congeners

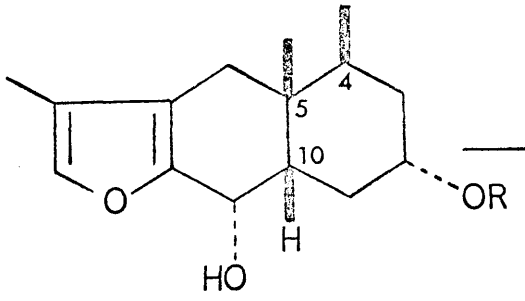
We had assumed that the marked differences between the furanoeremophilane isomers (23) and (26) implied a trans-ring fusion as in (26A). It was originally planned to confirm this by re-examining the hydrogenation of warburgin under various conditions in the hope of isolating the cis-fused epimer of tetrahydrowarburgin (17A) which could then be reduced to (23). As used in the degradation sequences above (17A) was homogeneous to GLC. We assumed that if the cis-fused epimer were formed it would be detectable by GLC. Attempts to obtain such a product were unsuccessful and will be only briefly discussed.

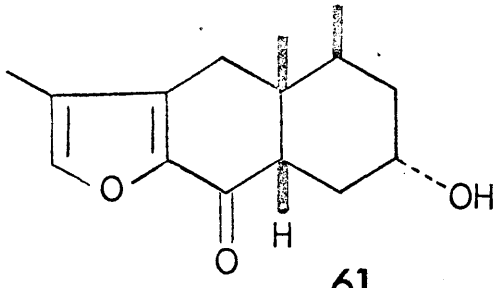
Hydrogenation of warburgin under basic conditions (5% sodium ethoxide in ethanol) with platinum oxide and palladium on charcoal gave, after remethylation, (17A) and a hydroxy-ester (55) exclusively as judged by GLC on the phases PEGA and SE-30. The steroid dienone (56) is reported to give the cis-fused A/B product under similar conditions.(46 [Hydroxy-ester (55) was isolated from the products of catalytic hydrogenation of warburgin with platinum oxide in neutral methanol and was also obtained by sodium borohydride reduction of tetrahydrowarburgin (17A.). It was adequately characterised by its infrared and mass spectra.]

Brief treatment of warburgin with zinc and acetic acid gave a complex mixture of products as judged by TLC. Preparative

TLC led to the separation of a component of similar polarity to (17A) This material sublimed as a solid and was homogeneous to GLC on the phase SE-30, having a slightly shorter retention than (17A). Only 1 mg was obtained in a pure state from 20 mg of reactant. The infrared spectrum (nujol mull) was similar to (17A) and showed ν_{CH} of furan at 3135cm^{-1}, $\nu_{C=O}$ of ester at 1723cm^{-1} and $\nu_{C=O}$ of saturated ketone at 1712cm^{-1}. Absorption in the ultraviolet was at 257 mμ (approximate ε, 5000) and was unchanged on the addition of base. We were at first hopeful that this might be the required cis-epimer of (17A). However the mass spectrum indicated a molecular ion m/e 274 and had no furan fragment "retro-Diels-Alder" base peak. Catalytic hydrogenation gave a product inseparable from (17A) on the phase SE-30. The conclusion was that the zinc reduction product was one of the dihydrowarburgins (57) or (58).

In the hope that alteration of the geometry of the dienone system might influence the steric course of catalytic hydrogenation in favour of the required cis-epimer, attempts were made to prepare the ethylene ketal of warburgin. These were not successful. However warburgin was converted to the thioketal at room temperature with ethanedithiol in ether and boron trifluoride catalyst. The product, made on a small scale, was characterised by its liquid film infrared spectrum which showed retention of the furan bands at 3180 and 1535cm^{-1} and the ester band at 1722cm^{-1}.

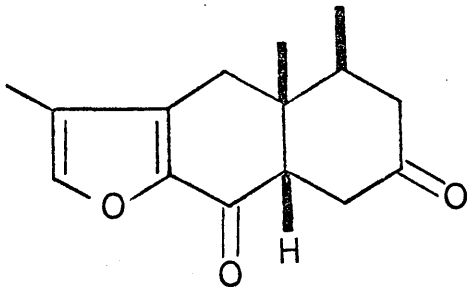




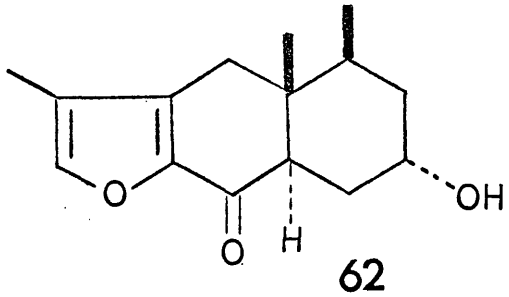
61

43 R = $CO.C(CH_3)=CH(CH_3)$ cis-

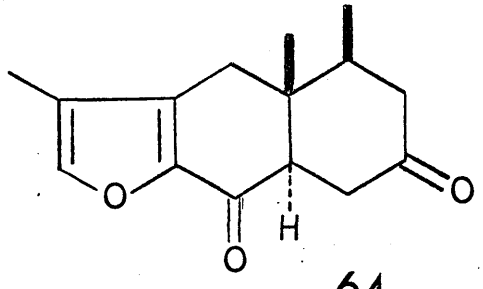
60 R = H



63



62



64

40% (23) + 60% (26A)

It was homogeneous to GLC (SE-30) and TLC and warburgin could be recovered in rather poor yield (GLC and TLC) by hydrolysis using the method of Pappas and Nace.[47] Catalytic hydrogenation under conditions sufficient to reduce warburgin resulted in recovery of starting material. Prolonged hydrogenation with platinum oxide in ethanol gave a major product (80% by GLC of the material recovered) which was not isolated but proved to be indistinguishable on four GLC phases from that obtained by hydrogenation of tetrahydrowarburgin thioketal. This product may be the desulphurised compound (59).

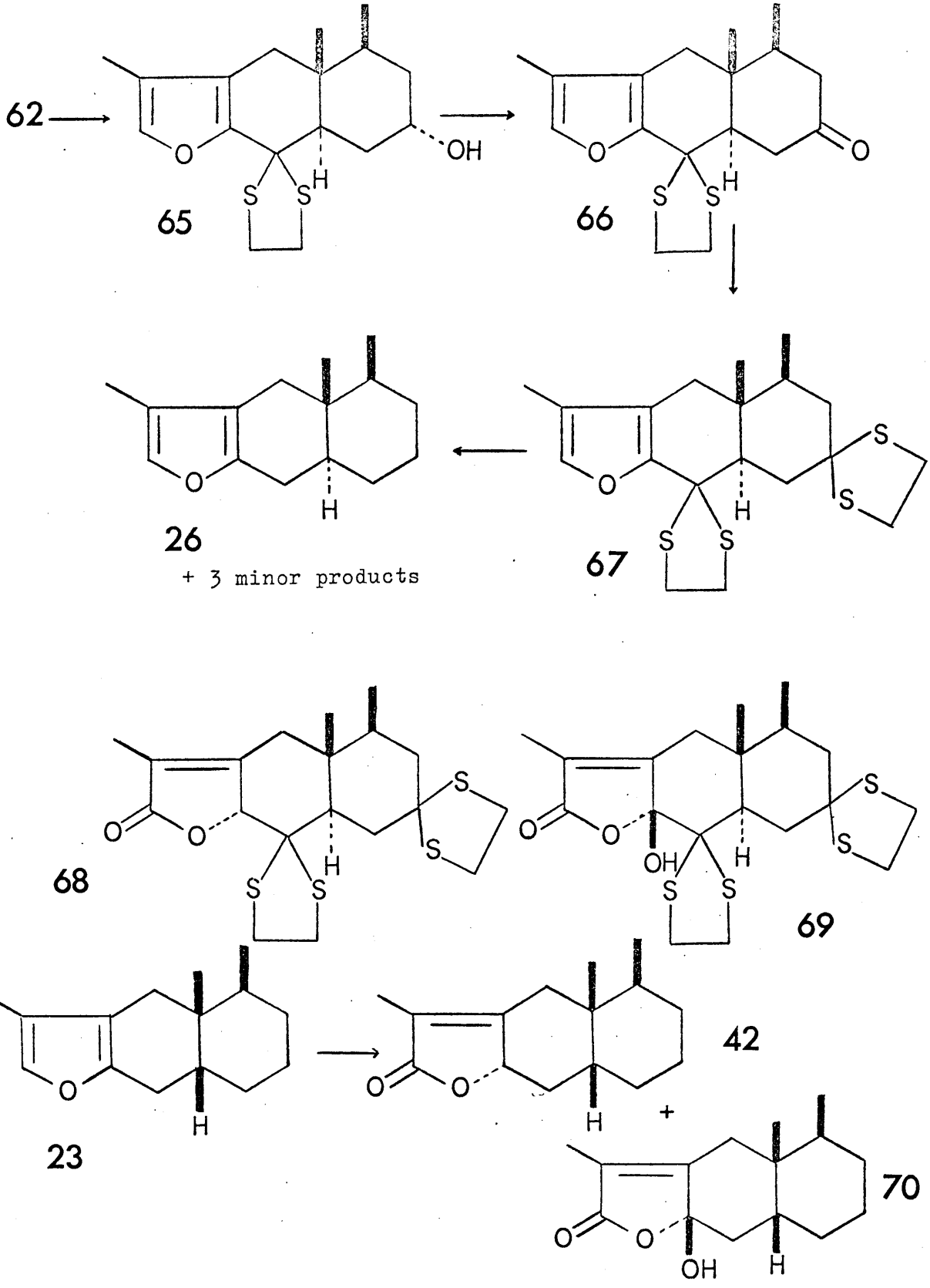
At this time Dr. L. Novotný again came to our assistance, generously providing a sample of furanopetasol (60), the hydrolysis product of the naturally occurring angelate ester furanopetasin (43).[37] The absolute stereochemical assignments at C-4, C-5 and C-10 as in formula (43) have been discussed (p.21).

Novotný *et al.* had reported that allylic oxidation of furanopetasol gave the *cis*-fused ketoalcohol (61).[37] We prepared this compound as described in good yield by shaking diol (60) with manganese dioxide in chloroform at room temperature over twenty hours. Equilibration of (61) in refluxing ethanolic sodium hydroxide gave a quantitative conversion to a new sharply-melting product. The elemental analysis and mass spectrum and a comparison of infrared and ultraviolet spectral data proved this product to be (62) the *trans*-fused isomer of (61). These

compounds were separable on TLC and the purity of each isomer could be assessed by this method. On GLC (Apiezon L) however injection of pure (61) gave two peaks, one of which corresponded to (62). Rather surprisingly injection of the pure trans-isomer also gave a measure of equilibration (ca.15%) to the cis-isomer. It was also found that melting (61) on the Kofler hot stage was sufficient to achieve substantial conversion to (62). This was confirmed by recovery of the melt for TLC, GLC and GC-MS.

The cis-dione (63) was prepared by chromium trioxide in acetone (Jones) oxidation[48] of ketoalcohol (61) as previously described.[37] In a similar manner the trans-dione (64) was obtained from ketoalcohol (62). Again the isomeric purity of the products could be established by TLC. The trans-isomer was thermodynamically the more stable. By GLC (1% QF-1 at 175°) injection of the pure cis-dione gave approximately 10% of the trans-isomer. Heating the melted cis-isomer and reinjection of the recovered material showed a significant conversion to the trans-form.

Huang-Minlon[25] reduction of trans-dione (64) in refluxing ethylene glycol gave a 60:40 mixture of the 10α-H and 10β-H furanoeremophilanes, (26A) and (23) respectively, as judged by GLC on the phase 10% Apiezon L. GC-MS confirmed that the products were solely the isomeric furanoeremophilanes. It must be assumed that a measure of equilibration to the cis-form occurs and that


62
OH
65
66
26
+ 3 minor products
67
68
69
23
42
+
70

its rate of reduction exceeds that of the trans-isomer.

In the interest of completeness it was decided to attempt a preparation of the pure trans-furanoeremophilane via the thioketal (65). After several failures this thioketal was obtained from (62) in poor yield by prolonged heating in ether-ethanedithiol with boron trifluoride etherate as catalyst under scrupulously oxygen-free conditions. The solid product was separated by preparative TLC. The infrared spectrum (nujol mull) had ν_{max} 3500 (broad OH band), 1710-1760 (weak absorption) and 1570 (furan)cm^{-1}. The thioketal protons appeared as a complex multiplet at 6.2-6.8τ in the NMR spectrum implying a dissymmetric environment for the thioketal methylenes. This can be contrasted with the thioacetal (38) where the four methylene protons appeared as a sharp singlet at 6.72 τ. The mass spectrum showed the molecular ion at m/e 324 corresponding to $C_{17}H_{24}O_2S_2$.

Controlled oxidation of (65) with chromium trioxide in pyridine under nitrogen gave the crystalline thioketal ketone (66) characterised by analysis, infrared and mass spectra. Further treatment with ethanedithiol in ether at room temperature again under nitrogen and with boron trifluoride etherate as catalyst gave the bisthioketal (67) as a colourless gum.

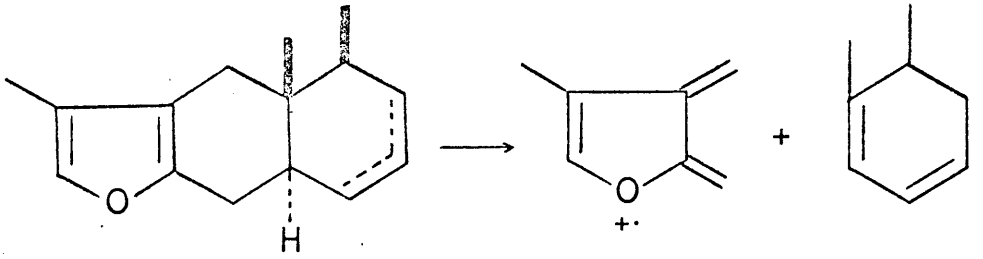
The chief difficulty in the preparation of compounds (65), (66) and (67) was the ready oxidation of the furan ring to a

butenolide. When ketoalcohol (62) was heated in air with ethanedithiol and boron trifluoride etherate the infrared spectrum of the product mixture showed a band at 1760cm^{-1} corresponding to an αβ-unsaturated γ-lactone. In the stage (66) to (67) when oxygen was not completely excluded at least two lactonic minor products formed. These were most probably (68) and (69) since the mass spectrum of the total reaction product in this instance showed ions of mass 16 and 32 units above the parent ion for compound (67).

A similar oxidation of furanoeremophilane (23) has been reported.[49] Solutions of (23) decomposed in air to a mixture of products including eremophilenolide (42). Catalytic oxidation of (23) over platinum gave both (42) and hydroxyeremophilenolide (70).

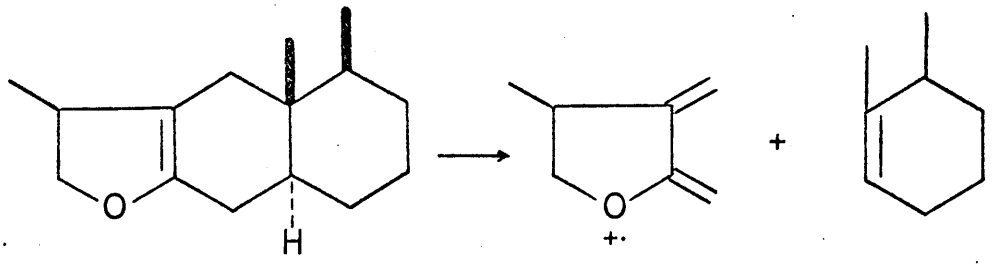
Attempts to form the cis-fused analogue of the bis-thioketal (67) either directly from dione (63) or via thioketalisation of (61) have been unsuccessful. They are briefly described in the experimental section (p. 59).

Raney nickel reduction of the bisthioketal (67) in dioxan gave four products. Analysis by GLC on the phases Apiezon L and Carbowax 20M, and by GC-MS, led us to conclude that there was approximately 80% of the trans-furanoeremophilane (26A) present. Two of the minor components, amounting to 10% and 4% of the total had molecular weight 216 which corresponds to $C_{15}H_{20}O$. The third,



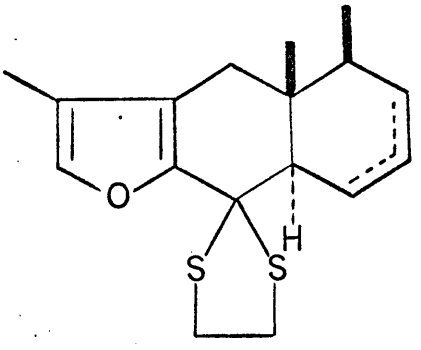
71

m/e 108

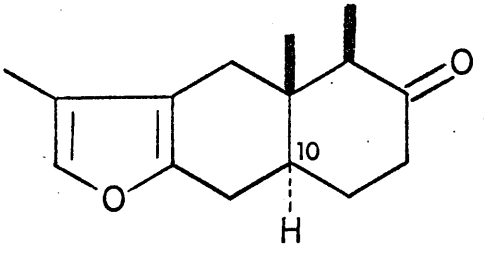


72

m/e 110



73



35

6% of the product, had molecular weight 220 corresponding to $C_{15}H_{24}O$. These may be dehydro- and dihydrofuranoeremophilanes respectively. The "dehydro" components had base peaks in the mass spectra at m/e 108 as do the furanoeremophilanes. This might imply that the extra double bond does not interfere with "retro-Diels-Alder" fragmentation as in (71). The "dihydro" compound $C_{15}H_{24}O$ had the base peak m/e 110. A tentative rationalisation would be that this compound is either the dihydrofuran (72) or that it can rearrange to (72) on electron impact.

The question of whether these are genuine by-products of the desulphurisation reaction, or whether they come from impurities in the sample of the bisthioketal (67) inevitably arises. The dehydro compounds, for example, could result from the presence of the dehydrothioketals (73) carried through from the first thioketalisation step (62)--> (65). The scale of the work was small and the thioketals tended to be unstable. However, as far as possible they were shown to be homogeneous at each stage by GLC and TLC. In the final step the bisthioketal was carefully examined by GLC on four phases and no evidence was obtained for the presence of impurities such as (73). We tend therefore to favour the supposition that (71) and (72) are genuine by-products, but are unable to provide an explanation for their formation. At this unsatisfactory stage the problem rests.

The major desulphurisation product was not further purified. However it was found to be indistinguishable from the furanohydrocarbon (26A) from tetrahydrowarburgin on four GLC phases. The two products had identical mass spectra (GC-MS). We consider these results to be sufficient proof of the 10α-H trans-stereochemistry in tetrahydrowarburgin and the compounds derived from it. Recently Professor Ourisson has informed us of confirmation of this point of stereochemistry in furanoligularanone (35) and its congeners by correlation with known compounds. No further details of this work are available at present.

Experimental Procedure‡

Materials used for column chromatography were Woelm neutral alumina (when necessary deactivated with water before use), and Mallinckrodt silicic acid. Merck "Kieselgel G" was used for thin layer chromatography (TLC) on 0.25 mm layers. Spots were detected by charring with ceric sulphate-sulphuric acid reagent. Preparative TLC was carried out on 0.7 mm layers of "Kieselgel H" or "HF_{254}". Bands have been detected by suppression of the fluorescence of "HF_{254}" or by the method of staining separate "lanes" with a destructive reagent. In specific cases non-destructive fluorescent dyes have been used e.g. 3-hydroxy-pyrene-5,8,10-trisulphonic acid sodium salt.

Analytical gas chromatograms unless otherwise stated have been run on 4' x 4 mm I.D. packed columns in Pye Argon chromatographs. Stationary phases denoted by the following abbreviations have been used:

SE-30	methyl-siloxane polymer
Ap.L	"Apiezon L"
QF-1	trifluoropropylmethyl silicone

‡ Generalisations made here also apply to the experimental discussions of the warburgiadione and ugandensolide sections.

PEGA	polyethyleneglycol adipate
"Carbowax" 20M	polyalkyleneglycol M.W.20,000
SAIB	sucrose acetate isobutyrate
CHDMS	cyclohexane dimethanol succinate
PVP	poly(vinylpyrrolidone)
JXR	methyl silicone
F-60	chlorophenyl methyl silicone
Z	ethyleneglycol succinate-phenylsiloxane (EGSP-Z)
DC 710	phenyl methyl silicone

Melting points were recorded on a Kofler block unless otherwise stated. "Petroleum ether" refers to the fraction of boiling point 40-60°. Organic extracts were dried with anhydrous magnesium sulphate.

Ultraviolet spectra were measured on an automatic-recording instrument (Unicam SP 800). Routine infrared spectra were measured on a Unicam SP 200 model and high resolution spectra on the SP 100 double beam spectrophotometer. Mass spectra were measured on an AEI MS9 spectrometer and also via combined gas chromatography-mass spectrometry (GC-MS) with an Atlas CH4 instrument (by Dr.C.J.W.Brooks in Dr.E.C. Horning's laboratory, Houston) and with an LKB 9000 instrument (Glasgow). Nuclear magnetic resonance (NMR) spectra‡ were determined on a Perkin Elmer

‡ NMR data quoted in the experimental sections denote only the major peaks in the spectra.

60 Mc/sec. instrument or a Varian HA 100 model equipped with a spin decoupler.

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TABLE (4)

Chromatography of Petroleum Extract (A)

(250 ml aliquots)

Fraction	Solvent	Vol. (litres)	Wt.(g)	Content
1- 8	pet. ether	2.0	8.9)	Oils, each
9-16	pet. ether - benzene (9:1)	2.0	6.0))	fraction complex
17-20	pet. ether - benzene (3:1)	1.0	1.5))	
21-24	pet. ether - benzene (1:1)	1.0	4.5	Concentrated in drimenol
25-28	benzene	1.0	3.2	Concentrated in warburgin
29-32	benzene	1.0	1.8	Concentrated in warburgiadione
33-36	benzene-ethyl acetate (1:1)	1.0	0.5	Complex
37-40	benzene-ethyl acetate (1:1)	1.0	1.1	Concentrated in ketol W.H./V
41-44	ethyl acetate	1.0	2.5	Ketols W.H./V and W.H./XIV
45-48	ethyl acetate - methanol (1:1)	1.0	1.0	Polar complex mixture

Recovery: 79%

Extraction of the First Consignment of Warburgia ugandensis heartwood: Chromatography of the Oils

Dried powdered heartwood of Warburgia ugandensis (2.1 kg) was extracted at room temperature for 3 days with petroleum ether (8 litres). Evaporation of the solvent in vacuo at 30° afforded a yellow oil [(A); 39 g]. This extract was chromatographed on alumina (600 g; grade III); 250 ml fractions were taken [table (4)]. Development of the chromatography was followed by TLC and by GLC (1% SE-30).

The first 20 fractions proved to be non-crystalline and complex in composition. From the fractions (21-24) eluted with benzene-petroleum ether (1:1) the sesquiterpene alcohol drimenol (7) crystallised (1.2 g) and was purified by crystallisation from petroleum ether and vacuum sublimation to m.p. 96-7°, $[\alpha]_D$-17° ($CHCl_3$). This material was identical by mixed m.p. determination and comparison of infrared (KCl disc) and mass spectra with an authentic sample of drimenol.

Crystalline warburgin (350 mg; m.p. 150-159°) separated from the first benzene fractions (25-28). Washing with petroleum ether, crystallisation from methanol and vacuum sublimation gave pale yellow prisms (m.p. 159-161°, $[\alpha]_D$ + 120° ($CHCl_3$). (Found: C, 70.42; H, 6.05. $C_{16}H_{16}O_4$ requires: C, 70.58; H, 5.92%). Warburgin proved to be sensitive to light and air. Solutions of

the compound darkened rapidly on standing.

Further elution with benzene (fractions 29-32) gave a yellow oil which on trituration with petroleum ether afforded warburgiadione (320 mg; m.p. 110-130°) recrystallised from methanol to yield yellow prisms, m.p. 127-8°, $[\alpha]_D$ + 25° ($CHCl_3$). (Found: C, 77.96; H, 7.73%. $C_{15}H_{18}O_2$ requires: C, 78.23; H, 7.88%).

Fractions 37-40 were rich in a compound (W.H./V) which failed to crystallise. The apparent molecular weight by GC-MS was 234 (76% of the base peak at m/e 97). The infrared spectrum of the crude material (liquid film) showed bands at 3500 (broad, ν_{OH}), 1670 (enone) and 1625cm^{-1}. The ultraviolet spectrum showed λ_{max} (mμ) 246 (ε, ca.7,000) and 282 (ε, ca.4,000).

Fractions 41-44 contained chiefly a second non crystalline component (W.H./XIV). Column chromatography on alumina (200g; grade II) using a benzene-ethyl acetate gradient achieved a further purification of W.H./XIV (1.3 g) in the ethyl acetate-benzene (1:1) fractions. GC-MS indicated a probable molecular ion, m/e 220 (4%) and base peak m/e 43. The infrared spectrum (liquid film) showed a strong ν_{OH} band centred at 3450cm^{-1} and carbonyl absorption at 1660cm^{-1}. The relatively high polarity of this compound on TLC suggested that it may be a diol (R_f 0.17 in the solvent system ethyl acetate-petroleum ether, 3:7. Compare W.H./V, R_f 0.25 and warburgiadione R_f 0.46).

Extraction of the wood was completed by treatment with 15 litres of ethanol at room temperature for 3 days. Evaporation of the solvent *in vacuo* at 50° gave a dark brown oil with semi-solid material (90 g). This was extracted twice with 250 ml portions of chloroform, leaving an insoluble, finely-divided brown solid (44 g). This material was highly polar (TLC), soluble in ethanol and sparingly soluble in water. It has not been further examined. The chloroform extract was washed with water, dried and the solvent evaporated (40° *in vacuo*) leaving a dark viscous oil [(B); 41 g]. TLC indicated an enhanced concentration of warburgin and warburgiadione when compared with extract (A). However considerable decomposition was evident from the high proportion of intractable material revealed by TLC.

The mother liquors from chromatography (A) concentrated in warburgin and warburgiadione, were combined with extract (B) and further separations were directed specifically at the isolation of these compounds. The total material (46 g) was chromatographed on alumina (800 g; grade II). Severe "streaking" of the zones, evidently due to decomposition, was accompanied by deactivation of the alumina. A similar petroleum ether-benzene-ethyl acetate gradient to that used in chromatography (A) [table (4)] afforded warburgin (2.50 g m.p. 145-160° after washing with petroleum ether), which crystallised from the benzene-petroleum ether (1:1) fractions.

Subsequent elution with benzene-petroleum ether (1:1) through benzene gave semi-crystalline mixtures concentrated in warburgin and warburgiadione (12.1 g). Elution with benzene-ethyl acetate (1:1) and ethyl acetate gave a total of 8.0 g dark oil of complex composition. Total recovery from this column was low (60%).

Further rechromatography of combined fractions on alumina afforded warburgin (310 mg; m.p. 145-160°) and warburgiadione (390 mg; m.p. 123-130°).

The relative inefficiency of separation was due to the closely similar polarities of the two compounds and to the instability of warburgin.

Hydrolysis and Remethylation of Warburgin

To a solution of warburgin (40 mg) in ethanol (5 ml) was added 10% aqueous potassium hydroxide (5 ml) and the mixture refluxed for 50 minutes. The solution was made slightly acid with hydrochloric acid and extracted with ether. The ethereal solution was washed, dried and evaporated giving non-crystalline acidic material (32 mg). TLC using the solvent system benzene-dioxan-acetic acid (90:25:4 by volume) indicated two products both giving red stains with 2:4-dinitrophenylhydrazine reagent [R_f values 0.45, 0.48; warburgin 0.70]. The total product had ν_{max} ($CHCl_3$) 2400-3500 (OH of CO_2H), 1740 (weak), 1698 and 1675cm^{-1}; λ_{max} (EtOH) 372, (EtOH/NaOH) 389 and (reacidified /HCl) 375 mμ.

Remethylation of the acidic products with ethereal diazomethane regenerated warburgin (TLC, characteristic fluorescence and staining and GLC on 1% SE-30) together with a second component (ca.30%) more polar, and just separable from warburgin (ethyl acetate-petroleum ether 3:7). Preparative TLC on a 0.5 mm thick layer of silica using a 50 cm development did not achieve complete separation but allowed isolation of a small amount of the new product [3 mg; (20) 4α-CH_3] which proved to be highly unstable to air and light; ν_{max} (CCl_4) 1734 and 1676cm^{-1}; λ_{max} (EtOH) 372 mμ (ε, ca. 20,000); mass spectral molecular ion m/e 272 corresponding to $C_{16}H_{16}O_4$.

Hydrogenation of Warburgin

Warburgin (1.05 g) was hydrogenated at room temperature and atmospheric pressure in ethyl acetate solution in the presence of 10% palladium on charcoal catalyst (250 mg). After 35 minutes hydrogen uptake had ceased. The colourless solid product tetrahydrowarburgin [(17); 980 mg] was recrystallised from ethyl acetate to m.p. 172-173.5°; $[\alpha]_D$ + 50° ($CHCl_3$); ν_{max} (CCl_4) 1730, 1721cm^{-1}; λ_{max} (EtOH) 255 mμ (ε,2650). 60 Mc./sec. NMR ($CDCl_3$) τ 9.33 (3H), 8.91 (3H, doublet J = 7c./sec.), 6.16 (3H), 2.07 (1H). Mass spectral molecular ion m/e 276 (47%), base peak m/e 152. (Found: C, 69.69; H, 7.02. $C_{16}H_{20}O_4$ requires: C, 69.55; H, 7.30%).

Warburgin (20 mg) was hydrogenated at room temperature and atmospheric pressure for 10 hours with platinum oxide catalyst (10 mg) in methanol solution. Colourless semi-solid (18 mg) was recovered. The major product was identified as tetrahydrowarburgin (TLC). A second more polar product (20-30%) was proved to be hydroxy ester (55) by comparison (TLC and GLC using 1% SE-30 at 150°) with a sample obtained by sodium borohydride reduction of tetrahydrowarburgin in methanol. Traces of minor polar products were evident from both reactions. Preparative TLC gave a colourless gum: ν_{max} (nujol) 3400 (broad, OH), 1720cm^{-1}. 60 Mc./sec. NMR ($CDCl_3$) τ 9.12 (3H), 8.77 (3H, doublet, J = 7 c./sec.), 8.30 (OH), 6.19 (3H), 6.15 (1H, multiplet), 2.15 (1H). Mass spectral molecular ion ($C_{16}H_{22}O_4$) m/e 278 (33%), base peak m/e 152.

Hydrolysis and Remethylation of Tetrahydrowarburgin (17)

Tetrahydrowarburgin (5 mg) was dissolved in ethanol (2 ml) and 10% aqueous potassium hydroxide added (1 ml). The solution was refluxed for 1 hour, cooled, made just acid with dilute hydrochloric acid, and extracted with ether; the extract was washed (water), dried and evaporated giving non-crystalline acidic material (3.5 mg). TLC with the solvent system benzene-dioxan-acetic acid (90:25:4) showed one product R_f 0.6 (tetrahydrowarburgin R_f 0.8). The crude product had λ_{max} 253 (neutral EtOH), 242 (EtOH/NaOH) and 253 mμ (reacidified/HCl). Methylation of the acidic product with ethereal diazomethane regenerated tetrahydrowarburgin identified by comparative TLC (ethyl acetate-petroleum ether, 3:7) and GLC (1% SE-30).

2,4-Dinitrophenylhydrazone of Warburgin

Warburgin (10 mg) was dissolved in ethanol (0.3 ml) and approximately 1.5 molar proportions of 2,4-dinitrophenylhydrazine reagent added in solution. (A stock solution contained 20 mg/ml reagent in 4% by volume sulphuric acid in ethanol). The mixture was warmed for 30 minutes. The scarlet mono-2,4-dinitrophenylhydrazone derivative separated on standing and was crystallised with difficulty from ethanol (5 mg; m.p. 250-260°), ν_{max} (nujol) 1720cm^{-1} (ester).

Diol (24)

A solution of tetrahydrowarburgin [(17); 860 mg] in dry tetrahydrofuran (20 ml) was added dropwise to a stirred suspension of lithium aluminium hydride (450 mg) in tetrahydrofuran (30 ml). After 8 hours' stirring at room temperature the mixture was decomposed with water (100 ml), made just acid with dilute hydrochloric acid and extracted with ether (6 x 50 ml). The extract was washed (water), dried and the ether evaporated to give the crude semi-solid diol [(24); 675 mg]. This material became discoloured in air. TLC indicated a major product (90%) having R_f 0.4 in the solvent system ethyl acetate-benzene (1:1); minor polar impurities were evident. The product was homogeneous on the GLC phases Apiezon L and SE-30 (t_R 6.9 min. on 1% SE-30 at 175°C; Rel. t_R 1.37 to n-C_{20} alkane). In the oxidation step described below the crude diol was used. A sample was purified by preparative layer separation as a colourless gum: $[\alpha]_D$ + 62° ($CHCl_3$) ν_{max} (CCl_4) 3624, 3615cm^{-1}; ν_{max} (liquid film) 1570cm^{-1} (furan). 60 Mc./sec. NMR ($CDCl_3$) τ 9.12 (3H), 8.90 (3H, doublet, J= 7 c./sec.) 6.15 (1H, multiplet), 5.52 (2H), 2.7 (furan CH obscured by $\underline{C}HCl_3$). Mass spectral molecular ion m/e 250 (32%), base peak m/e 124. A satisfactory analysis was not obtained. (Found: C, 71.31; H, 9.05. $C_{15}H_{22}O_3$ requires C, 71.97; H, 8.86%).

Ketoaldehyde (25) by Sarett Oxidation[27] of (24)

To a solution of the diol [(24); 500 mg] in pyridine (10 ml) was added slowly a suspension prepared from chromium trioxide (1.30 g) and pyridine (12 ml). The mixture was stirred for 18 hours at room temperature and then filtered. The residue was washed with pyridine. The combined pyridine solutions were added to water (100 ml) and extracted with ether. The extract was washed carefully with dilute hydrochloric acid and with water, dried, and the ether evaporated to give a colourless solid [(25); 380 mg] m.p. 123-133°. Crystallisation from ethyl acetate gave material of m.p. 133-134°; $[\alpha]_D$ + 58° ($CHCl_3$); ν_{max} (CCl_4) 2724, 1718, 1690cm^{-1}; λ_{max} (EtOH) 272 mμ (ε,2600): 60 Mc./sec. NMR ($CDCl_3$) τ 9.33 (3H), 8.91 (3H, doublet, J=7 c./sec.) 2.03 (1H), 0.01 (1H). Mass spectral molecular ion m/e 246 (71%), base peak m/e 122. (Found: C, 73.06; H, 7.33. $C_{15}H_{18}O_3$ requires: C, 73.15; H, 7.37%).

Furanohydrocarbon (26) by Huang-Minlon[25] Reduction of (25).

Ketoaldehyde [(25); 140 mg] was heated for 1 hour at 100° in ethylene glycol (8 ml) with 90% hydrazine hydrate (1 ml). Potassium hydroxide (1.6 g) was dissolved by warming in ethylene glycol (8 ml) and added to the ketoaldehyde hydrazone solution. The mixture was heated under reflux for 3 hours at bath temperature

180° and then concentrated to b.p. 190-195° by slow distillation. A further 0.3 ml 100% hydrazine hydrate was added and refluxing maintained at 195° (bath temperature) for 8 hours. The reaction mixture, distillate and condenser washings were added to water (50 ml), extracted with ether and the extract washed, dried and evaporated to give a discoloured oil (ca.130 mg, containing traces of ethylene glycol). TLC (benzene) indicated a major product (60%), R_f 0.7, and a more polar component of R_f 0.4. Preparative thin layer separation gave furanohydrocarbon [(26); 42 mg] as a fragrant colourless oil which decomposed slowly in air and did not differ in R_f value or staining characteristics from authentic cis-furanoeremophilane (23). It was however distinguished by GLC. [10% Apiezon L at 175°; gas flow 40 ml/min: (23), t_R 23.0 min.; (26), t_R 24.8 min; n-C_{16} alkane, t_R 14.6 min.]. The minor polar product, possibly representing incompletely reduced material, was also separated but proved to be unstable and was not characterised.

Trans-furanoeremophilane (26) had ν_{max} (liquid film, Unicam SP.100) 1648 (w), 1565 (w), 1462 (s), 1447 (s), 1383 (m), 1342 (m), 1134 (m), 1087 (s), 767 (m), 733 (s)cm^{-1}. 60 Mc./sec. NMR (CCl_4) τ, 9.32 (3H), 9.07 (3H, distorted doublet), 8.13 (3H, doublet J = 1.5 c./sec.) 3.05 (1H, multiplet). Mass spectrum, (GC-MS, Atlas CH4, admission via 6' 1% SE-30 at 125°) molecular ion ($C_{15}H_{22}O$) m/e 218 (40%), 122 (3%), 108 (base peak).

The infrared and mass spectra of cis-furanoeremophilane (23) were recorded under the same conditions as for the trans-isomer.

TABLE (5)

Hydrogenation Products of trans-(26) and cis-(23) Furanoeremophilanes

(Retention times are for 4' 1% SE-30 at 125°. Mass spectra were obtained by GC-MS.* Admission to the spectrometer was via a 6' 1% SE-30 column at 115°)

Reaction	t_R (min.)	Rel t_R	Mass spectra m/e (% of base peak)		
Hydrogenation of (26)	‡ 3.7 (5%)	0.46	208 (76%), 165 (100%)		
	9.2 (35%)	1.13	222 (51%), 163 (100%)	122 (25%)	98 (36%)
	10.9 (60%)	1.34	222 (51%), 98 (100%)	163 (91%)	
Hydrogenation of (23)	‡ 3.8 (2%)	0.47	208 (50%), 165 (100%)		
	9.2 (2%)	1.13	222 (61%) 163 (100%)	122 (76%)	98 (38%)
	10.0 (96%)	1.24	222 (18%) 122 (100%)	163 (47%)	
n-C_{16} alkane	8.1	1.00			

‡ Peaks incompletely resolved, evidently (GC-MS) mixtures of eremophilane isomers

* By CJWB using the modified Atlas CH4 instrument in Dr. E.C. Horning's laboratory, Institute for Lipid Research, Baylor University College of Medicine, Houston, Texas.

ν_{max} (liquid film) 1800 (w), 1766 (w), 1647 (w), 1565 (w), 1466 (s) 1447 (s), 1383 (m), 1148 (m), 1091 (s), 788 (m), 730 (s). Mass spectrum, molecular ion m/e 218 (23%); 122 (36%), 108 (base peak). The only significant difference in the mass spectra was the relative abundance of the ion m/e 122 (see Appendix).

Hydrogenation of trans-Furanoeremophilane (26)

Hydrogenation of trans-furanoeremophilane [(26); 10 mg] in acetic acid for 12 hours in the presence of platinum oxide catalyst (15 mg) afforded a mixture of products as judged by GLC. Examination by GC-MS [see table (5)] showed the two major products to be isomeric tetrahydrofuranoeremophilanes, distinguishable from an authentic sample of (23) both by GLC and GC-MS. At least two minor products proved to be eremophilanes (GC-MS).

Hydrogenation of cis-Furanoeremophilane (23)

cis-Furanoeremophilane was hydrogenated in the manner described for the trans-compound (26). The major product (96%) was, as previously reported, the tetrahydrofuranoeremophilane (30). A small amount of a mixture of eremophilanes was also present. In addition we detected approximately 2% of a component which proved to be a further isomer of (30).

Ketoaldehyde Thioacetal (38)

Treatment of ketoaldehyde [(25); 31 mg] with approximately 1.1 moles of ethanedithiol (13 mg) and boron trifluoride etherate (4 drops) in dry ether for 2 hours at room temperature gave a quantitative conversion to a single less polar product. Aqueous sodium hydroxide (1%;10 ml) was added to the reaction mixture which was then extracted with ether and the extract washed (water) and dried. Evaporation of the ether gave an oil (35 mg) which was purified by preparative TLC (ethyl acetate-benzene, 1:1) and obtained as a colourless gum [(38); 32 mg]: ν_{max} (CCl_4), 1718cm^{-1}. 60 Mc./sec. NMR (CCl_4) τ 9.35 (3H), 8.99 (3H, doublet, J=7 c./sec.), 6.72 (4H), 4.58 (1H), 2.79 (1H). Mass spectral molecular ion ($C_{17}H_{22}O_2S_2$) m/e 322 (base peak).

Furanoketone (35A)≡(35).

The ketoaldehyde thioacetal [(38); 27 mg] was dissolved in dioxan (5 ml); a suspension of freshly prepared Raney nickel[32] was added in dioxan (0.5 ml) and the mixture refluxed for 30 minutes. The Raney nickel was filtered off and washed with dioxan. The combined solution was evaporated to dryness under reduced pressure. TLC with ethyl acetate-benzene (1:19) as solvent indicated a major product (R_f 0.3), traces of starting material (R_f 0.2) and two minor polar components. [It had previously been observed

that treatment of (38) with Raney nickel in refluxing acetone for 6 hours led to recovery of starting material while refluxing in dioxan for more than 1 hour caused substantial decomposition]. Preparative TLC gave furanoketone [(35A); 8 mg] as an oil, which decomposed in air on standing: ν_{max} (KCl) 1711, 1650, 1563 (w)cm^{-1}. 60 Mc./sec. NMR (CCl_4) τ 9.36 (3H), 9.01 (3H, doublet J= 7c./sec.), 8.12 (3H, doublet J= 1.5 c./sec.), 3.03 (1H, multiplet). Mass spectral molecular ion ($C_{15}H_{20}O_2$) m/e 232 (39%), base peak (108).

Comparison of these data and of GLC behaviour on the phases 1% SE-30, 1% QF-1, 10% Apiezon L, 7% F60-1% Z and 2% Carbowax proved the identity of (35A) with furanoligularanone (35). "Seeding" (35A) with the authentic material and trituration with petroleum ether gave a solid. Vacuum sublimation gave m.p.84-88° undepressed when mixed with furanoligularanone.

Optical rotatory dispersion data provided further proof of identity. (35A): $[\phi]_{303}$ -4400°, $[\phi]_{266}$ + 6400°, a= -108 and furanoligularanone: $[\phi]_{304}$ -4770°, $[\phi]_{267}$ +6680°, a= -114.8.

Attempts to obtain a cis-fused isomer of Tetrahydrowarburgin (17).

(1) Catalytic hydrogenation of warburgin under basic conditions.

(a) Warburgin (5 mg) was hydrogenated in 2% potassium hydroxide in ethanol (5 ml) for 4 hours with 10% palladium on charcoal as catalyst. The products were methylated (ethereal diazomethane). GLC on 1% SE-30 and 1% PEGA showed the major product (95%) to be

indistinguishable from tetrahydrowarburgin as previously prepared.

(b) The reaction was repeated with platinum oxide catalyst for 90 minutes. Methylation of the products for GLC showed hydroxy ester (55) to be the main product with approximately 10% of tetrahydrowarburgin.

Retention data for 1% SE-30; 175°; 40 ml/min: t_R (17) 7.7 min. (55) 8.2 min., n C-20 alkane 4.7 min; and for 1% PEGA, 175°, 40 ml/min.: t_R (17) 19.6 min., (55) 24.0 min.

(2) Reduction of warburgin with zinc and acetic acid. (a) A solution of warburgin (10 mg) in 5 ml acetic acid-water (2:1) was stirred with zinc dust (100 mg) at room temperature for 20 minutes. After filtration, water (10 ml) was added and the solution extracted with ether. The extract was washed with sodium bicarbonate and with water, dried and the ether evaporated to give a colourless oil (10 mg) which became discoloured on standing in air. TLC indicated the absence of starting material in a complex mixture [6-8 products of polarity greater than warburgin: R_f 0.55 in ethyl acetate-petroleum ether (3:7)]. GLC on the phase 1% SE-30 showed two main peaks not fully resolved.

Retention data for 1% SE-30 at 175°C

	t_R (min.)	Rel.t_R
	6.8	1.41
	7.8	1.51
(n-C_{20})	4.9	1.00

The ultraviolet spectrum of the total product in ethanol had λ_{max} 220, 265 and 290 mμ. Addition of base (0.03 ml 4N sodium hydroxide to an approximately 0.004 M solution) produced an immediate yellow colouration accompanied by the increase with time (complete in 30 minutes) of a new maximum at 375 mμ at the expense of the 265 mμ absorption band. This shift was not reversed on reacidification.

(b) Warburgin (20 mg) was treated according to procedure (a) for 60 minutes. GLC (1% SE-30) indicated an enhancement of the component of relative t_R 1.41 which accounted for 70% of the recorded product. By TLC the mixture was again complex. Preparative TLC [ethyl acetate-petroleum ether (3:7)] allowed separation of a band at R_f 0.5 (2.0 mg) which corresponded to the major GLC component. This product which sublimed as a solid (120°/0.05 mm) was homogeneous by GLC (SE-30) and TLC and showed λ_{max} (ethanol) 257 mμ (ε, ca. 5000); ν_{max} (nujol) 3135 (furan CH), 1723, 1712cm^{-1}; mass spectral molecular ion m/e 274, corresponding to $C_{16}H_{18}O_4$. Catalytic hydrogenation (10% palladium on charcoal in ethyl acetate) of the dihydrowarburgin gave trans-tetrahydrowarburgin (17) as the only product (GLC on the phases 1% SE-30 and 1% PEGA).

Treatment of warburgin with zinc in ethanol at room temperature gave no reaction after 1 hour (TLC). Addition of 50% acetic acid gave incomplete reaction to product mixtures resembling those found in procedures (a) and (b) above.

(3) Catalytic hydrogenation of warburgin thioketal.

(a) Attempted formation of the ethylene ketal of warburgin using ethylene glycol, p-toluene sulphonic acid in refluxing benzene (Dean and Stark apparatus) for 4 hours resulted in decomposition (judged by TLC). Reaction with ethylene glycol and boron trifluoride etherate in ether with and without acetic acid at room temperature for up to 24 hours resulted in substantial recovery of starting material (TLC).

(b) To warburgin (10 mg) in ether (3 ml) boron trifluoride etherate (0.1 ml) and ethanedithiol (0.2 ml) were added. After 20 hours at room temperature, TLC indicated approximately 80% reaction to the thioketal [thioketal R_f 0.6, warburgin R_f 0.5 in ethyl acetate-petroleum ether (3:7)]. Water (5 ml) and 1 N sodium hydroxide (5 ml) were added and the product extracted with ether. Preparative TLC gave the thioketal as a pale yellow gum (6 mg) homogeneous on the GLC phase 1% SE-30 [t_R 9.0 min. at 225°C; gas flow 40 ml/mm] ν_{max} (film) 1725 (ester); 3180 and 1538cm^{-1} (furan). In a similar manner the thioketal from tetrahydrowarburgin was prepared as a colourless gum, ν_{max} (film) 1720 (ester) and 1550 (furan)cm^{-1}. [t_R 22.2 min. on 1% SE-30 at 200°C; 40 ml/min. gas flow].

Hydrolysis of warburgin thioketal (3 mg) in acetone (2 ml) with water (3 drops) mercuric chloride (8 mg) and cadmium carbonate

(5 mg) under nitrogen for 30 hours with stirring[47] resulted in considerable decomposition, but allowed the identification among the products of warburgin by TLC through its characteristic R_f value, fluorescence and staining and by GLC (1% SE-30).

Catalytic hydrogenation of warburgin thioketal (3 mg) in ethyl acetate for 1 hour with 10% palladium on charcoal as catalyst resulted in recovery of starting material unchanged (TLC). Hydrogenation over platinum oxide in ethyl acetate for 12 hours gave a major product which was not distinguishable from that obtained by similar treatment of tetrahydrowarburgin thioketal, when examined on the GLC phases: 1% SE-30, 1% QF-1, 1% PEGA all at 150° and 1% SAIB at 175°C. This product may be the furan ester (59).

Furanketoalcohols (61) and (62)

Furanopetasol [(60); 1.01 g] in chloroform (150 ml) was shaken for 24 hours at room temperature with manganese dioxide‡ (10.0 g). The manganese dioxide was filtered off and washed with chloroform. Evaporation of the solvent gave furanoketoalcohol [(61); 960 mg]. No starting material was recovered as judged by TLC. Crystallisation from ethyl acetate failed to produce a sharp m.p. (180-188°). ν_{max} (CCl_4) 3621 and 1680cm^{-1}; ν_{max} (nujol) 3200 (w) and 1535cm^{-1} (furan); λ_{max} (EtOH) 281 mμ (ε, 16,000); mass spectral molecular ion 248 (12%), base peak 163. (Found:

‡ The manganese dioxide used had been prepared several years earlier by a colleague according to the method of Attenburrow et al.[50]

C, 72.55; H, 7.86. $C_{15}H_{20}O_3$ requires: C, 72.55; H, 8.12%). [Reported physical data[37]; m.p. 188°; $[\alpha]_D^{20}$ + 58.0° (MeOH); ν_{max} 3600, 1678 and 1565cm^{-1}; λ_{max} 280mμ (ε, 21,000)].

Furanoketoalcohol [(61); 500 mg] was refluxed for 1 hour in 4% ethanolic sodium hydroxide (50 ml). The solution was concentrated by evaporation of the bulk of the ethanol, added to water (50 ml) and extracted with ether. The ether extract was washed (water), dried and evaporated to give the crystalline epimeric ketoalcohol [(62); 410 mg]; recrystallised from ethyl acetate to m.p. 170-1°; ν_{max} (CCl_4) 3621 and 1689cm^{-1}; λ_{max} (EtOH) 281 mμ (ε, 16,000); mass spectral molecular ion 248 (65%), base peak 163. (Found: C, 72.76; H, 8.33. $C_{15}H_{20}O_3$ requires: C, 72.55; H, 8.12%).

The epimeric furanoketoalcohols were separated on TLC with difficulty. It was necessary to develop a 15 cm analytical plate several times to achieve separation of the spots [e.g. after 10 developments in the system ethyl acetate-petroleum ether (3:7) the trans-isomer (62) had R_f 0.55 and the cis- (61) R_f 0.45]. GLC analysis of mixtures was complicated by a degree of cis-- trans-equilibration [see table (6)].

It was also observed that melted cis-isomer on a soda glass slide on the Kofler hot stage, left at 200° for 2 hours, on being recovered and examined by GLC and TLC showed substantial

epimerisation. That the product of this equilibration was the trans-isomer was confirmed by GC-MS.

Furanodiketones (63) and (64)

Furanoketoalcohol [(62); 100 mg] was dissolved in acetone (10 ml) and treated with a slight excess of chromium trioxide in sulphuric acid (Jones reagent)[48] at 0°C for 10 minutes. The reaction mixture was added to ice-water (50 ml) and extracted with ether; the extract was washed with water, dried and evaporated to give crude diketone [(64); 96 mg, m.p. 100-112°] recrystallised from ethyl acetate to m.p. 111-112°. ν_{max} (nujol) 1710, 1675 and 1540cm^{-1}; λ_{max} (EtOH) 280 mμ (ε, 15,000). (Found: C, 73.00; H, 7.32. $C_{15}H_{18}O_3$ requires: C, 73.14; H, 7.37%). The epimeric cis-diketone (63) was prepared in a similar manner from the ketoalcohol (61) and had m.p. 145-9° on recrystallisation from ethyl acetate (recorded m.p. 149°)[37]. As with the epimeric ketoalcohols the purity of the diketones could be established by TLC. By GLC (1% QF-1 at 175°) the pure cis-epimer showed about 10% conversion to the trans-form (64). Heating on the Kofler hot stage produced enhancement of the trans-peak.

Huang-Minlon[25] Reduction of Furanodiketone (64).

Furanodiketone [(64); 12 mg] was heated at 80° for 1 hour in ethylene glycol (2 ml) with 90% hydrazine hydrate (0.3 ml). Potassium hydroxide (150 mg) was added in ethylene glycol (1.5 ml),

the mixture refluxed at 180° for 3 hours, concentrated by distillation to b.p. 195° and refluxed for a further 8 hours. On working up as previously described [in the preparation of furanohydrocarbon (26)] an oil (10 mg) was recovered. Preparative TLC (5% ethyl acetate in petroleum ether as solvent) gave 6 mg of a mixture of furanohydrocarbons (26) and (23). GLC on 10% Apiezon L at 175° indicated a ratio of trans- to cis- of 3 to 2. The epimeric nature of the products was confirmed by GC-MS using a 10', 2% JXR column at 175° when, despite incomplete resolution, scanning through the distorted peak gave mass spectra which indicated (26) and (23) as the only products.

Thioketal (65)

Furanoketoalcohol [(62); 20 mg] was dissolved in dry ether (10 ml) together with ethanedithiol (2 ml). Redistilled boron trifluoride etherate (0.3 ml) was added and the mixture refluxed under nitrogen for 36 hours. (Traces of oxygen present in the nitrogen supply were removed using Fieser's solution).[51] The reaction mixture was diluted with ether (20 ml); 4% sodium hydroxide (30 ml) was added and the mixture allowed to stand for 3 hours, after which the layers were separated. The ether layer was washed twice with 4% sodium hydroxide and with water to neutrality, dried and the ether evaporated to give a colourless oil (150 mg).

Preparative TLC (developed in 3% methanol in chloroform) gave the thioketal (65) as a colourless solid m.p. 66-72° (45 mg). Impure starting material (70 mg) was also recovered, The product (65) had ν_{max} (nujol), 3500 (broad band), 1710-1760 (very weak absorption), 1570cm^{-1} (furan); λ_{max} (EtOH) 239 mμ (ε, 8,600); 60 Mc./sec. NMR ($CDCl_3$) τ 9.19 (3H), 9.05 (3H, distorted doublet), 8.38 (OH), 8.14 (3H, doublet J ca.2 c./sec.), 6.2-6.8 (5H, multiplet, Mass spectral molecular ion (corresponding to $C_{17}H_{24}O_2S_2$) m/e 324 Mass spectral molecular ion (corresponding to $C_{17}H_{24}O_2S_2$) m/e 324 (46%), base peak m/e 213. A satisfactory analysis was not obtained (Found: C, 62.61; H, 8.13. $C_{17}H_{24}O_2S_2$ requires: C, 62.93; H, 7.46%). The product was homogeneous to TLC by multiple elution in three separate solvent systems and to GLC on the phases 1% SE-30 (200°C), 1% QF-1 (200°C), 1% Apiezon L (200°C) and 1% CHDMS-2% PVP (225°).

Sarett Oxidation[27] of (65)

Thioketal [(65); 35mg] was dissolved in dry pyridine (2 ml) and a suspension prepared from chromium trioxide (150 mg) in dry pyridine (1 ml) was added. The mixture was left at room temperature under oxygen-free nitrogen for 14 hours, at which time TLC indicated complete reaction to (66). The chromium salts were filtered off and washed with ether-petroleum ether (1:1). The combined filtered solution was diluted with water (10 ml) and the organic layer separated. The aqueous pyridine layer was extracted

with ether-petroleum ether (1:1). The combined organic layers were washed with water to remove all traces of pyridine (washing with dilute acid caused decomposition) dried and the solvent evaporated. The semi-solid residue [(66; 29 mg] was purified further by preparative TLC (1% methanol in chloroform as solvent) and by crystallisation from ethyl acetate-petroleum ether to m.p. 145-150° (10 mg); ν_{max} (nujol) 1710cm^{-1}; λ_{max} (EtOH) 239 mμ (ε, 8,300); mass spectral molecular ion m/e 322 (60%) (corresponds to $C_{17}H_{22}O_2S_2$), base peak m/e 159. (Found: C, 63.03; H, 6.72: $C_{17}H_{22}O_2S_2$ requires: C, 63.32; H, 6.88%).

Bisthioketal (67)

Thioketalketone [(66); 7 mg] was dissolved in dry ether (2 ml) together with ethanedithiol (0.1 ml) and freshly distilled boron trifluoride etherate (0.1 ml). The solution was left for 14 hours at room temperature under oxygen-free nitrogen. The reaction mixture was diluted with ether and excess ethanedithiol was removed by washing with 4% sodium hydroxide. The ether layer was washed with water, dried and the solvent removed to give a colourless gum (5 mg). This material, which was homogeneous by TLC and GLC (1% SE-30 at 225°; 1% Apiezon L at 225°; 1% DC710 at 225°; 0.6% JXR-0.2% CHDMS at 225°), was characterised by its mass spectrum [molecular ion m/e 398 (38%) corresponding to $C_{19}H_{26}S_4O$, base peak m/e 61].

Attempts to obtain the Cis-fused Analogues of (65) and (67)

Reaction of the cis-furanoketoalcohol (61) under conditions analogous to those used in the formation of the thioketal (65) afforded a product not separated from (65) by GLC on the phase 1% QF-1. In addition the starting material recovered had been substantially converted (as determined by GLC) to the trans-epimer (62). Similar treatment of the cis-furanodiketone (63) gave as the major products two isomers tentatively identified as the cis- and trans-2-ketone mono-thioketals by GC-MS. No bis-thioketal (67) or its expected cis-fused analogue was observed by GLC (1% QF-1) from this reaction.

Raney Nickel Reduction of Bis-thioketal (67).

Bis-thioketal [(67); 4 mg] was dissolved in dioxan (1 ml) and added to a suspension of Raney nickel in dioxan (0.5 ml). The mixture was refluxed for 30 minutes, cooled and filtered. The Raney nickel residue was washed with dioxan (1 ml). The dioxan solution was carefully distilled under reduced pressure at 50° and the solution concentrated to 0.2 ml. This residual material was taken up in benzene-water (2:1; 10 ml) and the benzene layer separated, washed twice with water, dried and the solvent removed under reduced pressure, leaving an oil (3 mg) which was not further purified. Examination of this product

TABLE (7)

Products of the Raney Nickel Reduction of (67)

GLC on 10% Apiezon L at 175°; 40 ml/min

Products	t_R (min.)	
85%	24.8	(not separated from trans-furanoeremophilane)
15%	23.0	(not separated from cis-furanoeremophilane)
standard n-C_{16} alkane	14.6	

GLC on 10% 20M PEG at 150°; 40 ml/min* Mass Spectra**

Products	t_R (min.)	Parent m/e (% base)	Base peak
6%	11.0	220 (13)	110
80%	13.5	218 (40)	108
10%	15.7	216 (34)	108
4%	17.5	216 (44)	108

* Pure cis- and trans-furanoeremophilanes do not separate on this phase and have t_R 13.5 min.

** Admission to the spectrometer was via a 10 ft. 10% Carbowax column.

by GLC and by GC-MS led to the conclusion that trans-furanoeremophilane (26) accounted for 80% of the product. Initially GLC on the phase 10% Apiezon L at 175° suggested the presence of 15% cis-furanoeremophilane. This was disproved when GC-MS via the similar phase JXR revealed ions of mass 220 and 216 (corresponding to $C_{15}H_{24}O$ and $C_{15}H_{20}O$) in this 15% component. Analysis on the phase 10% Carbowax 20M achieved resolution of these probable dihydro and dehydro furano-eremophilanes [see Table (7) and discussion p. 31].

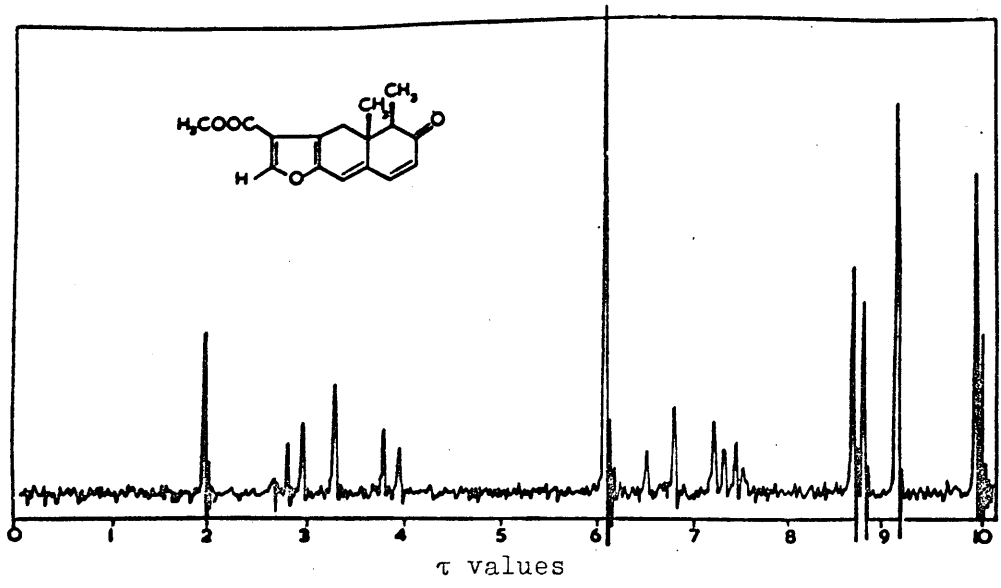
The Constitution of Warburgiadione

Proposed Structure

A second new crystalline sesquiterpenoid, named warburgiadione, was isolated from the oils obtained by solvent extraction of the first consignment of Warburgia ugandensis heartwood. Warburgiadione was eluted after warburgin in the benzene fractions from chromatography on alumina of petroleum extract (A) (p. 37). Further chromatography of ethanol extract (B) (p. 39) and of combined mother liquors containing these compounds gave a total of 0.63g of the yellow crystalline warburgiadione (in crude state). In contrast to warburgin, warburgiadione proved to be stable to air and light and could be crystallised or sublimed without serious loss through decomposition.

Elemental analysis indicated the molecular formula $C_{15}H_{18}O_2$. This was confirmed by the mass spectrum (Appendix) which had the molecular ion at m/e 230. The infrared spectrum of a carbon tetrachloride solution showed two bands of approximately equal intensity at 1686 and 1659cm^{-1}. Both were assigned to conjugated carbonyl functions. A weaker band at 1614cm^{-1} was assigned to the $\nu_{C=C}$ frequency of a double bond. Absorption at 292 mμ (ε, 21,500) in the ultraviolet spectrum suggested an extended chromophoric system.

Fig.(7) 60 Mc./sec. NMR Spectra of Warburgin (16A) and Warburgiadione (74) ($CDCl_3$)



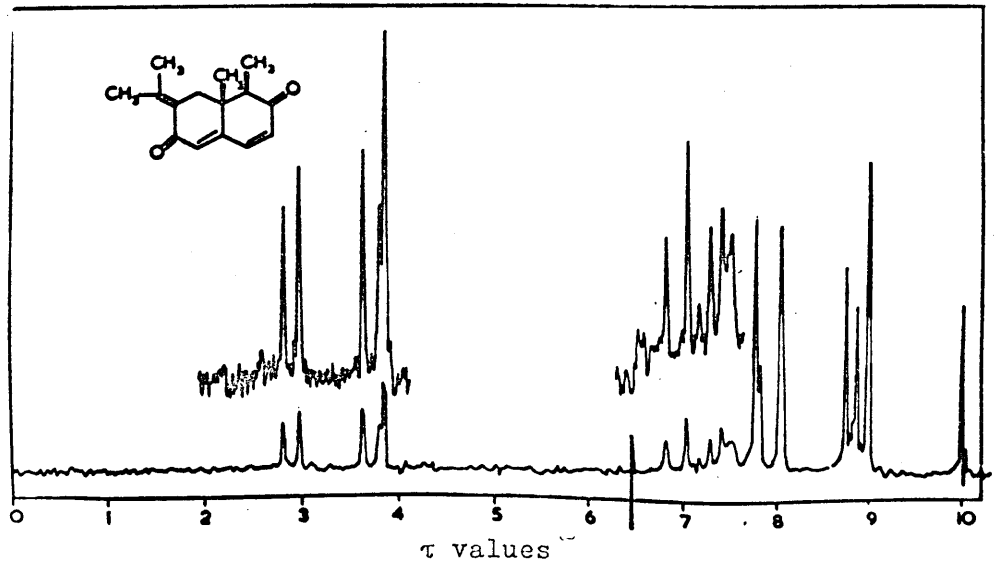
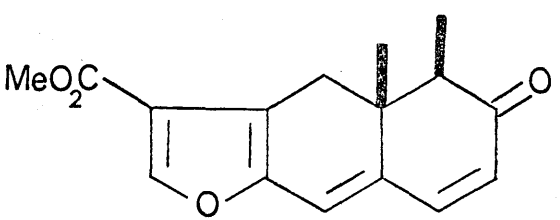


TABLE (8)

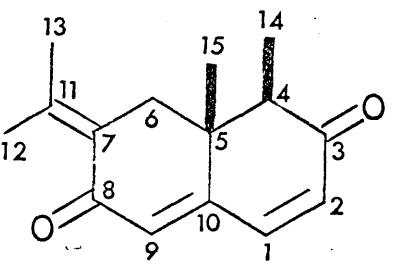
Comparative Data from 100 Mc./sec. NMR Spectra

	Warburgiadione (74) τ	Warburgiadione (74) peak pattern[a]	Warburgin (16A) τ	Warburgin (16A) peak pattern
1 H at C-1	3.10	d, J = 10.0	3.06	d, J = 10.0
1 H at C-2	3.89	d, J = 10.0	4.02	d, J = 10.0
1 H at C-9	4.03	s	3.45	s
1 H at C-6 (βH)	7.06	d, J_{gem}=13.6	6.80	d, J_{gem}=17.0
1 H at C-6 (αH)	7.65	dm, J_{gem}=13.6	7.42	d, J_{gem}=17.0
1 H at C-4	7.44	q, J = 6.8	7.47	q, J = 6.5
3 H at C-14	8.85	d, J = 6.8	8.81	d, J = 6.5
3 H at C-15	9.03	s	9.21	s
3 H at C-12	7.85	d, J = 2.0	-	
3 H at C-13	8.12	d, J = 1.4	-	

[a] J = Values in c./sec. Peak patterns denoted: s = singlet d = doublet; dm = doublet of multiplets; q = quartet.



16A



74

Comparison of the NMR spectrum of warburgiadione with that of warburgin (16A) revealed important points of structural similarity. These observations to be discussed below, taken in conjunction with the above spectroscopic data and an assumed biogenetic relationship with warburgin, allowed deduction of structure (74) (excluding stereochemistry) for warburgiadione. Fig.(7) shows the 60 Mc./sec. NMR spectra of the two compounds while the data in table (8) are taken from the 100 Mc./sec. spectra. Double irradiation experiments, carried out subsequently, confirmed all the couplings assigned.

The spectrum of warburgiadione [table (8)] had a singlet at 9.03 τ and a doublet at 8.85 τ (J = 6.8 c./sec.) which showed the presence of tertiary and secondary methyl groups respectively. As in the spectrum of warburgin the secondary methyl was coupled with a deshielded methine, which afforded a quartet at 7.44 τ. A large coupling (13.6 c./sec.) between signals at 7.65 and 7.06 τ suggested a deshielded methylene function in a dissymmetric environment. However, in contrast to warburgin, the higher field signal (7.65 τ) was further coupled and appeared rather as a doublet of multiplets. Of the three low field protons two, at 3.89 and 3.10 τ were coupled, with J = 10.0 c./sec., and were assigned respectively to the α- and β- protons of an enone. The third was a sharp singlet at 4.03 τ and could thus be regarded

as due to the ethylenic proton of a trisubstituted double bond. A significant difference in the spectra of the two compounds was the presence of a probable isopropylidene function in warburgiadione, indicated by two low field methyl groups at 7.85 and 8.12 τ. We assigned the lower field isopropylidene methyl signal (7.85 τ) to the C-12 methyl group adjacent to the C-8 carbonyl. Both the C-12 and C-13 methyl signals were split and coupled to the higher field (7.65 τ) C-6 proton. Discussion of the stereochemical information to be deduced from the tabulated NMR data will be postponed until a later stage in the argument.

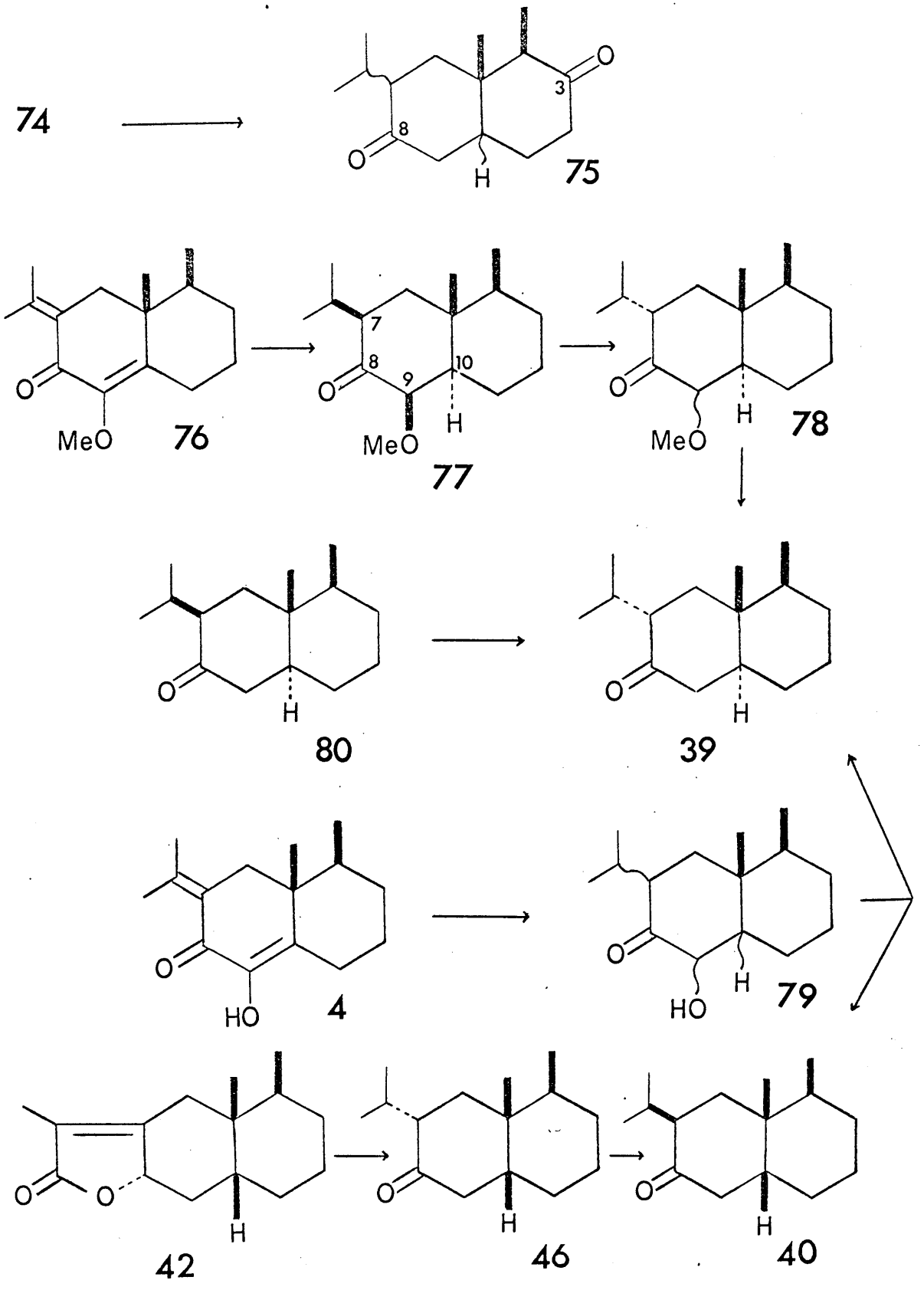
The conjugated chromophore based on the Δ^1-3-one has a calculated λ_{max} value of 286 mμ[52] [215 mμ (parent enone) + 2 x 18 mμ (γ- and δ-substituents) + 30 mμ (conjugated double bond) + 5 (exocyclic double bond)]. This value was in reasonably good agreement with the observed λ_{max} 292 mμ.

Catalytic hydrogenation of warburgiadione with 10% palladium on charcoal in ethyl acetate gave a colourless distillable oil (75) for which elemental analysis indicated the formula $C_{15}H_{24}O_2$, corresponding to the uptake of three moles of hydrogen. The infrared spectrum in carbon tetrachloride showed only one carbonyl band at 1716cm^{-1} with an apparent extinction coefficient (ε^a) of 800 suggesting the superposition of the $\nu_{C=O}$ bands of two

saturated ketone functions. Examination of the product by TLC and by GLC on the phases SE-30 and QF-1 revealed two components in a ratio (estimated from GLC) of 3:2.

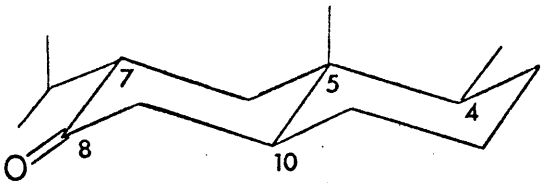
Initial GLC evidence seemed to indicate (if the elemental analysis of the mixture were discounted) that one component might be a hydroxy ketone formed by overhydrogenation, since Jones oxidation[48] led to the virtual removal of one of the peaks. Acetylation had a similar effect, but no peak due to an acetate could be detected: moreover there was only a weak hydroxyl band in the infrared spectrum. That their apparent reactions were due to selective decomposition of one component became clear from later results. Thus treatment of the hydrogenation products with 0.2 N sodium hydroxide in methanol at 60° for one hour again removed the "labile" peak on GLC. TLC examination of the products of the three reactions cited showed spots corresponding in R_f value to the original components, with an enhancement of the less polar spot in each case.

When GC-MS became available the hydrogenation reaction was re-examined: both products showed the molecular ion m/e 236 (corresponding to $C_{15}H_{24}O_2$) and were then assumed to be hexahydrodiketone isomers. The previous observations pointed to the lability of one of these under both acid and basic conditions.


74
75
76
77
78
80
39
4
79
42
46
40
MeO
HO
H
O
3
7
8
9
10

C-8 eremophilanones, as previously observed (p. 21) have been the subject of considerable interest.[24,33,36] Hydrogenation of hydroxyeremophilone methyl ether (76) is reported to give chiefly the 7β, 9β-trans-fused isomer (77) characterised by a strong positive Cotton effect.[33] Isomerisation of (77) with base gave (78) which exhibited a marked reduction in the amplitude of the Cotton effect. Demethoxylation and reoxidation gave (39). It was later observed that hydrogenation of hydroxyeremophilone (4) itself gave a mixture of tetrahydro-isomers (79).[24] Acetylation and deacetoxylation of (79) gave a 1:1 mixture of the cis-fused (40) and trans-fused (39) both of which were base-stable. The base-labile isomers (46) and (80) have also been reported; by degradation of eremophilenolide (42)[24] and by synthesis respectively.[33] The preferred conformations of the thermodynamically stable C-8 eremophilanones are considered to be (81) and (82) in which the isopropyl and C-4 methyl groups are equatorial.[36]

In view of the above results the observation of two isomeric hexahydrowarburgiadiones and the lability of at least one of these to base, are not unexpected. The presence of the C-3 keto group may cause the predicted thermodynamic stabilities of 3,8-diketo systems to be different from the simpler C-8 eremophilanones. It only remains to observe that since we have not



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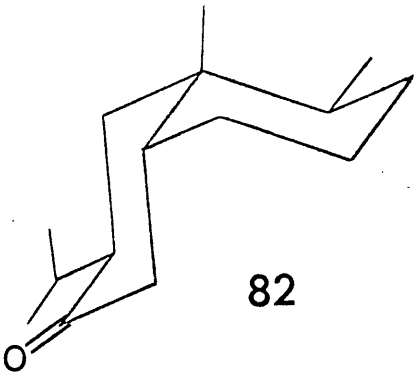

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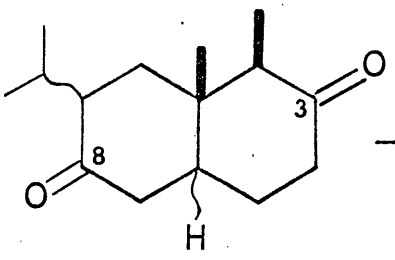
74 →



75

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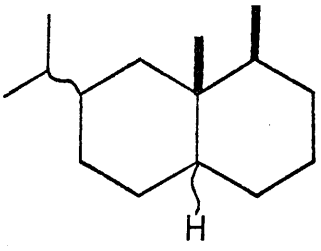

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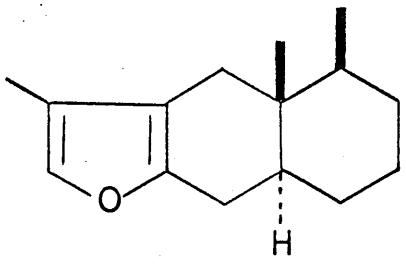


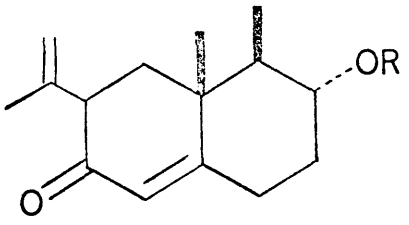
26A

separated and individually examined the hydrogenation products, speculation as to their nature is hardly justified. Fortunately this was not to be a critical point in the structural proof of warburgiadione.

Huang-Minlon reduction[25] of the crude diketone mixture (75) gave in poor yield hydrocarbon (83). This product was apparently homogeneous to GLC on the phase SE-30 but this observation does not exclude the possibility of the presence of hydrocarbon isomers. The mass spectrum had the molecular ion at m/e 208 which corresponds to $C_{15}H_{28}$. The spectrum was similar to that obtained from the mixture of eremophilane isomers from the catalytic hydrogenation of trans-furanoeremophilane (26A). It is however sufficient to regard the conversion of warburgiadione (74) through (75) to a hydrocarbon of formula $C_{15}H_{28}$ as confirmation that the seven double bond equivalents of warburgiadione consist of three double bonds, two ketone functions and two rings.

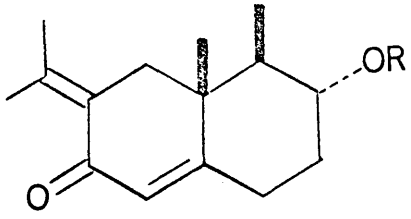
Proof of Structure (74) for Warburgiadione

We considered that eremophilane sesquiterpenoids of the petasin (8) type could be used as starting materials in a partial synthesis of warburgiadione. Petasin and S-petasin (84) have been isolated from Petasites hybridus (L) Compositae.[53-55]



8 R = CO.C(CH_3)=CH(CH_3) cis-

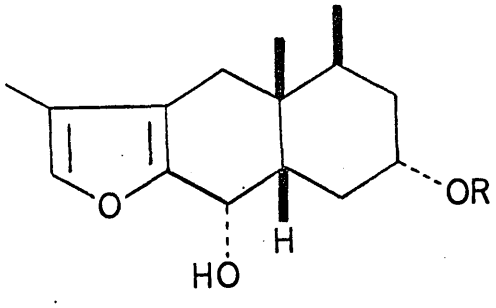
84 R = CO.CH = CH(SCH_3) cis-



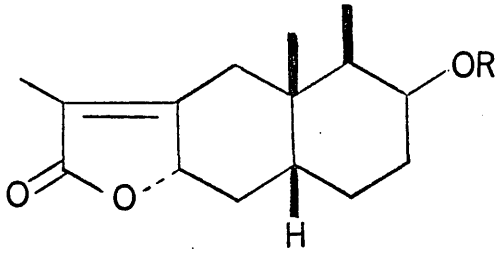
85 R = CO.C(CH_3)=CH(CH_3) cis-

86 R = CO.CH = CH(SCH_3) cis-

88 R = CO.C(CH_3)=CH(CH_3) trans-



43 R = CO.C(CH_3)=CH(CH_3) cis-



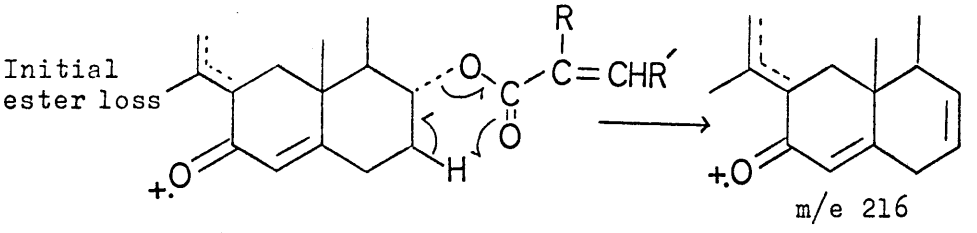
87 R = CO.C(CH_3)=CH(CH_3) cis-

The structures were established by the Swiss workers Aebi and Waaler.[56] Absolute configurational assignments as (8) and (84) were later made by Aebi and Djerassi[34] and by Herbst and Djerassi.[35] The isopropylidene analogues isopetasin (85) and iso-S-petasin (86) were also isolated. However these may be artefacts of the separation procedure, since the ready isomerisation of (8) to (85) has been demonstrated.[56]

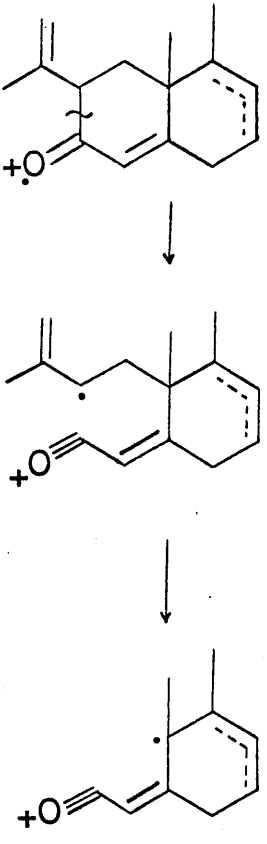
Petasin and its congeners were originally reported as constituents of Petasites hybridus of Swiss origin. The Prague group of Šorm, working with varieties of the same species from Czechoslovak sources, isolated eremophilane types having furan and α,β-unsaturated-γ-lactone groupings as typified by furanopetasin (43)[37] and petasitolide A (87).[49] In a later study on the chemotaxonomy of Petasites species, Novotný et al. examined P. hybridus plants from several locations in Europe.[57] They found an approximately equal distribution of the furanoid and isopropenyl (petasin) types but the two did not co-occur. It seemed therefore that our chance of finding petasin in a Scottish variety of P. hybridus was close to even. In the event, the hardest part of the work was locating the plants in the first place. A source was eventually found on a rubbish dump in rather boggy ground![‡]

‡ We thank Mr. A.M. Grant, Ministry of Agriculture and Fisheries, Motherwell, Lanarkshire, for locating the site and for assistance in the collection of rhizomes.

Fig.(9) Suggested Mass Spectral Fragmentation of the Petasin Group of Esters (8), (84), (85) and (86)



Origin of m/e 148
from (8) and (84)



Origin of m/e 161
from (85) and (86)

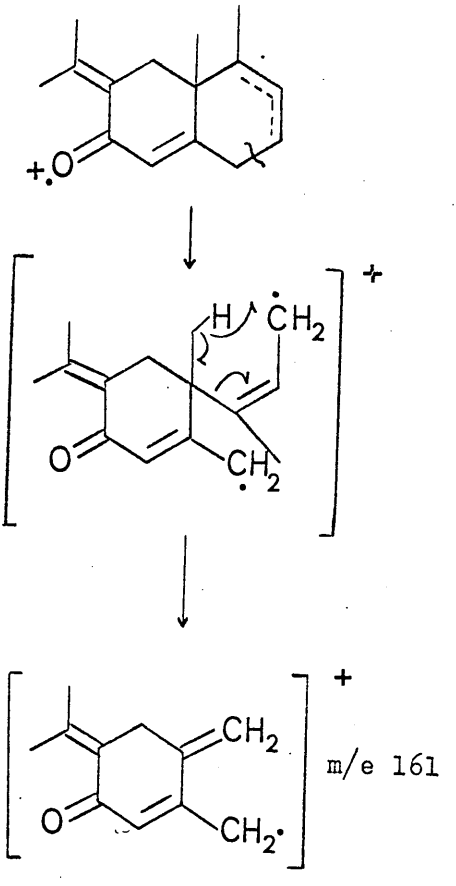


Fig (8)

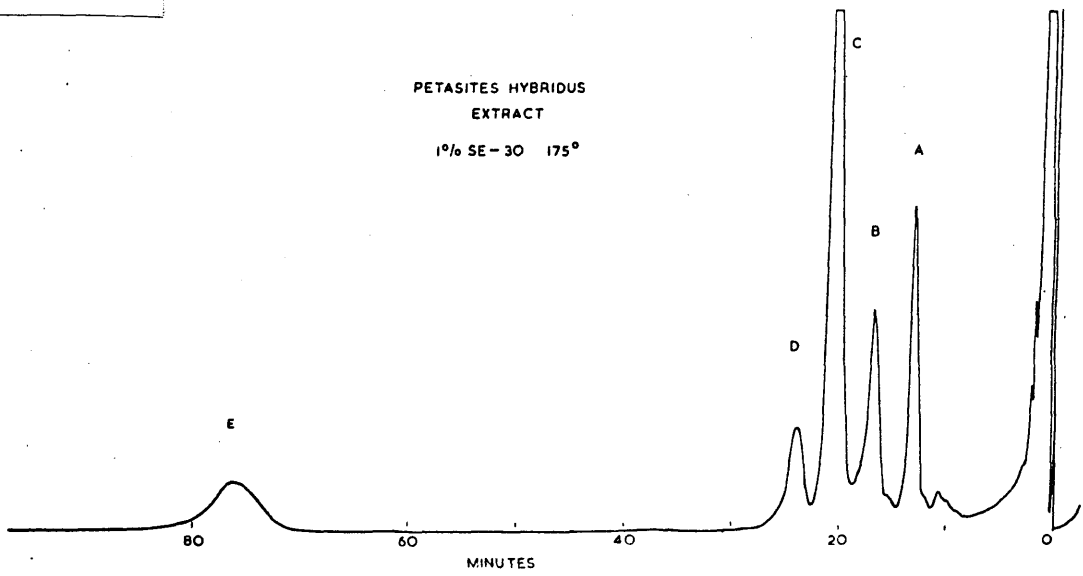


Table (9)

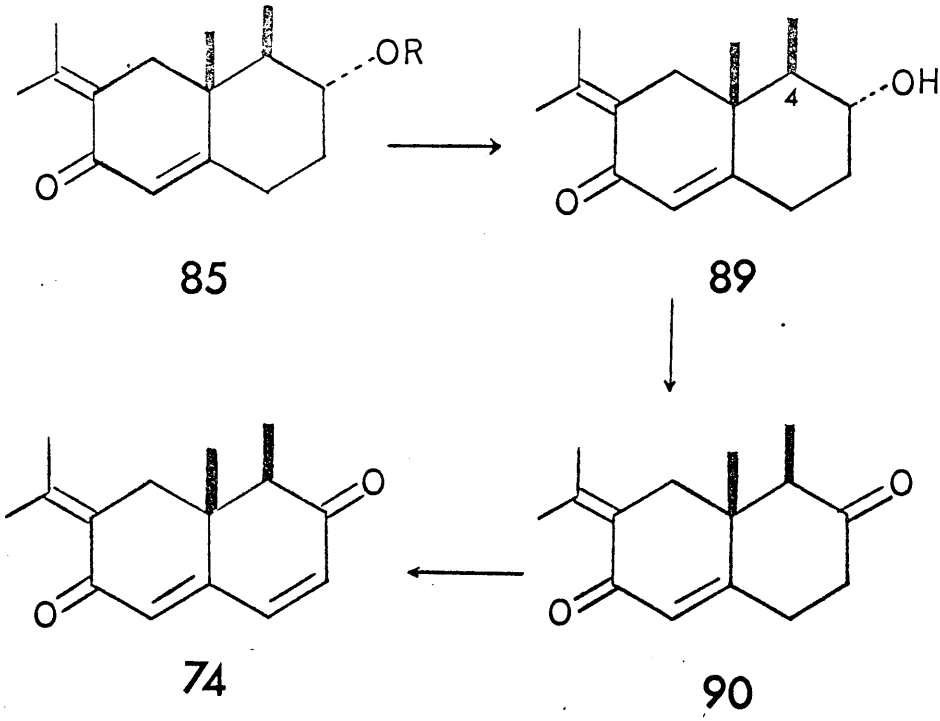
GC-MS of Petasites hybridus Extract

Peak	$t_{R_{SE-30}}$ (min.)	Parent ion (m/e)	Base peak (m/e)	Identity
A	13.2	(~330)	185	?
B	16.8	316	148	Petasin
C	20.5	316	161	Isopetasin
D	23.5	316	161	? Isomer
E	76.0	334	161	Iso-S-petasin

A small scale ethanol extract of the fresh rhizomes was made for preliminary examination by GLC on the phase 1% SE-30. This showed the sesquiterpenoid region to be relatively uncomplicated. A portion of the oil was sublimed for GC-MS. Fig.(8) shows the gas chromatogram of the sublimed extract. It should be noted that sublimation, which may not have been complete, altered the relative proportions of components (B) and (C). Petasin (B) was in fact the major sesquiterpenoid constituent of the total extract. Mass spectral scans of peaks (A)-(E) were made and the results are summarised in table (9). The mass spectra of components (B) and (C), which were inferred to be petasin and isopetasin respectively, are reproduced in the Appendix. Both showed losses of 100 mass units which were expected for the elimination of the angelic acid moieties from the molecular ions [fig.(9)]. The base peaks of the two spectra were different, m/e 148 for petasin and m/e 161 for isopetasin. A tentative explanation of these fragmentations (kindly proposed by Dr. A. McCormick) is also given in fig. (9). Component (D) appeared to be a third isomer and also showed a loss of 100 mass units from the parent ion. The base peak of the spectrum was at m/e 161 as for isopetasin. (D) may be the tiglate analogue (88).
[A tiglate isomer of petasitolide A (87) has been isolated and is considered to be an artefact of the isolation procedure.[49]].
Peak (E) in later extracts was resolved into two components,

probably S- and iso-S-petasin (84) and (86). In these cases the initial ester loss was 118 mass units which corresponds to elimination of β-methylthioacrylic acid.

The above GLC and GC-MS evidence appeared to confirm the presence of the petasin type in our variety of P. hybridus and a large scale extraction was carried out. An initial ethanolic extract was made from 6.5 kg of milled fresh rhizomes at room temperature for six days. This extract was partitioned between chloroform and water and afforded 46 g of oil from the chloroform layer. Chromatography on silica gel resulted in the concentration of petasin with smaller amounts of isopetasin (total 15.1 g) in fractions eluted with ethyl acetate-petroleum ether (1:4). Advantage was then taken of the ready isomerisation of petasin to isopetasin on alumina described by Aebi and Waaler.(56 One third of the petasin-isopetasin mixture (5.0 g) was taken up in petroleum ether and allowed to stand over grade I neutral alumina on a column for twenty-four hours. Elution with ether gave 3.1 g of crude isopetasin (85). Melting point, optical rotation, infrared and ultraviolet data for a pure sample were in good agreement with those reported.(56 The infrared spectrum (measured for a carbon tetrachloride solution) showed the ester carbonyl absorption at 1716cm^{-1}, unsaturated ketone at 1667cm^{-1} and double bond at 1630cm^{-1}.



85 89 74 90

The ultraviolet spectrum in ethanol had λ_{max} 242 mμ (ε, 15,500) and 280 mμ (ε, 7,900). Alkaline hydrolysis of isopetasin gave isopetasol (89), the common hydrolysis product of the petasin-type esters.[56] Oxidation of isopetasol with chromium trioxide in acetone gave isopetasone (90). Both isopetasol and isopetasone had been reported previously. Our physical data for these compounds were consistent with those reported.[56]

The Conversion of Isopetasone (90) to Warburgiadione (74).

Treatment of isopetasol and isopetasone with manganese dioxide in chloroform for twenty-four hours at room temperature resulted in recovery of starting material in each case (TLC). Isopetasone was treated with selenium dioxide in refluxing t-butyl alcohol and a trace of pyridine under nitrogen for seven hours. TLC indicated several polar products, traces of starting material and no evidence of warburgiadione.

Isopetasone was successfully dehydrogenated to warburgiadione using 2,3-dichloro-5,6-dicyanobenzoquinone (D.D.Q.)[58] in refluxing dioxan. The reaction was carried out with 1.1 moles of reagent under nitrogen for ten hours. GLC showed a ratio of starting material to product of approximately 2:3. TLC indicated a small amount of decomposition to polar components. The product was separated by preparative TLC and was crystallised from methanol. This sample was proved to be identical with the naturally-occurring

warburgiadione by the undepressed mixed melting point and by comparison of ultraviolet, infrared (KCl disc) mass spectra and GLC behaviour.

Optical rotation data were in sufficiently close agreement to confirm the stereochemical identity of the two samples. Provided no epimerisation has taken place at C-4 in the transformation of isopetasol through isopetasone to warburgiadione which would require conversion of an equatorial to an axial methyl group, then the absolute configuration of warburgiadione is as represented in formula (74).

Assignment of the NMR Signals due to the C-6 protons of Warburgiadione and Warburgin.

In the 100 Mc./sec. NMR spectrum of warburgiadione [see table (8)] spin decoupling experiments revealed a homoallylic coupling of the higher field C-6 proton (7.65 τ) with the protons of the C-12 and C-13 methyl groups. As expected the transoid 12-H - 6-H coupling (2.0 c./sec.) was greater than the cisoid 13-H - 6-H coupling (1.4 c./sec.).[59,60] The magnitude of such a coupling also depends on the angle between the plane of the 7-11 double bond and the C-6 to hydrogen bond in formula (74). The coupling will be greatest when this angle is close to 90° and a minimum when it is close to zero.[59,60] From models the preferred conformation of (74) appears to be that in which the 6β-proton is in the plane of the 7-11 double bond. The higher

field doublet of multiplets may then be assigned to the 6α-axial proton.

This assignment is supported by the data for warburgin (16A). In the spectrum of warburgin [table (8)] the geminal C-6 protons appear at 6.80 and 7.42 τ as doublets with J = 17.0 c./sec. The higher field doublet is of lower intensity indicating a weak secondary coupling. This signal may be assigned to the 6α-axial proton coupled to the protons of the 5β-angular methyl group. Such a coupling would be a maximum when the C-6 to hydrogen and C-5 to C-15 bonds are at an angle of 180°.(61

Experimental

Warburgiadione (74)

The isolation of warburgiadione from the oils obtained by solvent extraction of the first consignment of *Warburgia ugandensis* heartwood has been described (pp.37-40). The pure material, yellow prisms from methanol, had m.p. 127-8°; $[\alpha]_D$ + 25° ($CHCl_3$); ν_{max} (CCl_4) 1686, 1659 and 1614cm.$^{-1}$; λ_{max} (EtOH) 292 mμ (ε, 21,500); mass spectral molecular ion m/e 230 (100%). (Found: C, 77.96; H, 7.73%. $C_{15}H_{18}O_2$ requires: C, 78.23; H, 7.88%).

Catalytic Hydrogenation of Warburgiadione (74)

Warburgiadione (55 mg) in ethyl acetate (10 ml) was hydrogenated for 30 minutes with 10% palladium on charcoal catalyst (50 mg). A colourless, fragrant oil [(75); 50 mg] was recovered. Micro-distillation (sublimation tube) at 110°-120° with a vacuum of 0.2 mm Hg gave 40 mg of colourless oil which showed ν_{max} (CCl_4) 1716cm^{-1} (ε^a *ca.* 800) and weak ν_{OH} (*ca.* 3500-3600cm^{-1}). (Found: C, 76.16; H, 10.29. $C_{15}H_{24}O_2$ requires: C, 76.23; H, 10.24%). TLC with the solvent system, 2% methanol in chloroform, showed one main component which gave a yellow colouration with 2,4-dinitrophenylhydrazine (2,4-d.n.p.) reagent [R_f 0.75 and not separated from warburgiadione (orange stain with 2,4-d.n.p.)]. Minor polar products were also evident. TLC with the solvent

system, ethyl acetate-petroleum ether, 1:1, revealed two main components both of which gave yellow 2,4-d.n.p. stains (R_f values 0.45, 0.50; warburgiadione R_f 0.50).

GLC using 1% SE-30 and 5% QF-1 showed two components in a ratio of 3:2. Retention data for 1% SE-30 at 150°, with 40 ml./min. gas flow: minor product, t_R 11.5 min; major product, t_R 12.6 min.; n-C_{18} alkane, t_R 12.4 min. Retention data for 5% QF-1 at 175° with 40 ml./min. gas flow: minor product, t_R 11.2 min.; major product, t_R 12.8 min.; n-C_{26} alkane, t_R 11.2 min.

GC-MS, with admission to the mass spectrometer via a 10' 1% QF-1 column, showed that both components had the molecular ion at m/e 236 corresponding to $C_{15}H_{24}O_2$: minor component m/e 236 (35%), 194 (22%), 124 (31%), 122 (base peak, 100%); major component m/e 236 (77%), 194 (base peak, 100%), 124 (97%), 122 (57%).

When the time of hydrogenation was extended to 90 minutes no significant alteration in the product ratio was observed when compared, by GLC and TLC, with the mixture after 30 minutes.

Reactions with Isomer Mixture (75)

(a) Acetylation conditions.

Diketone [(75); ca. 1 mg] was left for 36 hours at room temperature in a small test tube with pyridine (1 drop) and acetic anhydride (2 drops). The solution was evaporated slowly

during reaction time in a vacuum desiccator. GLC (SE-30 and QF-1) revealed the original component of shorter retention (see above) and traces only of the second peak. Further minor products were observed but none amounted to more than 5% of the total recorded product. TLC (ethyl acetate-petroleum ether 1:1) indicated enhanced concentration of the component of R_f 0.50 (see above) with ca. 30% of a spot R_f 0.45 which also gave a yellow stain with 2,4-d.n.p. reagent

(b) Jones oxidation.[48]

To diketone [(75); 5 mg] in acetone at 0°C was added 2 drops of a solution of Jones oxidant (chromium trioxide in aqueous sulphuric acid). After 90 minutes stirring at ice temperature the reaction was worked up as previously described (p. 55). TLC and GLC (SE-30 and QF-1) revealed closely similar results to the observations under (a) above. In this case decomposition evident from streaking of polar material was shown by TLC.

(c) Base treatment.

Diketone [(75); 5 mg] was heated in methanolic sodium hydroxide (1 ml; 0.2 N) at 60° for 1 hour. The reaction mixture was diluted with water and extracted with ether. The ether extract was washed (water) and dried. Again the results judged by GLC and TLC as above were similar to those observed in (a) and (b), i.e. the component of longer retention on GLC had virtually

disappeared while TLC apparently indicated the two original spots with enhancement in concentration of that of R_f 0.50. Decomposition to polar material was not significant in this case.

Huang-Minlon[25] Reduction of Diketone (75)

The total hydrogenation product, crude diketone [(75); 30 mg] was dissolved in ethylene glycol (3 ml) with 90% hydrazine hydrate (0.3 ml) and heated at 60° for 2 hours. Potassium hydroxide (300 mg) in ethylene glycol (3 ml) was added and the solution heated at 180° (bath temperature) for 6 hours then concentrated slightly by distillation at 195-200° during 3 hours. The distillate was combined with the bulk of the solution, diluted with petroleum ether (20 ml) and water (50 ml) and the layers separated. The aqueous layer was re-extracted with petroleum ether. The combined petroleum ether extracts were washed with water, dried and the solvent was removed at room temperature. This total material was chromatographed on alumina (grade II; 0.5 g) and two fractions were taken. An oil (6 mg) was eluted with petroleum ether (10 ml); further oily material (11 mg) was eluted with ether. TLC (developed in petroleum ether) showed the presence of a hydrocarbon, R_f 0.75 (weakly stained with ceric sulphate) and a ketonic polar component (stained with 2,4-d.n.p. reagent), R_f 0.05, in the petroleum ether fraction. The hydrocarbon constituent was absent from the ether fraction. GLC on the phase 1% SE-30 at 100°

appeared to show the eremophilane (83) peak (*ca.* 75% of the total petroleum ether fraction) to be homogeneous (t_R 6.1 min.); two further components of t_R 16.3 min. and 19.6 min. were also recorded. The hydrocarbon component was not distinguishable under the above GLC conditions from the composite eremophilane peaks obtained from *trans*- and from *cis*-furanoeremophilane [p.47 and table (5)]. GC-MS indicated the molecular ion at m/e 208 corresponding to $C_{15}H_{28}$. It should be noted that the mass spectra of the eremophilanes from warburgiadione, *cis*- (23) and *trans*-furanoeremophilane (26) were essentially similar.

TABLE (10)

Chromatography of Extract (A) from Petasites hybridus L.

Fraction	Solvent	Total vol. (litres)	Wt. (g.)	
1-4	petroleum ether	2.0	1.5	
5-8	ethyl acetate-petroleum ether (1:49)	2.0	0.2	
9-12	" (1:19)	2.0	0.1	
13-16	" (1:9)	2.0	2.5	
17-24	" (1:4)	4.0	15.1	petasin/ isopetasin
25-28	" (1:1)	2.0	3.9	
29-34	ethyl acetate	3.0	9.4	
		Recovery	71%	

Extraction of the Rhizomes of Petasites hybridus L., Isolation of Isopetasin

Petasites hybridus L. plants were collected from a site near Motherwell (Lanarkshire) in early Spring. Fresh rhizomes (6.5 kg) were milled in ethanol and allowed to stand for 6 days at room temperature in ethanol (30 litres). The solution was concentrated to 3 litres using a "cyclone" evaporator and then the solvent completely removed (40° *in vacuo*) leaving a tar-water mixture. This was shaken with chloroform (2.0 litres) and water (500 ml). The chloroform layer was separated, washed with water (250 ml), dried and the solvent evaporated (30° *in vacuo*) to give a brown oil [(A); 46 g].

The total oil (A) was chromatographed on commercial grade chromatographic silica (1800 g) and 34 fractions of 500 ml were taken using a rough gradient elution with petroleum ether-ethyl acetate [table (10)]. Chromatography was directed specifically at the isolation of petasin (8) and isopetasin (85), the major sesquiterpenoid constituents, and its progress was followed by TLC and by GLC (1% SE-30). Fractions which did not contain these compounds were not further purified. Petasin and smaller amounts of isopetasin were the major components of 8 fractions eluted with ethyl acetate-petroleum ether (1:4). A portion (5.0 g) of these combined fractions was taken up in petroleum ether and allowed to stand over alumina (grade I; 100 g)

on a column for 24 hours. Elution with ether afforded crude semi-solid isopetasin (3.1 g) recrystallised twice from methanol to m.p. 90-94° (1.20 g). An analytical sample from methanol had m.p. 95-98°; $[\alpha]_D$ + 26° ($CHCl_3$); ν_{max} (CCl_4) 1716, 1667, 1630cm^{-1}; λ_{max} (EtOH) 242 mμ (ε, 15,500), 280 mμ (ε, 7,900); λ_{max} (cyclohexane) 228 mμ (ε, 17,900), 269 mμ (ε, 7,200). (Found: C, 75.97; H, 8.90. $C_{20}H_{28}O_3$ requires: C, 75.91; H, 8.92%). Aebi and Waaler[56] quote: m.p. 96-98°; $[\alpha]_D^{21}$ + 28° ($CHCl_3$); ν_{max} (CCl_4) 1714, 1667cm^{-1}; λ_{max} (EtOH) 245 mμ (ε, 13,550), 280 mμ (ε, 7,460); λ_{max} (cyclohexane) 228 mμ (ε, 16,800), 268 mμ (ε, 7,490).

Isopetasol (89)

Isopetasin (1.0 g) was dissolved in ethanol (20 ml) and potassium hydroxide (0.5 g) in ethanol/water (1:1; 20 ml) was added. The solution was refluxed for 2 hours, concentrated by evaporation of the bulk of the solvent under reduced pressure, diluted with water (100 ml) and extracted with ether. The ether extract was washed with dilute hydrochloric acid (1 N) and with water, dried and the ether evaporated to give a discoloured semi-solid (702 mg). This product was recrystallised from ethyl acetate-ether to give isopetasol [(89); 424 mg] m.p. 120-126°. A sample for analysis was crystallised from ether to m.p. 126-7°; $[\alpha]_D$ + 105° ($CHCl_3$); ν_{max} (CCl_4) 3634, 1669, 1630cm^{-1};

λ_{max} (EtOH) 246 mμ (ε, 12,900), 280 mμ (ε, 7,700). (Found: C, 76.88; H, 9.52. $C_{15}H_{22}O_2$ requires: C, 76.88; H, 9.46%) [reported[56 m.p. 126-7°; $[\alpha]_D^{21}$ + 110° ($CHCl_3$)].

Isopetasone (90)

Isopetasol [(89); 380 mg] was dissolved in acetone (20 ml) at ice temperature. Jones reagent[48 (1.0 ml) was added slowly with stirring over 10 minutes, the temperature being kept below 5°C. After 15 minutes the reaction mixture was diluted with water (50 ml) and extracted with ether. The ether extract was washed with water to neutrality, dried and the solvent removed to give a solid product (340 mg). Recrystallisation from methanol gave isopetasone [(90); 225 mg] of m.p. 112-115°. A sample for analysis had m.p. 113-115°; $[\alpha]_D$ + 31° ($CHCl_3$); ν_{max} (CCl_4) 1724, 1669, 1630cm^{-1}; λ_{max} (EtOH) 244 mμ (ε, 12,300), 281 mμ (ε, 7,000). (Found C, 77.57; H, 8.55. $C_{15}H_{20}O_2$ requires: C, 77.55; H, 8.68%). (Reported[56 m.p. 115-117°; $[\alpha]_D$ + 33.3° $CHCl_3$)].

Preliminary Attempts to Synthesise Warburgiadione

Isopetasone [(90); 5 mg] and manganese dioxide (50 mg) were shaken for 24 hours at room temperature in chloroform (5 ml). TLC indicated recovery of starting material. Under the same conditions isopetasol (89) was also unchanged.

Isopetasone [(90); 5 mg] was refluxed in t-butyl alcohol (5 ml) with selenium dioxide (10 mg) and pyridine (1 drop) under nitrogen for 7 hours. Ethanol was added, the solution filtered and evaporated. TLC of the residue (2% methanol in chloroform) indicated the presence of starting material and unidentified polar products.

D.D.Q. Dehydrogenation[58] of Isopetasone (90) to Warburgiadione (74)

Isopetasone [(90); 50 mg] was dissolved in AnalaR grade dioxan (5 ml) with 2,3-dicyano-5,6-dichlorobenzoquinone (D.D.Q.) (ca. 1.1 mole, 55 mg) and heated under oxygen-free nitrogen at 110° bath temperature for 10 hours. The reaction mixture was cooled, filtered and the dioxan evaporated under reduced pressure. The residue was taken up in benzene and eluted from a short column of alumina (2 g; grade IV) with benzene (50 ml). A yellow oil (36 mg) was recovered. Examination of this product by GLC (1% CHDMS - 2% PVP) indicated a 60:40 ratio of warburgiadione (74) to isopetasone (90). The two compounds were separated by preparative TLC (using the solvent system ethyl acetate-petroleum ether, 3:7) and crude warburgiadione, was obtained as a solid (15 mg) m.p. 105-120°. Recrystallisation from methanol gave 9 mg m.p. 122-6°, $[\alpha]_D$ + 29° ($CHCl_3$). This material was proved to be identical with natural warburgiadione by mixed m.p.; also by comparison of infrared (KCl disc), ultra-violet and mass spectra and of GLC data under the following

conditions: 1% QF-1, 175°, 40 ml/min., t_R 7.7 min. (isopetasone t_R 9.8 min.); 1% SE-30, 150°, 40 ml/min., t_R 7.4 min. (isopetasone 8.2 min.): 1% CHDMS-2% PVP, 175°, 40 ml/min., t_R 11.9 min. (isopetasone t_R 14.3 min.).

The Constitution of Ugandensolide and Ugandensidial

We received a fresh supply of *Warburgia ugandensis* heartwood, again through the Tropical Products Institute of London. This wood was paler in colour and less aromatic than the first. Unfortunately we have no information as to the season of collection or place of origin in Uganda of either consignment. The two heartwood oils were compared by GLC and on the phase SE-30 the second sample, although complex, appeared to show warburgin and two new major components of longer retention. The phase QF-1 resolved the peak originally assigned to warburgin and revealed a third major constituent in the second oil. Chromatography was then directed towards the isolation of these three compounds. Two of them, which we have named ugandensolide and ugandensidial have been identified. The third referred to as W.H./XII has not been obtained in pure state.

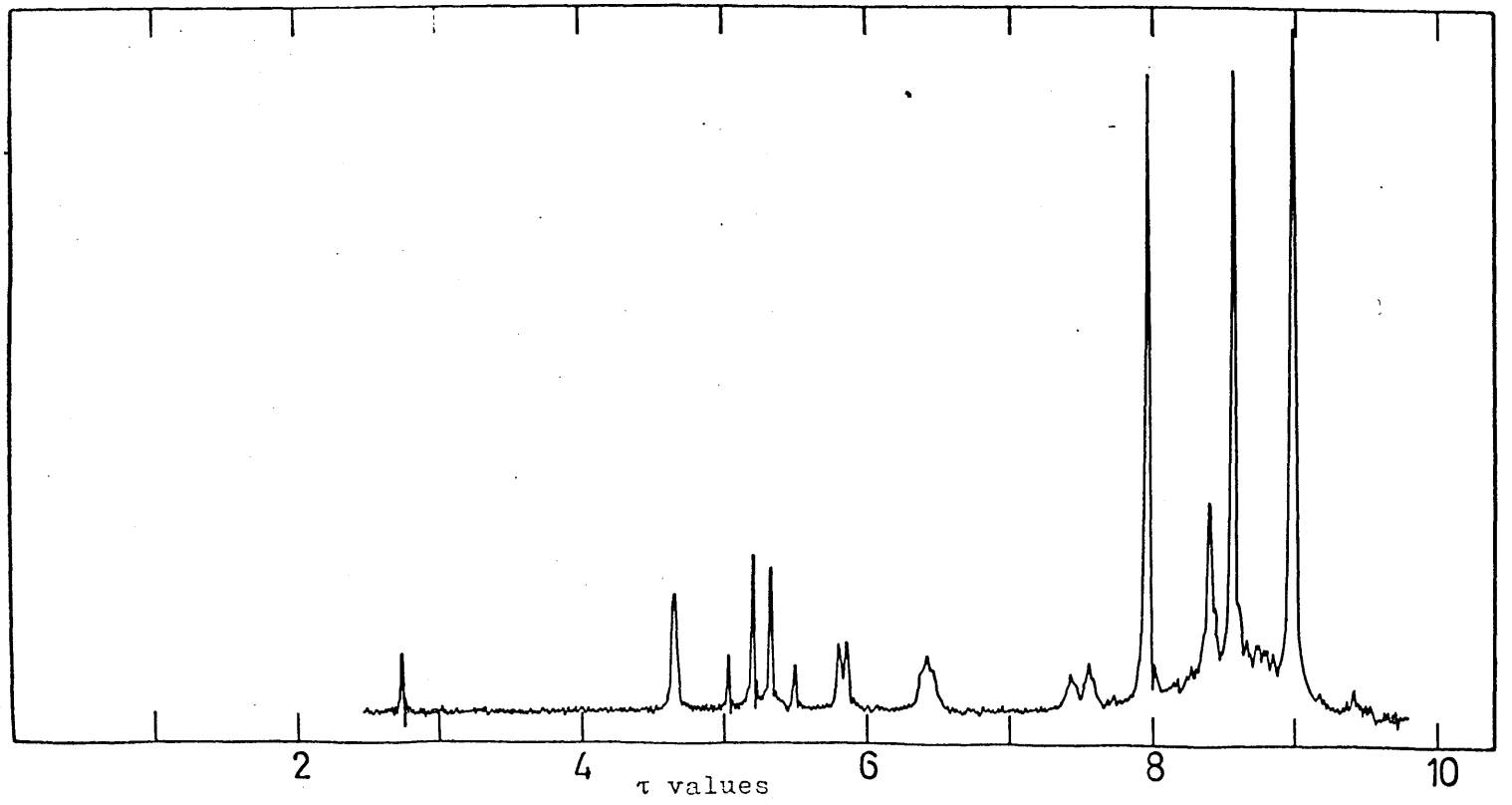
Ethanol extraction of the powdered heartwood, chloroform-water partition and chromatography of the resulting oil is detailed in the experimental section (pp. 102-7). Ugandensolide and ugandensidial were obtained after successive column chromatography on silicic acid and alumina. Both proved to be stable and highly crystalline. The component W.H./XII appeared to be thermally labile and was not present in significant amount in extracts from which the solvents had been removed at steam

temperature using a "cyclone" evaporator. It was concentrated as a rather unstable discoloured oil by chromatography on silicic acid of an extract obtained by removal of solvents under milder conditions. W.H./XII had apparent molecular weight (GC-MS) of 328, ν_{max} (film) 1750s.cm^{-1} and weak ultraviolet absorption at 225 mμ. It has not been further characterised or fully purified,

Structural Proposals for Ugandensolide

Ugandensolide crystallised as colourless needles from ethanol with melting point 218° and $[\alpha]_D$ + 23° ($CHCl_3$) Elemental analysis and the mass spectrum indicated the formula $C_{17}H_{24}O_5$. The infrared spectrum in carbon tetrachloride showed a hydroxyl band at 3600cm^{-1} (unaltered on dilution) and carbonyl bands at 1734 (tentatively assigned to an acetate), 1769s. and 1751ms.cm^{-1}. In chloroform solution these bands appeared at 1750, 1760 (shoulder of slightly weaker intensity) and 1731cm^{-1} (acetate). Similar complex shapes and solvent dependence of the carbonyl absorption have been reported in studies of γ-lactones.(62,63 Where applicable Fermi resonance involving the first overtone of the $\gamma_{CH(\alpha\text{-to } C=O)}$ frequency and $\nu_{C=O}$ has been proposed as an explanation.(62 The reasons for complexity in the carbonyl region in other cases are not fully understood.(63 In the ultraviolet region ugandensolide showed absorption in absolute ethanol at λ_{max} 214 mμ (ε,13,000) (intensified in 0.1 N sodium

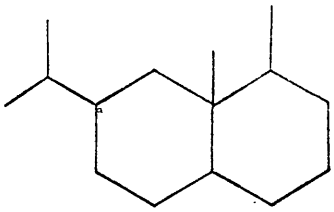
Fig.(10) 100 Mc./sec. NMR Spectrum of Ugandensolide ($CDCl_3$)



hydroxide). Taken in conjunction with the infrared data this suggested the presence of an αβ-unsaturated γ-lactone function.

The 100 Mc./sec. NMR spectrum[fig.(10)] afforded considerable structural information. The presence of an acetate was supported by a peak at 7.97 τ (3H). The hydroxyl proton at 6.43 τ was coupled, with J = 5.5 c./sec., to a proton at 5.83 τ, which proved the hydroxyl to be secondary. (Exchange of the hydroxyl with deuterium oxide gave a singlet at 5.83 τ). Three tertiary methyl groups were apparent from signals at 9.00 and 8.98 τ (6H) and 8.57 τ. A single proton at 4.66 τ, a broadened singlet, was assigned to that on the carbon bearing the acetate (-C<u>H</u>-OAc). Two doublets at 5.39 τ (1H) and 5.15 τ (1H), strongly coupled with J = 17.0 c./sec. were assigned to the lactone methylene protons (-O-C<u>H</u>$_2$-) evidently in a dissymmetric environment. A doublet of multiplets due to a single proton at 7.47 τ with a coupling constant of approximately 12 c./sec. could not at first be explained.

On the above rationale there were no olefinic protons and the butenolide was assumed to have a tetrasubstituted double bond. This is consistent with the resistance of ugandensolide to catalytic hydrogenation. It was recovered unchanged (as determined by GLC) after treatment in ethanol over



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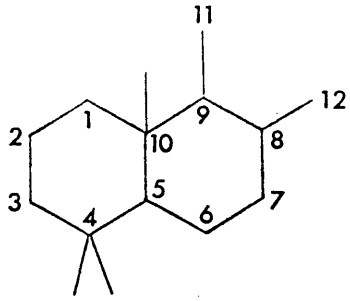

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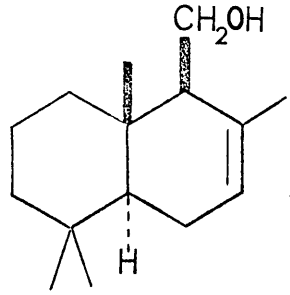

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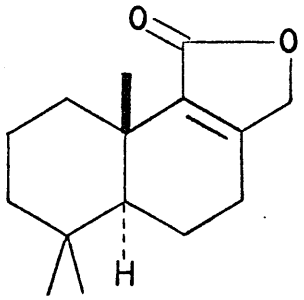

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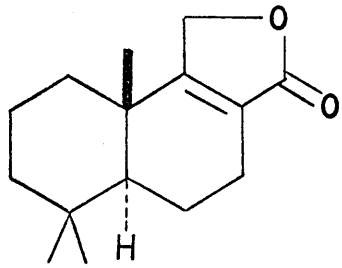

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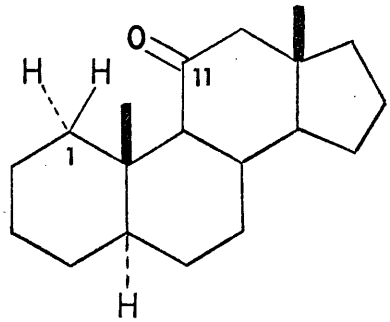


93

C-1α- 9.22τ
C-1β- 7.55τ
} J = 12 c./sec.

platinum oxide catalyst at 40° under a pressure of four atmospheres. In acetic acid again with platinum oxide catalyst, 60% starting material was recovered together with two unidentified products. Of the six double bond equivalents implied by the formula, $C_{17}H_{24}O_5$, the butenolide and acetate accounted for four, leaving two carbocyclic rings. The absence of secondary and vinylic methyl groups virtually excluded the possible eremophilane (1) carbon skeleton. Three angular methyl groups and a butenolide seemed best accommodated in a bicyclofarnesane (2) structure. The isolation of drimenol (7) from extraction of the first supply of heartwood lent biogenetic support to this theory. We therefore assigned to ugandensolide, as a working hypothesis, the basic isodrimenin (91) or confertifolin (92)[64] skeleton with additional secondary hydroxyl and acetate functions.

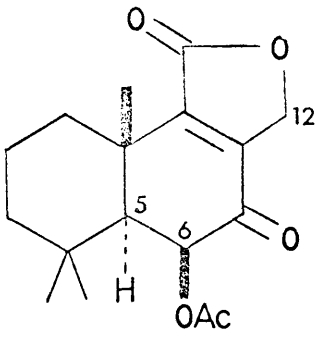
The doublet of multiplets at 7.47 τ, unassigned in the above discussion, was shown by double irradiation to be coupled to an unseen resonance at 8.72 τ (J ca.12c./sec.). We observed similar doublets in the spectra of butenolide degradation products of ugandensolide. Assuming the isodrimenin skeleton, the lower field doublet could be assigned to the C-1β equatorial proton in the plane of, and deshielded by, the lactone carbonyl. The α-proton, out of this plane, is then slightly shielded, accounting for the signal at 8.72 τ. Similar data are recorded for

5α-androstan-11-one (93) in which a coupling of 12 c./sec. between the C-1β proton at 7.55 τ and the shielded 1α proton at 9.22 τ is observed.[65] The spectrum of isodrimenin also shows a diffuse doublet at 7.52 τ with J ca. 13 c./sec.

The C-10 angular methyl resonance in isodrimenin is at 8.92 τ. In ugandensolide this methyl signal is shifted to 8.57 τ implying a marked deshielding due either to the acetate or hydroxyl functions. The lactone methylene in isodrimenin appears as a sharp singlet at 5.58 τ. The coupling of 17.0 c./sec. between these protons in ugandensolide was again presumed to be due to the effect of the acetate or hydroxyl functions.

Oxidation of Ugandensolide and Proof of the Gross Structure

Oxidation of ugandensolide with chromium trioxide in acetone-sulphuric acid (Jones)[48] gave a ketone of molecular formula $C_{17}H_{22}O_5$ (analysis and mass spectrum). Absorption at 1698cm^{-1} in the infrared suggested a conjugated carbonyl function. This received support from the ultraviolet absorption at 250 mμ (ε, 10,000). In the NMR spectrum the secondary acetate proton (-CHOAc) appeared as a doublet at 4.11 τ with J = 2.0 c./sec. proved, by double irradiation, to be coupling with a proton at 8.13 τ. This latter signal was clearly distinguishable from the saturated methylene resonances and was assigned to the 5α-(ring



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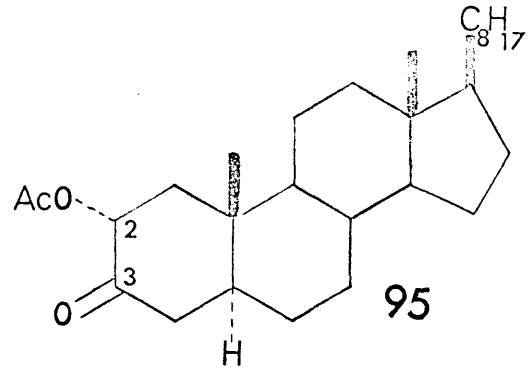
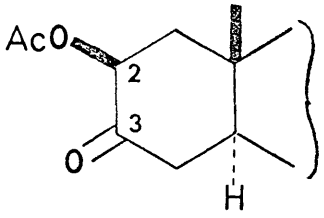


95

ν_{max}

1759cm^{-1}

1740cm^{-1}



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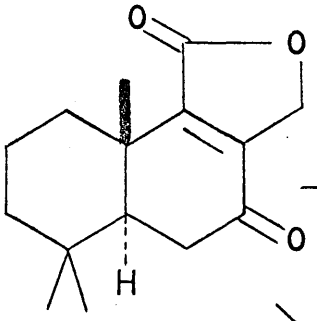
1758cm^{-1}

1743cm^{-1}



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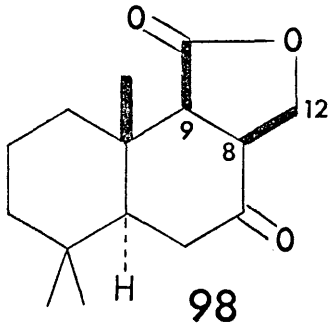

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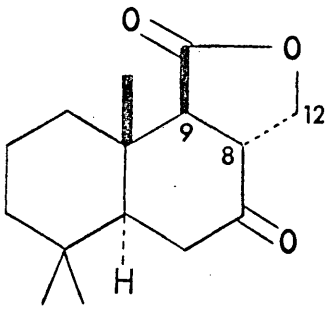

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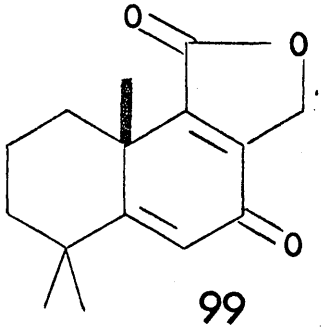

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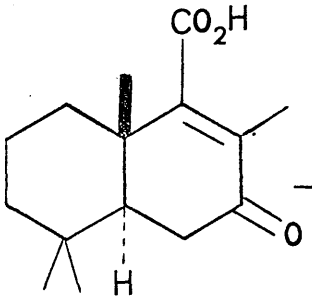

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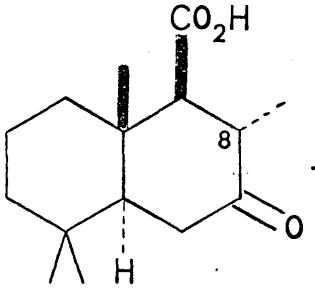

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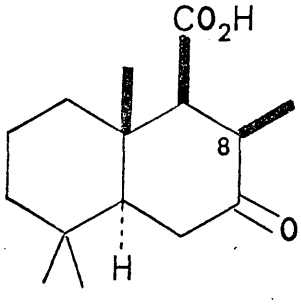


103

junction) proton. If we assume the basic isodrimenin structure and absolute stereochemistry, these observations strongly support structure (94) for this ketone. The assignment of the C-6β-axial acetate is indicated by the weak coupling (2.0 c./sec.) between the 5α- and 6α- protons consistent with a dihedral angle of about 60°.(66

There were, however, several interesting features in the physical data recorded for (94) some of which were at first difficult to reconcile with the assigned structure. In the carbonyl region of the solution infrared spectrum, bands at 1761 and 1774cm^{-1} were assigned to the acetate and lactone respectively. The rise of 27cm^{-1} in the acetate frequency when compared with ugandensolide can be accounted for by interaction between the ketone and acetate function as in 2α-acetoxycholestan -3-one (95) and the 2β-acetoxy-3-one (96).(67 In the ultraviolet spectrum of ketone (94) a hypsochromic shift in base from λ_{max} 250 mμ (ε, 10,000) to λ_{max} 245 mμ (ε, 8,500) contrasts with the observation for oxoisodrimenin (97) which shows a bathochromic shift from 247 mμ (ε, 10,600) to 259 mμ (ε, 5,600).(64 The ultraviolet spectrum of (94) is briefly discussed at a later stage (p. 97).

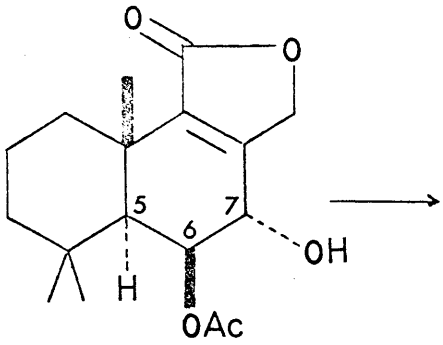
The signal due to the lactone methylene in the NMR spectrum of (94) appeared as a singlet at 5.10 τ. The dissymmetry

at this position in ugandensolide was therefore evidently mainly due to the effect of the hydroxyl function. The most significant feature of the NMR spectrum of the ketoacetate was the additional deshielding of the C-10 angular methyl group to 8,35 τ. In ugandensolide this signal was at 8.57 τ and in isodrimenin at 8.92 τ. However if Zürcher's rules[68] are applied using isodrimenin as the parent compound a value of 8.46 τ is predicted for the angular methyl resonance in (94) [8.92 - 0.183 (6β-OAc) - 0.275 (7-keto group) = 8.46] which is in moderately good agreement with the observed value.

We considered that a correlation of the ketoacetate (94) with either oxoisodrimenin (97) (obtained by oxidation of isodrimenin) or its zinc and acetic acid reduction product (98)[64] should be feasible and would thus provide a simple proof of structure (94). Reaction of (94) in refluxing acetic acid with zinc dust gave a single crystalline product of molecular formula $C_{15}H_{22}O_3$ (analysis and mass spectrum) with infrared maxima in carbon tetrachloride at 1712 (cyclohexanone) and 1778cm^{-1} (butanolide). This product proved to be identical with a sample of dihydro-oxoisodrimenin‡ by mixed melting point determination and comparison of infrared and mass spectra, optical rotation and GLC data.

‡ A sample of dihydrooxoisodrimenin [originally assigned structure (98)] was kindly supplied by Dr. J.D.Connolly of this Department.

Appel et al. had assigned a cis-fused lactone structure (98) to dihydro-oxoisodrimenin on the basis of its ready dehydrogenation with selenium dioxide to the dienone (99).[64] The NMR spectrum of dihydro-oxoisodrimenin revealed a coupling of 12.5 c./sec. between the C-8 and C-9 ring junction protons. The C-9 proton gave a sharp doublet at 7.16 τ. The C-8 proton at 6.70 τ appeared as a complex multiplet due to additional coupling with the C-12 methylene at 5.54 τ. Irradiation at this methylene resonance gave a doublet with J = 12.5 c./sec. for the C-8 proton. The dihedral angle between the C-8 and C-9 protons in the cis-fused lactone (98) is close to zero. From the Karplus equation the coupling constant should therefore have a maximum value of 10 c./sec.[66] The observed coupling of 12.5 c./sec. better fits the trans-fused structure (100) implying an epimerisation at C-8 under the reaction conditions. This is supported by the coupling of 12.0 c./sec. between the C-8 and C-9 protons of the 8α-methyl ketoacid (102). (The signal due to the C-8 proton was complex, however irradiation at the C-8 methyl resonance collapsed the multiplet due to the C-8 β-proton to a doublet with J = 12.0 c./sec.). Ketoacid (102) is the major product of zinc and acetic acid reduction of the unsaturated compound (101).[11] The 8β-methyl isomer (103) was also isolated in small amount from the reaction products. While we have not isolated an analogous labile lactone from the reduction of



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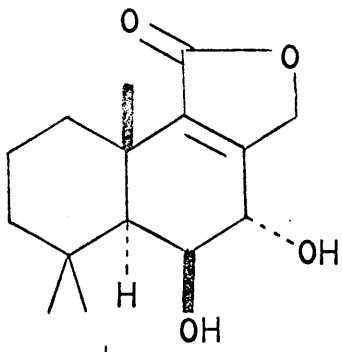

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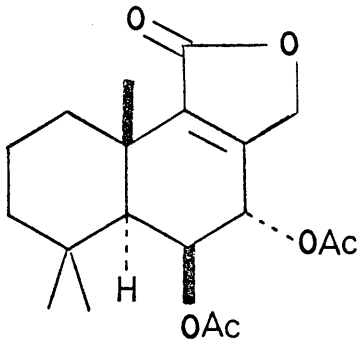

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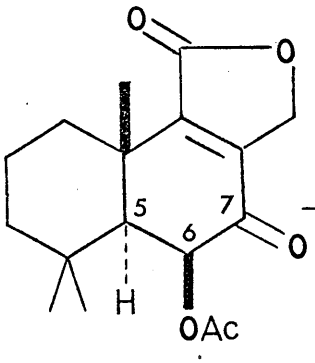

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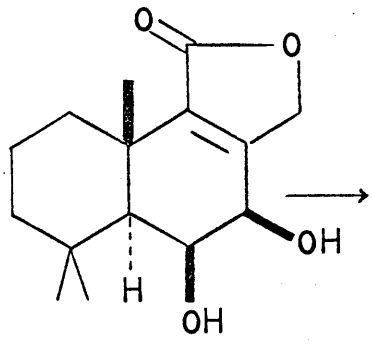

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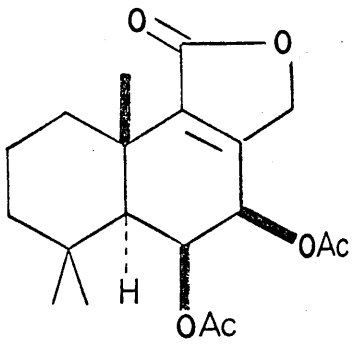


108

ketoacetate (94) we feel that the NMR evidence favours the trans-structure (100) for dihydro-oxoisodrimenin.

Regardless of the configuration of dihydro-oxoisodrimenin at C-8, the identity of our product with that obtained from isodrimenin together with the physical data cited determined the structure and absolute configuration of the ketoacetate as (94) and of ugandensolide as (104) or its C-7 epimer.

Stereochemistry at C-7 in Ugandensolide

The following evidence permits assignment of a C-7α-hydroxyl configuration to ugandensolide as (104).

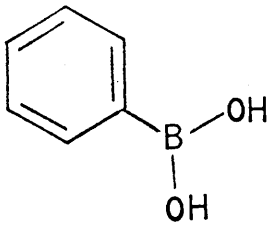
Hydrolysis of ugandensolide with sulphuric acid in methanol gave a diol (105), $C_{15}H_{22}O_4$ (analysis and mass spectrum). The infrared spectrum in chloroform showed two bands due to the lactone in the carbonyl region at ν_{max} 1761 and 1751cm^{-1}. Reacetylation with acetic anhydride in pyridine gave the diacetate (106) also obtained by acetylation of (104). Treatment of the ketoacetate (94) with sodium borohydride in methanol resulted in reduction and hydrolysis to a second diol (107) characterised by elemental analysis, infrared and mass spectra. Acetylation of (107) gave the diacetate (108). Examination of the crystallisation mother liquors from diol (107), by acetylation of a portion for GLC, suggested that the isomeric diol (105) accounted for not more than 5% of the total hydride reduction product.

The distinction between diols (105) and (107) could have

been due to their isomeric nature at either or both the C-6 and C-7 positions. However the NMR spectra of the corresponding diacetates showed no significant coupling between the C-5α- and C-6 protons proving the axial nature of the C-6-hydroxy-substituent and indicating that no epimerisation had taken place at C-6 in the borohydride reduction of (94). The diols (105) and (107) were therefore epimeric at C-7. Hydride attack on the ketoacetate (94) would be expected to occur predominantly from the less hindered α-face leading to the cis-6β,7β-diol (107). Ugandensolide (104), diol (105) and diacetate (106) therefore have the trans-6β,7α-stereochemistry implied in the formulae.

The coupling constants between the C-6α- and C-7 protons in the NMR spectra of diacetates (106) and (108) are as expected for the dihedral angles estimated from models.[66] Thus for compound (106), where the angle (6α-H - 7β-H) is substantially greater than 60°, coupling is weak (ca. 1 c./sec.) and for (108), where the angle (6α-H - 7α-H) is correspondingly less than 60°, the observed coupling is 5.0 c./sec.

Evidence supporting the stereochemical assignments to diols (105) and (107) was obtained from their reaction with phenylboric acid (109). Solutions of the diols in pyridine were treated with 1.5 molar equivalents of phenylboric acid reagent in acetone at room temperature for three hours.[72] The mixtures were examined directly by GLC (SE-30) and by GC-MS.



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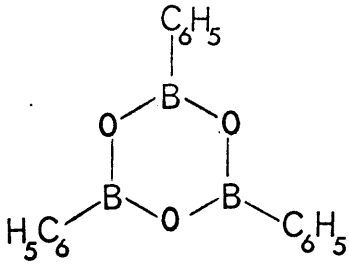

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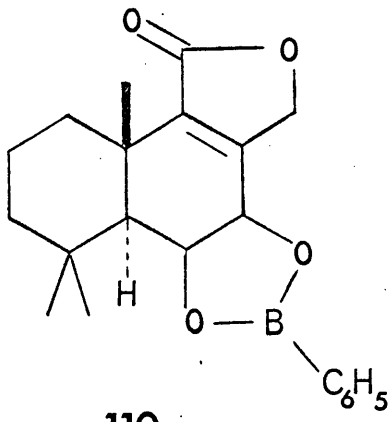
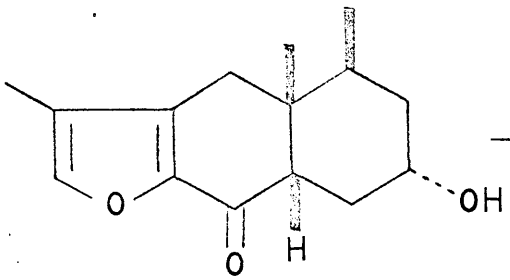


110

No attempt was made to isolate the products. The cyclic borate ester (110) was formed from the cis-6β,7β-diol (107) and was identified by GC-MS. No corresponding product was apparent (GLC) from reaction of the trans-diol (105) under the same conditions, although starting material was also absent. Rapid increase of GLC column temperature revealed a distorted peak which may be attributable to a bis-borate ester. This failure to form a cyclic ester is consistent with the diaxial geometry assigned to diol (105).

Peaks corresponding to reagent were recorded by GLC of the above reaction mixtures.[‡] GC-MS indicated that those were due to the trimeric anhydride (111).

‡ One "hazard" in the direct injection of unreacted phenylboric acid to GLC was its apparent sequestration by the GLC column. A pool of reagent appeared to build up so that subsequent injection of the free diol (107) led to derivative formation in situ. In fact a colleague using the same column on the following day found it impossible to record single peaks from the injection of pure steroid alcohols, implying that mono-borate esters were also readily formed on the column. The phenylboric acid could be flushed from the column by injection of propane-1,3-diol.(72



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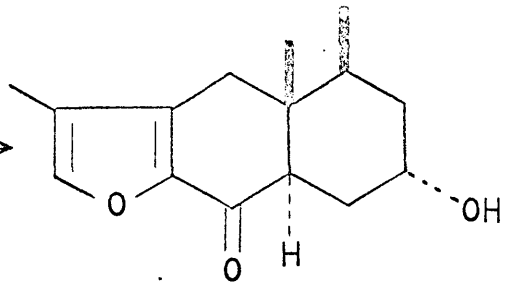

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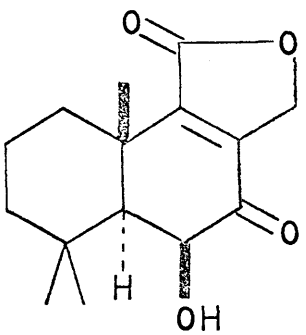

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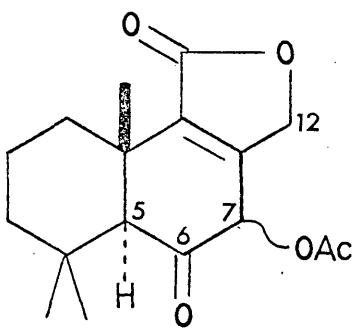

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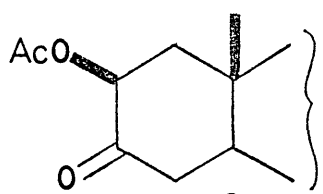

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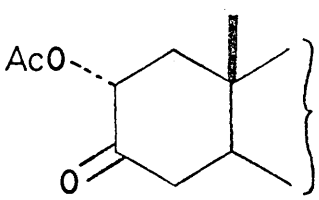

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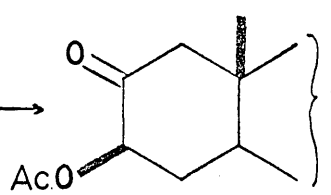

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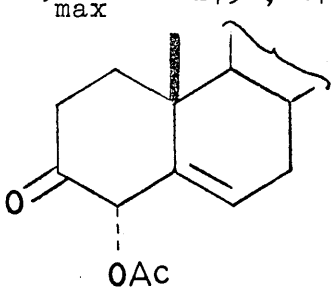
ν_{max} 1758, 1743cm^{-1}; 1759,1740cm^{-1}; 1756, 1735cm^{-1}.



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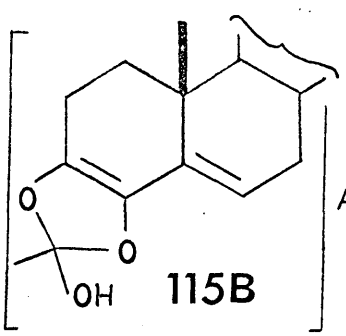

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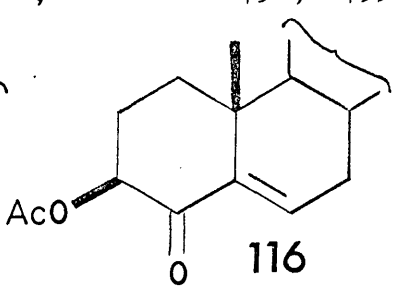

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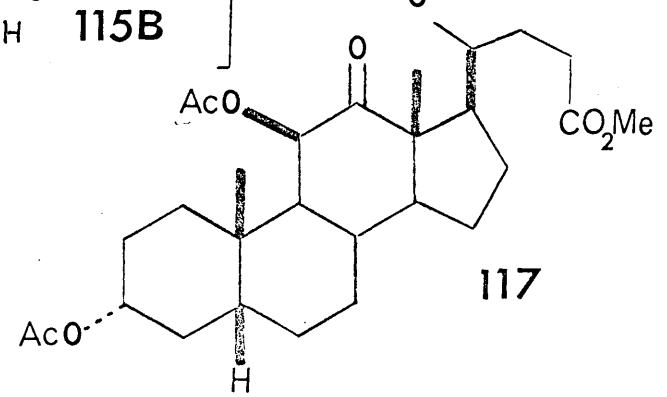

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Rearrangement of the Ketoacetate (94)

GLC of the ketoacetate (94) on the phase 1% QF-1 at 200° revealed an "impurity" of 15% which was proved to be isomeric with (94) by GC-MS. Repeated crystallisation did not reduce the relative amount of this second component. Convincing proof that its presence was due to a thermal isomerisation came from injection of (94) into the volatilisation zone, kept at 240°, at zero gas flow. After thirty seconds the gas flow was restored and it was found that the original ratio of 85:15 had become 30:70.

We had previously observed isomerisation under GLC conditions of the cis-furanoketoalcohol (61) to the trans-isomer (62). We inferred that an explanation of the above results might be the conversion of (94) to its C-6 equatorial acetate isomer.

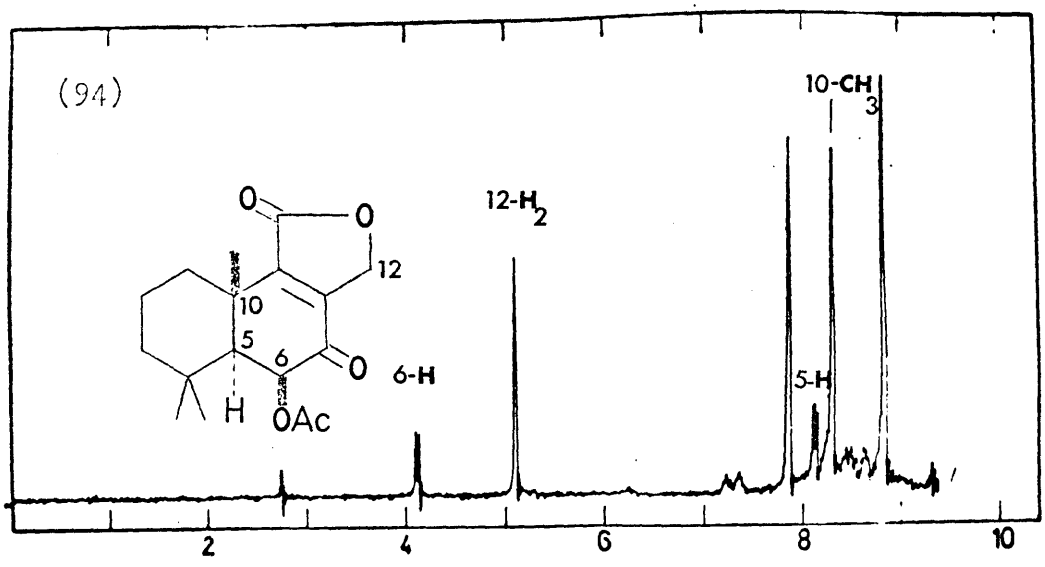
Several attempts to reproduce this isomerisation on a preparative scale were made. Treatment of (94) under mild basic conditions gave hydrolysis to ketol (112) and some decomposition. Reacetylation returned (94) unchanged (GLC). Refluxing in sulphuric acid-methanol resulted in slow hydrolysis to (112). Again acetylation regenerated the original ketoacetate. Pyrolysis in a sealed capillary tube for one minute at 150° produced nearly complete isomerisation but with substantial decomposition. The ketoacetate was also heated in various high boiling solvents but

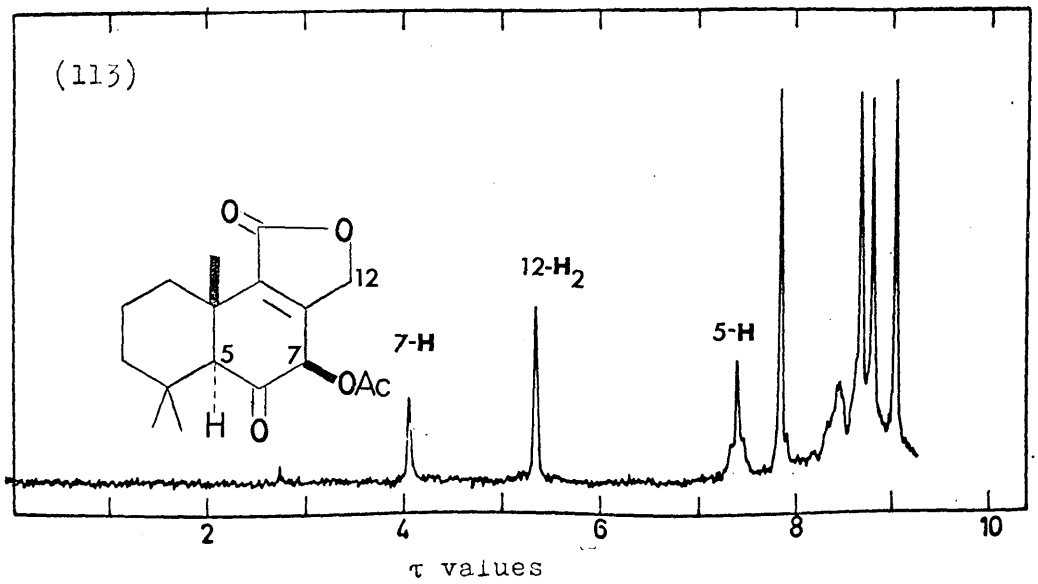
no clearly-defined increase in isomer content was observed on GLC. Preparative GLC was considered as a possible means of isolating the required isomer. At the time all the available instruments had metal columns and collection systems, and it was found that decomposition occurred under such conditions.

We finally turned to an approach based on the successful pyrolysis under GLC conditions. The ketoacetate was evenly absorbed on to GLC support [1.5 g QF-1 packing from a discarded column was used for 250 mg of compound (94)], placed in a Pyrex glass tube, sealed under a vacuum of 0.05 mm and heated at 175° for forty-five minutes. Examination of the products by GLC indicated that the original ratio of (94): isomer of 85:15 had become 45:55. Preparative TLC enabled separation of starting material from the slightly more polar product. Decomposition was relatively slight. Recovered starting material was pyrolysed again and after the two cycles a total of 70 mg of almost pure crystalline isomer was isolated. The yield, based on recovered starting material, was 45%.

The product of pyrolysis had the molecular formula $C_{17}H_{22}O_5$ (analysis and mass spectrum) and was therefore isomeric with (94). It was, however, immediately obvious that it was not the anticipated C-6 epimer. The infrared spectrum in carbon

Fig.(11) 100 Mc./sec. NMR Spectra of Ketoacetates (94) and (113) ($CDCl_3$)





tetrachloride showed bands at 1773, 1768 and 1745cm^{-1}, showing the absence of a conjugated ketone function. This was confirmed by ultraviolet absorption at 210 mμ (ε, 11,000) which corresponded to the αβ-unsaturated butenolide chromophore alone. The rearranged ketoacetate (113) seemed a possible structure. Non-bonded dipolar interaction between the ketone and acetate could account for the high values of their infrared carbonyl frequencies. Ketoacetates (95) and (96)[67] provide analogies for this despite their greater conformational flexibility. There was no obvious driving force for the transformation (94) to (113). Nevertheless a comparison of the NMR spectra of the two compounds convinced us that the isomer (113) was in fact the product [fig.(11)]. The 5α-proton signal a doublet at 8.13 τ in the spectrum of (94), was in (113) a singlet at 7.39 τ superposed on the doublet of multiplets due to the C-1β proton. A slightly broadened singlet at 4.05 τ was assigned to the C-7 proton coupling with the C-12 lactone methylene protons at 5.35 τ (confirmed by double irradiation). The geminal methyl signals in the spectrum of (113) had different chemical shifts due to the anisotropy of the C-6 ketone function. No confident assignment of the three tertiary methyl resonances at 8.68, 8.81 and 9.04 τ could be made.

Rearrangement of 2α-acetoxy-cholestan-3-one (95) to the 3β-acetoxy-2-one (114) in 20% yield occurs on active alumina.[67]

TABLE (11)

Ultraviolet Spectra of Isodrimenin (91) Derivatives

	Neutral ethanol	Basic solution	Reacidified
7-ketone (97)[64]	247 (10,600) →	259 (5,600)	Reversed
Δ^5, 7-ketone (99)[64]	248 (14,800)	unaltered	-
6β-acetoxy-7-ketone (94)	250 (10,000) →	230 (5,000) ↓ 245 (8,500 after 30 min.)	224 (5,000) 250 (2,500)
7β-acetoxy-6-ketone (113)	210 (11,000) →	yellow solution 243 (13,000 after 10 min.) 455 (1,500)	yellow discharged 223 (11,000) 252 (4,000) 350 (1,500)
6β-ol-7-ketone (112)	246 (10,000) →	228 (5,500) ↓ 245 [7,000 after 30 min. (increasing)] faint yellow	yellow discharged 223 (5,000) 245 (4,000)

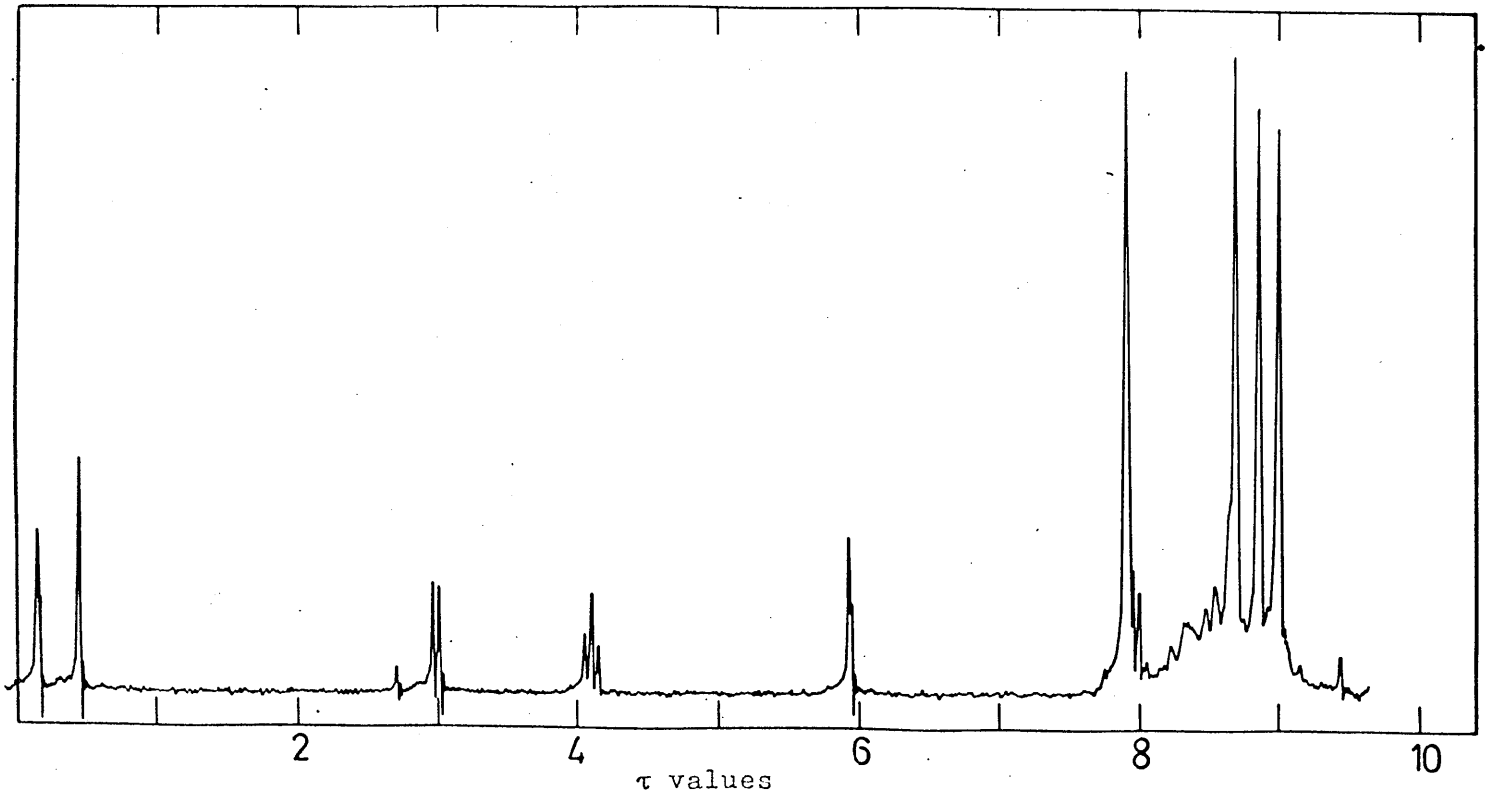
Similarly 4α-acetoxy-Δ^5-cholesten-3-one (115) is isomerised to the 3β-acetoxy-4-one (116).[67b] In both cases the conversion is considered to occur by enolisation followed by acyl migration probably via a cyclic intermediate such as (115B). If reaction of (94) to (113) goes via an intermediate of type (115B) assignment of configuration at C-7 is not justified in (113), although chemical evidence discussed below (p.98) favours a β-oriented acetate function.

The ultraviolet spectra of ketoacetates (94) and (113) [table (11)] revealed some remarkable shifts in basic ethanol and on reacidification. We had previously observed that mild base treatment of (94) gave ketol (112) accompanied by decomposition to unidentified polar products. Shifts in the spectra of (94) and (112) after base treatment are then probably due to the same species. We are unable to interpret the initial hypsochromic shifts in both spectra on the addition of base. The appearance of absorption at 245 mμ on basification in the spectrum of (113) may be due to hydrolysis followed by ketone-alcohol exchange. However an interpretation of the other observation - the intense yellow colouration and the bands appearing on reacidification - awaits a more detailed investigation. These changes are almost certainly due to the presence of more than one species since we have observed extensive decomposition of (113) under hydrolytic conditions.

There proved to be quite striking differences in the chemical reactivity of the two ketoacetates (94) and (113). Thus attempted hydrolysis of (113) with sulphuric acid in methanol gave a complex mixture of products under conditions which effected partial hydrolysis of (94) to (112) without any significant decomposition. Treatment of (113) with sodium borohydride in methanol also gave complex products while under similar conditions (94) was smoothly converted to diol (107).

Two attempts to deacetoxylate the 6-keto-7-acetate (113) were made. Reaction with chromous chloride in aqueous acetic acid gave a complex mixture. Prolonged treatment in refluxing acetic acid with zinc resulted in recovery of starting material unchanged as judged by TLC, GLC and infrared. Reaction of (94) with zinc and acetic acid caused deacetoxylation and hydrogenation to (100). The failure of the isomer (113) to undergo deacetoxylation could be taken as evidence that the acetate function of (113) is equatorial (7β-). Elimination of the function α- to the ketone in reactions of this type occurs most readily if this substituent is axial, or can adopt a conformation in which it is perpendicular to the plane of the C=O double bond.[69] Thus while methyl 3α,11β-diacetoxy-12-ketocholanate (117) is deacetoxylated in 64% yield after seven hours with zinc in refluxing acetic acid, the 11α-epimer does not react under these conditions, and requires treatment for twenty-four hours before a 28% yield is achieved.[70]

Fig.(12) 100 Mc./sec. NMR Spectrum of Ugandensidial ($CDCl_3$)



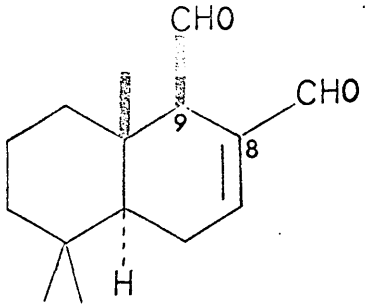
Ugandensidial

GLC of the total heartwood extract suggested that the compound to be discussed here, named ugandensidial, was one of the major components of the oil. It was apparently also present (by GLC) in much smaller amount in the first sample of heartwood examined.

Ugandensidial crystallised as colourless needles from ether-petroleum ether with melting point 137-140°; $[\alpha]_D$ - 407° ($CHCl_3$). The molecular formula from elemental analysis was $C_{17}H_{24}O_5$. This was confirmed by the mass spectrum although the molecular ion was extremely weak being less than 1% of the base peak. Single proton resonances at 0.24 and 0.54 τ in the NMR spectrum [fig.(12)] led to the conclusion that the compound was a dialdehyde. Carbonyl bands in the infrared spectrum at 1746, 1724 and 1698cm^{-1} were assigned to acetate, saturated aldehyde and conjugated aldehyde functions respectively. The presence of an intramolecularly hydrogen bonded hydroxyl function was indicated by a band at 3469cm^{-1} (for carbon tetrachloride solution) unaffected by dilution. The ultraviolet spectrum in absolute ethanol showed an inflection at 225 mμ (ε, ca. 1,500), and a possible maximum at ca. 206 mμ (ε, 4,500). In cyclohexane, maximal absorption was at 219 mμ (ε, 14,000).

Returning to the NMR spectrum,‡ the presence of three tertiary methyl groups (8.69, 8.85 and 9.00 τ) and an acetate

‡ Double irradiation experiments confirmed all the couplings discussed.



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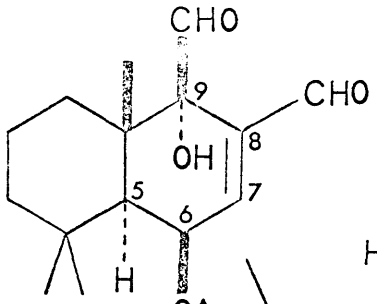

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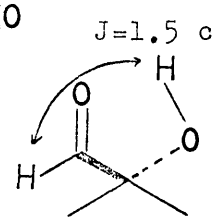
J=1.5 c./sec.



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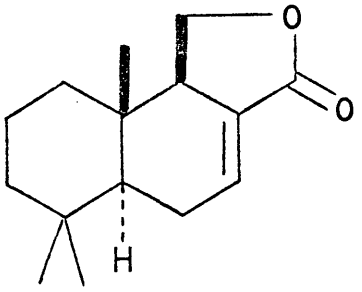

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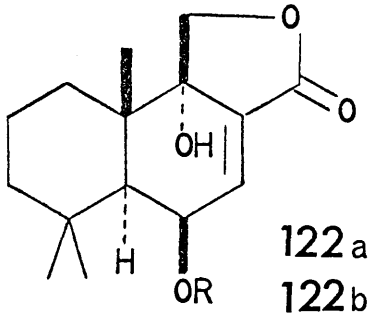
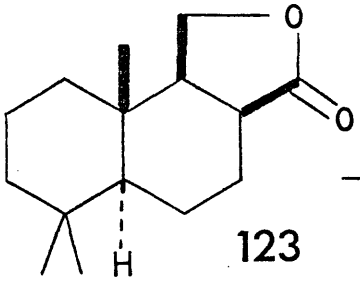


122a R = Ac

122b R = H



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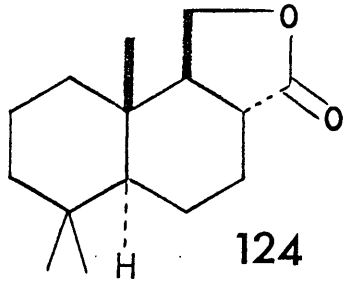

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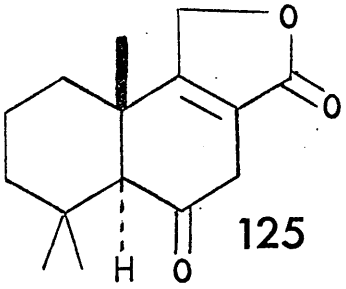

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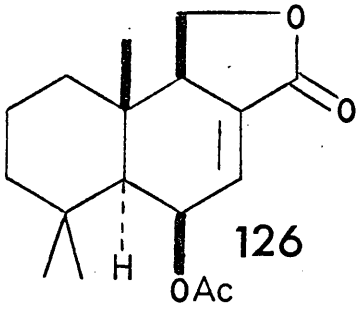

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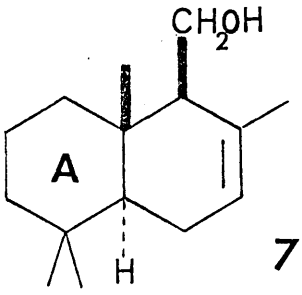

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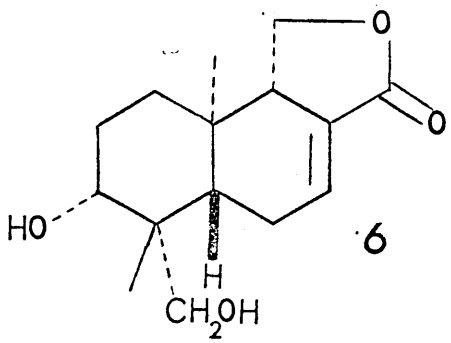


6

(7.91 τ) suggested that ugandensidial was related to ugandensolide. Conjugated and non-conjugated aldehyde functions could then only be accommodated at C-8 and C-9 as in polygodial (118).[12] The acetate and hydroxyl functions were assigned as represented in formula (119). The C-6 proton appeared as a triplet at 4.10 τ due to identical couplings of 5.0 c./sec. with each of the C-5α- (7.97 τ) and C-7 ethylenic protons (2.99 τ). The magnitude of the coupling constant between the protons at C-5 and C-6 made probable the assignment of the C-6 acetate as axial.[66]

The most striking feature of the NMR spectrum was a coupling of 1.5 c./sec. between the hydroxyl proton at 5.93 τ and the lower field aldehyde resonance (0.24τ). This seemed best explained by a coupling with the proton of the tertiary aldehyde function in the planar but "non-W" conformation (120) which also accounted for the strong intramolecular hydrogen bonding of the hydroxyl function.

One possible objection to structure (119) for ugandensidial was the absence of intense ultraviolet absorption in ethanol comparable with that recorded for polygodial [(118), λ_{max} 231 mμ (ε, 11,800)]. It is possible that distortion from planarity of the unsaturated aldehyde chromophore affects this absorption.

We obtained an unexpected confirmation of structure (119) when we learned, by chance, and prior to publication, of the work of Canonica *et al*. This group had isolated and proved the structure

and absolute configuration of cinnamolide (121), cinnamosmolide (122a) and cinnamodial (119) from Cinnamosma fragrans (Baillon), another member of the family Canellaceae.[71] The identity of cinnamodial with ugandensidial was established by direct comparison of physical data. We are indebted to Dr. Jommi of Milan who informed us of his results and recorded mixed melting points and infrared spectra of the two compounds.

The constitution of cinnamodial (ugandensidial) was proved[71] when an internal Cannizzaro reaction with hot aqueous sodium hydroxide gave (122b), the hydrolysis product of cinnamosmolide. In turn (122a) was correlated with the known dihydroconfertifolin (123) and isodihydroconfertifolin (124).[64]

Recently we have been informed by Dr. Jommi of the identification of two further constituents of Cinnamosma fragrans, (125) and (126).

Ugandensolide, ugandensidial (cinnamodial) and the other extractives from Cinnamosma fragrans (Baillon) are presumably formed in nature by oxidation of the biogenetic parent drimenol (7).[11] It is noteworthy that, in contract to iresin (6)[10] and its analogues, as yet no examples of the drimane type oxygenated in ring A have been found.

TABLE (13)

Heartwood Constituents of Warburgia ugandensis

Compound	m.p.	TLC R_f values	GLC (1% SE-30; 40 ml/min.)			Mass Spectra	
		EtOAc-petrol (3:7)	^tR (mins) 150°	175°	R_{Index}	Parent ion	Base peak
Drimenol	96-7°	0.70	7.5	-	1725	222 (10%)	109
Warburgiadione	127-8°	0.46	14.3	-	1855	230	230
W.H./V	-	0.25?	16.7	-	1890	234 (76%)	97
W.H./XIV	-	0.16	19.1	-	1920	220? (4%)	43
*Ugandensidial	137-140°	0.35 (tailing)	-	14.0	2120	308 (1%)	43
†Warburgin	159-161°	0.53	-	14.0	2120	272	272
W.H./XII	-	0.70?	-	21.7	2225	328?	43
Ugandensolide	218°	0.17	-	25.4	2265	308 (5%)	43

* Ugandensidial (R_{Index} 2925); † warburgin (R_{Index} 2760) on 1% QF-1.

Experimental

Extraction of the Second Consignment of Warburgia ugandensis heartwood.

Dried powdered heartwood (6.5 kg) was extracted with ethanol (35 litres) for 6 days at room temperature. A portion of this solution (2 litres) was evaporated (50° *in vacuo*) and the residue taken up in chloroform (2 x 150 ml) leaving insoluble solid (1.4 g). The chloroform extract was evaporated (40° *in vacuo*) and the residue dissolved in ether (300 ml), washed with water (100 ml) and dried. Evaporation of the ether (room temperature *in vacuo*) gave a dark red oil [(C); 6.0 g]. A GLC (1% SE-30) comparison of (C) with a similar extract made from the first batch of heartwood [see p. 39 and table (13)] showed two major peaks of greater retention than warburgin (W.H./XII. R_{Index} 225, and ugandensolide R_{Index} 2265). Subsequent GLC on the phase 1% QF-1 resolved the peak attributed to warburgin and revealed a third major component, ugandensidial also apparently present (GLC) though previously undetected, in the first extract. Only a small amount of warburgin was apparent by GLC and TLC. Its presence has not been rigorously proved by isolation.

The remaining ethanol solution (about 33 litres) was concentrated using a "cyclone" evaporator (at steam temperature). The residue yielded an amorphous brown solid (26 g)

insoluble in chloroform. The final oil [(D): 90 g] was dark red and extremely viscous. Chloroform was exchanged for ether at the final stage because an ether solution could be dried more efficiently. There would appear to be no objection to omitting chloroform and carrying out the initial partition with ether or benzene.

The component W.H./XII, present in oil (C), was absent in (D). It must be assumed that W.H./XII decomposed under the more vigorous conditions of working-up (use of the "cyclone" evaporator).

Chromatographic separations on oils (C) and (D) were directed at the isolation of W.H./XII, ugandensolide and ugandensidial, i.e. fractions not containing these compounds were set aside.

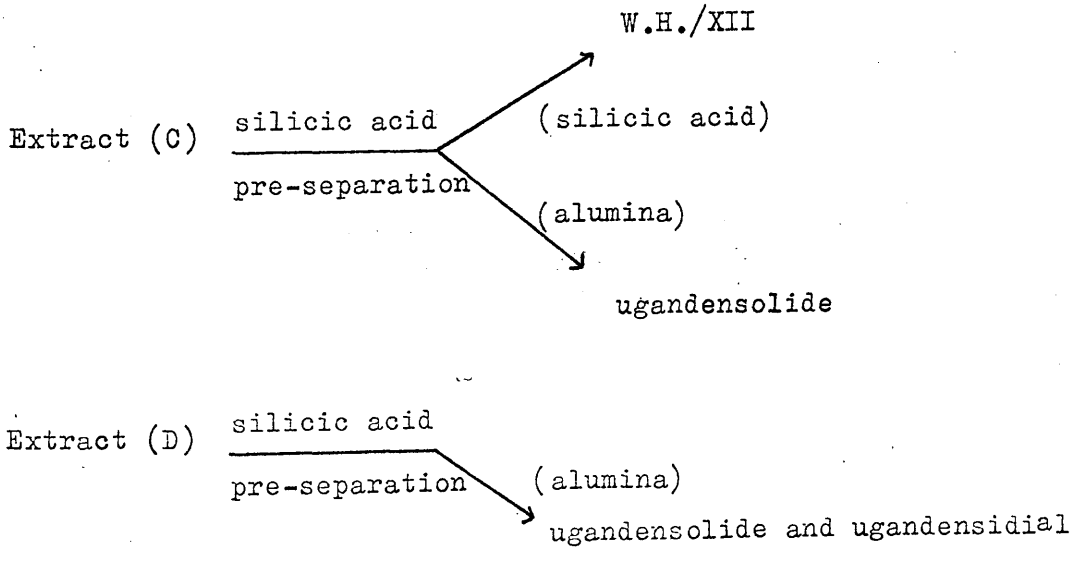


TABLE (14)

Chromatography of Oil (C) on Silicic Acid.

Fraction	Solvent	Vol. (litres)	Wt. (g)	Content
1	Petroleum ether	0.5	0.35	
2	benzene	0.5	0.31	
3	benzene-chloroform (1:1)	0.5	0.26	
4	chloroform	0.3	0.41	ugandensidial
5	chloroform	0.3	1.16	ugandensidial, XII
6	chloroform	0.3	2.20	ugandensidial, ugandensolide; traces XII.
7	chloroform	0.3	0.30	
8	ethyl acetate	0.5	0.30	
		Recovery	88%	

Chromatography of Oil (C): Isolation of W.H./XII and ugandensolide

Extract (C) (6.0 g) was chromatographed on silicic acid (100 g). 8 fractions were taken [table (14)]. W.H./XII was concentrated (GLC) in fraction 5. This material was re-chromatographed on silicic acid (40 g) using an ethyl acetate (250 ml) into petroleum ether (500 ml) gradient elution with automatic fraction collection. 50 fractions of 10 ml were taken at a flow rate of about 1 ml/min. The bulk of the material was eluted in fractions taken with ethyl acetate-petroleum ether (1:3 to 1:1) as judged by TLC using the "chess plate" technique.‡ W.H./XII (505 mg, crude oil) was eluted by ethyl acetate-petroleum ether (1:3 to 3:7) through 5 fractions. It could not be crystallised and was rather unstable. The impure material showed strong absorption in the infrared at 1750cm^{-1} (liquid film) and weak ultraviolet absorption at 225 mμ (ethanol solution). By GC-MS the molecular ion was apparently at m/e 328, base peak 43.

‡ Squares of 0.5-1.0cm are drawn on ordinary silica TLC plates. Each fraction, without concentration, is then spotted in a square and the plate sprayed. Fractions which do not stain are considered to be blank and are ignored. Assuming that 10 μg is detectable from an application of 50 μl, this indicates 2 mg of material in a 10 ml fraction. The method allowed a rapid evaluation of a large number of fractions.

Fraction 6 (2.2 g) containing chiefly ugandensolide and ugandensidial was rechromatographed on alumina (120 g: grade III) using an ethyl acetate (500 ml) into petroleum ether (500 ml) gradient elution and automatic fraction collection (60 x 15 ml fractions; flow rate ca. 1 ml/min.). 7 fractions taken with ethyl acetate-petroleum ether, 1:1 to 3:2, were appreciably concentrated in ugandensolide, (415 mg oil). After standing overnight at 0° crystals separated (110 mg). This material was washed with ether-petroleum ether and recrystallised twice from ethanol to m.p. 218° $[\alpha]_D$ + 23° ($CHCl_3$); colourless needles. (Found, C: 66.12; H: 7.79%. $C_{17}H_{24}O_5$ requires C: 66.21; H: 7.84%). Ugandensidial appeared in 25 fractions from 35% to 75% ethyl acetate in petroleum ether and was not appreciably concentrated in any. This tailing effect was similar to that observed on silica gel TLC with petroleum ether-ethyl acetate solvent systems.

Chromatography of Oil (D): Isolation of ugandensolide and ugandensidial

Extract (D) (90 g) was chromatographed on silicic acid (500 g). 8 fractions of 2 litres were taken using the same solvents as in the silicic acid separation of (C) [table (14)]. Despite the different ratio of adsorbent to material the result was similar, the bulk being eluted in the chloroform fractions

TABLE (15)

Chromatography on Alumina: Isolation of ugandensolide and ugandensidial.

Fraction	Solvent	Total vol. (litres)	Wt. (g)	Content
1-4	5%, 10%, 15%, 25% ethyl acetate in petroleum ether	4.0	1.2	
5-12	25%, 30% ethyl acetate in petroleum ether	2.0	3.0	
13-20	30% ethyl acetate in petroleum ether	2.0	6.3	ugandensolide separated
21-24	30% ethyl acetate in petroleum ether	1.0	0.8	
25-28	50% ethyl acetate in petroleum ether	1.0	2.7	ugandensidial separated
29-32	ethyl acetate	1.0	0.3	
		Recovery	75%	

4 (15.1 g), 5 (19.2 g) and 6 (20.6 g). Ugandensidial appeared in fractions 4, 5 and 6 while ugandensolide was the major component of 6.

Fraction 6 (19.2 g) was rechromatographed on alumina (1 kg ; grade III). A careful ethyl acetate-petroleum ether gradient elution [table (15)] was directed specifically at the isolation of ugandensolide and ugandensidial. Elution with 5% to 30% ethyl acetate in petroleum ether through 12 fractions gave complex oils (4.2 g). Further elution with 30% ethyl acetate in petroleum ether gave ugandensolide through 6 fractions of 250 ml (6.3 g total oil). Trituration with ether-petroleum ether gave solid material (4.55 g). Two crystallisations from ethanol yielded 2.1 g; m.p. 214-219°.

Ugandensidial was again present in small amounts in all fractions from 30% to 100% ethyl acetate in petroleum ether but was the major component of 4 fractions taken with 50% ethyl acetate (2.7 g, crude solid). This material was triturated with ether-petroleum ether and allowed to stand at 0° overnight when ugandensidial separated as discoloured needles (480 mg). Crystallisations from ether-petroleum ether and ethyl acetate-petroleum ether gave m.p. 137-140°; $[\alpha]_D$ - 407° ($CHCl_3$). (Found. C: 66.45; H: 7.85. $C_{17}H_{24}O_5$ requires C: 66.21; H: 7.84%). Sublimation at 120°/0.1 mm returned ugandensidial

unchanged (GLC and TLC). Elemental analysis of the sublimed material: found C: 66.09; H: 7.80%.

Attempted Catalytic Hydrogenation of Ugandensolide

Ugandensolide was recovered unchanged (as judged by GLC on the phase 1% SE-30, and TLC with the system 3% methanol in chloroform) after attempts to hydrogenate under the following conditions:

(a) in ethyl acetate with 10% palladium on charcoal catalyst for 6 hours;

(b) in methanol with platinum oxide catalyst for 14 hours;

(c) in ethanol at 45°C under a pressure of 4 atmospheres with platinum oxide catalyst for 24 hours.

Hydrogenation of ugandensolide in acetic acid with platinum oxide catalyst for 20 hours gave a mixture containing an estimated 60% of starting material (GLC; 1% SE-30). Two other components, each approximately 20% of the recorded products were also observed: [t_R (min.) on 1% SE-30 at 175° with gas flow 40 ml/min.: 13.6 (20%), 16.0 (ugandensolide, 60%), 17.5 (20%)]. TLC (10% methanol in chloroform) showed spots of R_f 0.70 (ugandensolide), 0.65 and 0.05. Some streaking of polar material was also evident. GC-MS on the products of hydrogenation gave inconclusive results.

Hydrolysis of Ugandensolide

(a) Alkaline conditions

Ugandensolide (10 mg) was refluxed with potassium hydroxide in 95% ethanol (0.1 N; 5 ml) for 30 minutes. The solution was made just acid (hydrochloric acid), diluted with water (10 ml) and extracted with ether. The ether extract was washed (water) and dried yielding a discoloured oil (6 mg). TLC (ethyl acetate-petroleum ether, 1:1) showed at least 3 unidentified polar products together with streaking on the plate.

(b) Acid Conditions [Diol (105)]

Ugandensolide (60 mg) was dissolved in methanol (10 ml) with dilute sulphuric acid (6 N; 0.2 ml) and stirred at 60° for 18 hours when TLC (ethyl acetate-petroleum ether, 1:1) showed approximately 20% reaction to diol (105), R_f 0.15; ugandensolide R_f 0.45. Sulphuric acid (6 N; 0.2 ml) and water (0.3 ml) were added and the mixture was refluxed for a further 48 hours when greater than 90% reaction to (105) was estimated. The solution was concentrated by evaporation of the bulk of the solvent *in vacuo* and diluted with water (10 ml). This was extracted with ether, washed (water) and dried. Evaporation of the ether gave crude solid diol (36 mg). Crystallisation from ethanol gave colourless needles of m.p. 260-264° (sealed capillary); ν_{max} ($CHCl_3$) 1761, 1751cm^{-1}; ν_{max} (nujol) 3250-3500, 1725cm^{-1}. (Found: C, 67.51; H, 8.34%. $C_{15}H_{22}O_4$

requires: C, 67.65; H, 8.33%).

A small scale reacetylation of the diol (105) with pyridine-acetic anhydride (1:2) at 60° for 18 hours gave a single product not distinguishable by GLC (1% QF-1) and TLC (ethyl acetate-petroleum ether, 1:1) from that obtained below (106) by acetylation of ugandensolide.

Acetylation of Ugandensolide [diacetate (106)]

Ugandensolide (50 mg) was heated at 60° in pyridine (1 ml) and acetic anhydride (2 ml) for 20 hours. TLC (ethyl acetate-petroleum ether, 1:1) indicated that reaction to the acetate (106) was complete. The bulk of the solvents was removed *in vacuo* and the mixture diluted with water and extracted with ether. The ethereal solution was washed (aqueous sodium bicarbonate and water), dried and evaporated to give an oil [(106); 55 mg]. This was crystallised with difficulty from petroleum ether-ethyl acetate as colourless prisms and had m.p. 104-5°; ν_{max} (CCl_4) 1769, 1752cm^{-1}. 100 Mc./sec. NMR ($CDCl_3$) τ values: 8.97 (6 H), 8.51 (3 H), 7.95 (3 H), 7.90 (3 H), 7.44 (1 H, doublet of multiplets J *ca.* 13 c./sec.), 5.36 (2 H, incompletely resolved doublet), 4.84 (1 H, broad singlet), 4.45 (1H, broad singlet).(Found: C, 65.18; H, 7.36%. $C_{19}H_{26}O_6$ requires: C, 65.13; H, 7.48%).

Ketoacetate (94)

Ugandensolide (1.20 g) was dissolved in acetone (15 ml). Chromium trioxide in sulphuric acid (Jones reagent)[48] (1.5 ml) was added over 1 hour with stirring at 0°. Reaction was continued for a further 2 hours at 0°. Addition to ice and water (100 ml) was followed by ether extraction. The ethereal solution was washed (water) and dried. Evaporation of the ether gave a solid product [(94); 1.15 g]. One crystallisation from a small volume of ethanol gave 0.90 g of m.p. 133-138° An analytical sample from ethanol, colourless prisms, had m.p. 135-138°; ν_{max} (CCl_4) 1774, 1761 and 1698cm^{-1}; λ_{max} (EtOH) 250 mμ (ε, 10,000); λ_{max} (0.05 N NaOH in EtOH) initially 230 mμ (ε, 5,000) shifting to 245 mμ (ε, 8,500 after 30 minutes); λ_{max} (reacidified - HCl) 224 mμ (ε, ca. 5,000) and 250 mμ (ε, ca. 2,500). 100 Mc./sec. NMR ($CDCl_3$) τ values: 8.89 (6 H), 8.35 (3 H), 8.13 (1 H, doublet J = 2.0 c./sec.), 7.90 (3 H), 7.29 (1 H, doublet of multiplets J ca. 13 c./sec.), 5.10 (2 H), 4.11 (1 H, doublet J = 2.0 c./sec.). Mass spectral molecular ion ($C_{17}H_{22}O_5$) at m/e 306 (14%), base peak m/e 43.

(Found: C, 66.39; H, 7.02%. $C_{17}H_{22}O_5$ requires: C, 66.65; H, 7.24%).

Sodium Borohydride Reduction of Ketoacetate (94) to Diol (107)

Ketoacetate [(94); 180 mg) was dissolved in methanol (10 ml). Sodium borohydride (200 mg) was added in portions over 30 minutes. The mixture was stirred for 20 hours at room temperature. Water (50 ml) was added and the solution extracted with ether (without prior acidification), washed (water) and dried. Evaporation of the ether gave a colourless solid [(107); 140 mg], recrystallised as colourless needles from methanol to m.p. 240-245° (sealed capillary); ν_{max} ($CHCl_3$) 1760 (shoulder), 1751cm^{-1}. Mass spectral molecular ion ($C_{15}H_{22}O_4$) at m/e 266 (base peak). (Found: C, 67.51; H, 8.34%. $C_{15}H_{22}O_4$ requires: C, 67.65; H, 8.33%).

Acetylation of Diol (107) and the Crystallisation Liquors from (107)

Diol [(107); 25 mg] was dissolved in pyridine (0.5 ml) with acetic anhydride (1 ml) and heated for 20 hours at 60°. The solvents were removed *in vacuo*. Water (10 ml) was added and the mixture extracted with ether. The ethereal solution was washed (aqueous sodium bicarbonate and water), dried and evaporated to give a colourless solid [(108); 28 mg]. This was recrystallised from methanol as colourless needles m.p. 222-223°. ν_{max} (CCl_4) 1771 and 1750cm^{-1}. 100 Mc./sec. NMR ($CDCl_3$) τ values: 8.99, 8.96 (6 H); 8.43 (3 H), 7.96 (3 H), 7.92 (3 H), 7.47 (1 H, doublet of multiplets, J *ca.* 13 c./sec.), 5.37 (2 H, incompletely resolved doublet), 4.27 (1 H, incompletely resolved

doublet, J ca. 5 c./sec.), 4.09 (1 H, doublet J = 5.0 c./sec.) (Found: C, 65.08; H, 7.56%. $C_{19}H_{26}O_6$ requires: C, 65.13; H, 7.48%).

GLC data for the trans-6β,7α-diol (105), the cis-6β,7β-diol (107) and their diacetates (106) and (108) on the phase 1% QF-1 at 175° with gas flow 40 ml/min. were as follows:

Sample	t_R (min.)	Relative t_R
ugandensolide (104)	13.0	1.0
diols (105) and (107)	11.5	0.89
diacetate (106)	16.1	1.24
diacetate (108)	17.9	1.38

Acetylation of the crystallisation liquors from the cis-diol (108) (ca. 1/3 of the total borohydride product) gave 80% cis-diacetate (108), 15% of a component not distinguishable from trans-diacetate (106), and 5% of an unidentified component as judged by GLC under the above conditions.

Zinc and Acetic Reduction of Ketoacetate (94)

Ketoacetate [(94); 200 mg] was dissolved in acetic acid (15 ml) and zinc dust (400 mg) added. The mixture was refluxed for 2 hours then filtered when cold. Sodium bicarbonate (aqueous and solid) was added to make the solution just basic. This was then extracted with ether and the ethereal solution washed (water) and dried. Evaporation of the solvent gave a colourless solid product [(100); 170 mg]. This was recrystallised from ether and from benzene-petroleum ether to m.p. 123-126°; $[\alpha]_D$ - 119°

(benzene) ν_{max} (CCl_4) 1778, 1712cm^{-1}. 100 Mc./sec. NMR spectrum ($CDCl_3$) τ values: 9.14 (3 H) 9.07 (6 H), 7.54 (2 H, complex multiplet), 7.16 (1 H, doublet J = 12.5 c./sec.), 6.70 (1 H, complex multiplet), 5.54 (2 H, complex multiplet). Mass spectral molecular ion ($C_{15}H_{22}O_3$) at m/e 250 (3%), base peak m/e 85. (Found: C, 71.91; H, 8.73%. $C_{15}H_{22}O_3$ requires: C, 71.97; H, 8.86%). This reduction was also carried out on a small scale at room temperature and aliquots were separately worked up for GLC examination. The results suggested that reaction was complete, after 30 minutes at room temperature followed by 30 minutes at 60°, to a component not distinguishable from the keto-lactone (100). Two intermediates observed after 30 minutes at room temperature remain unidentified. At this point the estimated content of the reaction mixture was starting material, 48% (t_R 20.4 and 15.2 min.) final product 14% (t_R 11.2 min.), unidentified components 33% (t_R 6.5 min.) and 5% (t_R 12.3 min.). Retention times are quoted for 1% QF-1 at 175° with gas flow 40 ml/min.

Identity of the ketone (100) with dihydro-oxoisodrimenin[64] was proved by their undepressed mixed m.p., by comparison of infra-red (KCl disc) and mass spectra and of optical rotation data. The two samples were also found to have identical GLC behaviour under the following conditions: 5% QF-1, 5% Ap.L, 2% Carbowax 20 M, all at 200°C. Data quoted for dihydro-oxoisodrimenin are m.p. 124-126°; $[\alpha]_D$ -115° (benzene); ν_{max} (CCl_4) 1781s and 1716ms cm^{-1}; λ_{max} (EtOH) 282 mμ (ε, 30).

Treatment of Diols (105) and (107) with Phenylboric Acid

A stock solution of phenylboric acid (10 mg/ml of acetone) was prepared. Solutions of the diols (105) and (107) in pyridine in concentrations suitable for GLC were made up, and 1.5 molar equivalents of phenylboric acid solution added to each. After allowing to stand for 2 hours at room temperature the reaction mixtures were examined directly by GLC, and later GC-MS, on the phase 1% SE-30 at 200°C. In both cases the free diol was absent and peaks corresponding to excess reagent ["trimer"(111)] were recorded. GLC and GC-MS results are summarised below. The cyclic borate ester (110) was formed from diol (107). No corresponding component was recorded from the reaction of (105). However rapid increase of the column temperature in this case revealed a distorted peak of markedly longer retention than cyclic ester (110) which may be attributable to a bis-ester.

Difficulties arising from the apparent sequestering of phenylboric acid on GLC columns are discussed on p. 93.

GLC and GC-MS data for the phenylboric acid reactions: (GLC retention times were measured on a 4' 1% SE-30 column at 200° with gas flow 40 ml/min. Admission to the mass spectrometer was via a 10' 1% SE-30 column)

Sample	t_R (min.)	GC-MS Scans
Reagent ["trimer"(111)]	7.5	parent m/e 312 (100%)
Diols (105) and (107)	5.3	
Cyclic borate ester from diol (107)	19.9	parent m/e 352 (65%) base peak m/e 41

Hydrolysis of Ketoacetate (94) to Ketol (112)

Ketoacetate [(94); 60 mg) was dissolved in methanol (5 ml). Sulphuric acid (6 N; 0.5 ml) and water (0.3 ml) were added and the mixture refluxed for 48 hours when TLC (with the solvent system 2% methanol in chloroform) showed greater than 90% reaction to a more polar component (112). R_f values: (94) 0.80 and (112) 0.60. The reaction mixture was diluted with water (15 ml) and extracted with ether. The ethereal solution was washed (water), dried and evaporated giving a solid product [(112); 35 mg], recrystallised from methanol to m.p. 142-145°. ν_{max} (CCl_4) 1775, 1691 and 3579cm^{-1} (ν_{OH} unaffected on dilution). Mass spectral molecular ion ($C_{15}H_{20}O_4$)at m/e 264 (50%), base peak m/e 41.

TABLE (16)

GLC of Ketoacetate (94) and Conversion to Isomer (113)

(GLC on 1% QF-1 at 200°C and 40 ml/min. gas flow.
Gas flow off for time T minutes after injection to pre-heater at 240°)

T (min.)	Estimated Ratio (94):(113)	
0	85 : 15	
0.5	30 : 70	
2.0	20 : 80	
8.0	5 : 95	(decomposition evident; minor peaks of shorter retention)

Conversion of the 6β-acetoxy-7-ketone (94) to the 7β-acetoxy-6-ketone (113)

Injection of pure (TLC) ketoacetate (94) on 1% QF-1 at 200° resulted in partial conversion to the isomer (113). GC-MS confirmed the isomeric nature of the two peaks recorded. That the ratio of (94) to (113) was thermally dependent was shown by injection into the pre-heater zone (240°) at zero gas flow [see table (16)]. The progress of subsequent attempts to prepare the isomer (113) could be followed by GLC.

Preliminary attempts to obtain ketoacetate (113)

(a) Mild alkaline conditions.

Ketoacetate [(94); 5 mg] was dissolved in methanol (0.5 ml). Saturated aqueous sodium bicarbonate (0.1 ml) was added to half of this solution. To the remainder, sodium hydroxide (0.1 N; 0.1 ml) was added. Each mixture was shaken for 1.5 hours at room temperature, then diluted with water and extracted with ether. The extracts were washed (water), dried and evaporated. GLC (1% QF-1 at 175°) showed in the case of the bicarbonate treatment, starting material and approximately 50% conversion to a component not separated from ketol (112). Sodium hydroxide treatment effected almost complete hydrolysis. TLC (2% methanol in chloroform) revealed decomposition to polar products not recorded under the above GLC conditions. Reacetylation (pyridine - acetic anhydride, 1:2, at 60° overnight)

in each case regenerated ketoacetate (94) unchanged (GLC).

(b) Acid conditions.

Refluxing ketoacetate (94) in aqueous methanol in the presence of sulphuric acid, as described above, gave ketol (112). Acetylation regenerated (94) (GLC).

(c) Heating (94) in high-boiling solvents.

Ketoacetate (94) was heated at bath temperatures 160-170° in the following solvents for 30 minutes and the mixtures examined directly by GLC (1% QF-1 at 200°C): ethylene glycol, n-hexanol, dimethylformamide. It was observed that injection of (94) to GLC in ethylene glycol affected the ratio of (94) to (113) recorded when compared with injection in methanol. In no case was further definite enhancement of the peak due to (113) on heating observed.

(d) GLC on the Perkin-Elmer 451 instrument.

This instrument may be used for preparative work. The system however is an all metal one and it was found that (94) decomposed totally under these conditions.

(e) Pyrolysis.

Ketoacetate [(94); 2 mg] in a sealed capillary tube was heated at 150° for 30 seconds. The material immediately darkened on melting. Recovery for GLC indicated near to complete conversion to (113). However TLC revealed extensive decomposition

to polar products not recorded on GLC (1% QF-1, 200°C).

Pyrolysis of Ketoacetate (94) on GLC packing

Several trial reactions were made before proceeding to the large scale pyrolysis described below.

Ketoacetate [(94); 250 mg] was dissolved in ether (50 ml) and used 5% QF-1 packing from a discarded column (1.5 g) was added. The solvent was evaporated (rotary evaporator) and (94) was thus evenly absorbed on the packing. The mixture was placed in a Pyrex glass tube which was sealed under a vacuum of 0.05 mm. The tube was heated for 45 minutes at 175°. The products (295 mg), contaminated with QF-1 phase, were recovered by elution with ether. GLC (1% QF-1, 200°) indicated a marked enhancement in concentration of isomer (113). TLC (ethyl acetate-petroleum ether, 1:2) indicated two major components; starting material; R_f 0.45 and product (113), R_f 0.30 together with at least three minor components, not recorded under the above GLC conditions and estimated as 10% of the total material recovered. (N.B. QF-1 phase was not detectable by TLC). Preparative TLC allowed the separation of impure starting material (150 mg) and product (55 mg). The recovered starting material was recycled under the same conditions of pyrolysis and crude product (30 mg) and starting material (90 mg) were recovered in a similar manner to that described above.

Crystalline ketoacetate [(113); 70 mg] was obtained after a further preparative TLC purification of the combined products of the two pyrolysis cycles. Crystallisation from ethanol gave colourless needles of m.p. 179-181°; ν_{max} (CCl_4) 1773, 1768 and 1745cm^{-1}; λ_{max} (absolute EtOH) 210 mμ (ε, 11,000); λ_{max} (0.05N NaOH in EtOH) 243 mμ (ε, 13,000 after 10 min.) and 455 mμ (ε, ca. 1,500); λ_{max} (reacidified - HCl) 223 mμ (ε, ca. 11,000) 252 mμ (ε, ca. 4,000) and 347 mμ (ε, ca. 2,000). Mass spectral molecular ion ($C_{17}H_{22}O_5$) at m/e 306 (base peak), 100 Mc./sec. NMR ($CDCl_3$) τ values: 9.04 (3 H), 8.81 (3 H), 8.68 (3 H), 7.83 (3 H), 7.39 (1 H), 7.38 (1 H, doublet of multiplets J, ca. 12 c./sec.), 5.35 (2 H, slightly broadened singlet), 4.05 (1 H, slightly broadened singlet). (Found: C, 66.48; H, 6.99%. $C_{17}H_{22}O_5$ requires C, 66.65; H, 7.24%).

Attempted Reactions with 7β-acetoxy-6-ketone (113)

(A) Ketoacetate (10 mg) was refluxed for 6 hours in acetic acid (2 ml) with zinc dust (150 mg). The reaction mixture was worked up as previously described. Crystalline material (7 mg) was recovered and proved to be unchanged starting material by comparison of TLC, GLC and infrared data.

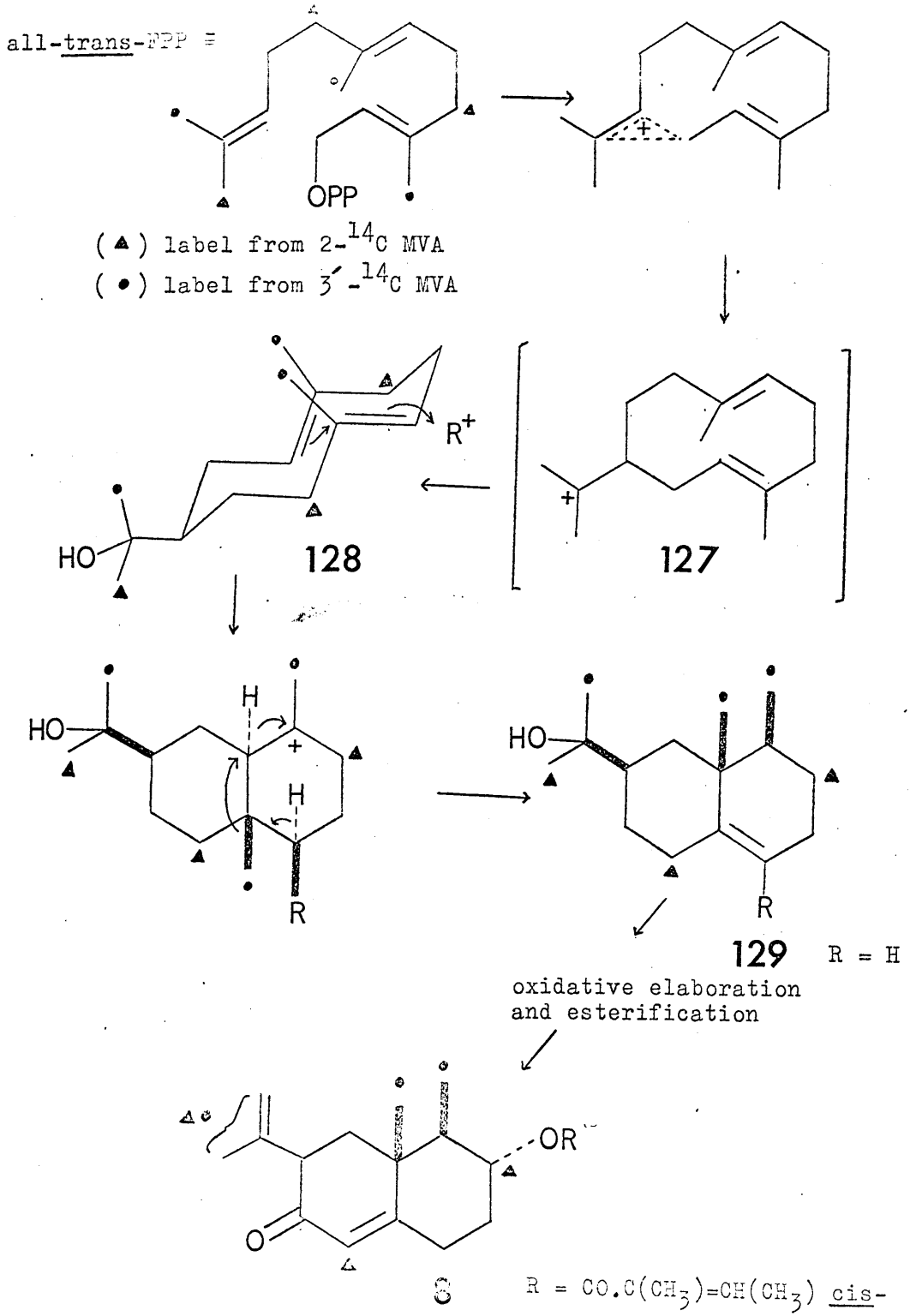
(B) Chromous chloride was prepared by slowly eluting aqueous chromic chloride from a zinc amalgam column. Ketoacetate (5 mg) in acetic acid (2 ml) with aqueous chromous chloride (1 N; 0.5 ml)

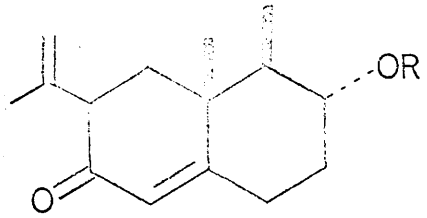
was stirred under nitrogen at 50° for 20 hours. The mixture was made just basic with aqueous sodium bicarbonate and extracted with ether. The extract was washed (water), dried and evaporated yielding a colourless gum (3 mg). Both TLC (ethyl acetate-petroleum ether, 1:2) and GLC (1% QF-1 at 200°) indicated a complex mixture of products together with starting material. GLC data suggested that starting material amounted to 30% of the recorded product.

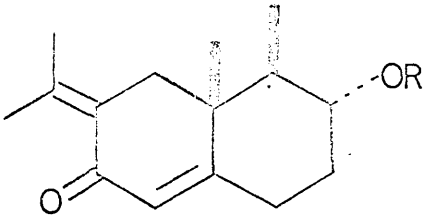
(C) To a stirred solution of ketoacetate (5 mg) in methanol (2 ml) sodium borohydride (5 mg) was added. An immediate yellow colouration, discharged after about one hour, developed in the solution. The reaction was left for 12 hours before work up as previously described for the reduction of the isomeric compound (94). The products were complex as judged by TLC (ethyl acetate-petroleum ether, 1:1) and GLC (1% QF-1 at 200°). No starting material or component corresponding to the diol (107) was detected.

(D) A solution of ketoacetate (5 mg) in methanol with sulphuric acid (6 N; 0.1 ml) was refluxed for 2 hours. Recovery of the products revealed no starting material in a complex mixture (TLC).

Fig.(14)


all-trans-FPP =
OPP
(▲) label from 2-^{14}C MVA
(●) label from 3'-^{14}C MVA
R+
HO
128
127
HO
H
H
R
HO
R
129
R = H
oxidative elaboration
and esterification
OR
O
8
R = CO.C(CH3)=CH(CH3) cis-





8 R = CO.C(CH_3)=CH(CH_3) cis-

84 R = CO.CH = CH(SCH_3) cis-

85 R = CO.C(CH_3)=CH(CH_3) cis-

86 R = CO.CH = CH(SCH_3) cis-

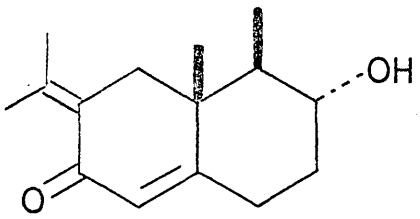


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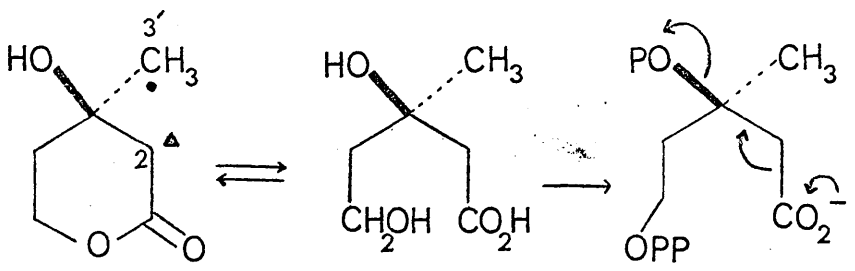
Fig.(13)



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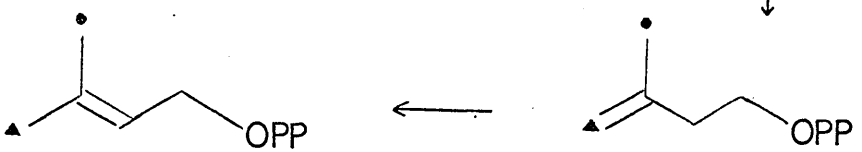
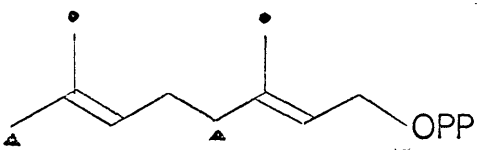


dimethylallyl pyrophosphate
DMAPP

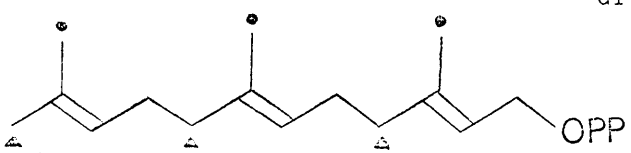
isopentenyl pyrophosphate
IPP

DMAPP + IPP ⟶



geranyl pyrophosphate
GPP

GPP + IPP ⟶



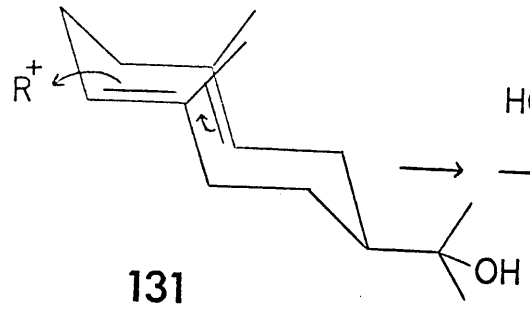
farnesyl
pyrophosphate
FPP

Biosynthetic Studies with the Eremophilane Sesquiterpenes of Petasites hybridus (L) Compositae

Introduction

Earlier work with the plant Petasites hybridus (discussed in the section on warburgiadione) had shown that the petasin-type esters were the main sesquiterpenoid constituents of the rhizomes. It was envisaged that these esters [petasin (8), isopetasin (85), S-petasin (84) and iso-S-petasin (86)] could be collectively isolated as their common hydrolysis product isopetasol (89).[56] This convenient circumstance, together with the availability and rapid growth characteristics of the plant, led us to undertake biosynthetic experiments directed at the elucidation of a part of the pathway to the eremophilanes. The problem has been one of classical interest since the observation by Penfold and Simonsen[9] that the eremophilanes were not derivable by application of the simple "isoprene rule".

This section of the thesis is intended to be a review of the main objectives and preliminary results in this necessarily long-term project. It should be stressed that the writer has been directly involved only in a part of the work discussed here. Many of the results have been obtained by Dr. J.A. Zabkiewicz who, for much of the time, has had little more than moral support from the present author.



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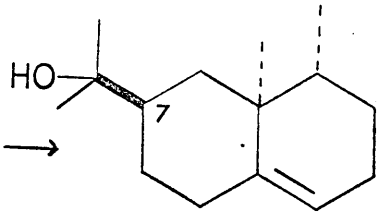

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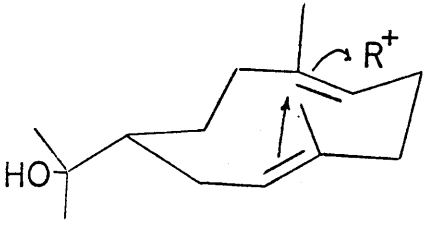

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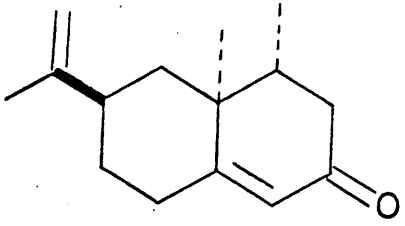

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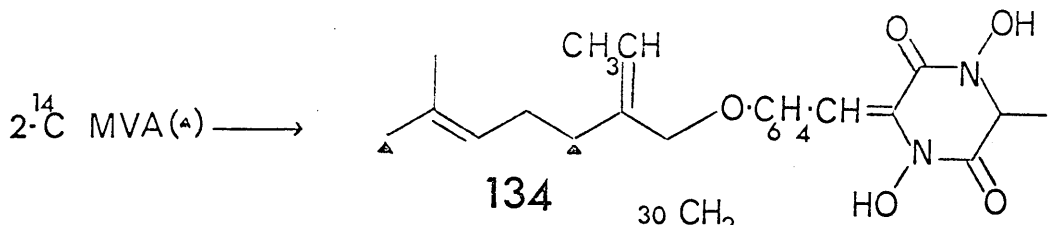
Hypothetical schemes of sesquiterpene biogenesis are now extensively detailed. The possible intermediacy of large rings of the type (127) in fig. (14) was suggested by Ruzicka[73] and by Barton and de Mayo.[78] This concept was later extended by Hendrickson[74] who derived most of the known classes of sesquiterpene carbon skeleton by carbonium ion mechanisms from farnesol as primary precursor. Recently this field has been re-assessed by Parker, Roberts and Ramage[75] in a most able review.

It is generally agreed that the eremophilanes are probably derived by methyl migration from a eudalenoid precursor,[33,73,74,75] although direct experimental evidence has not been obtained. Fig.(13) shows the now accepted biogenetic route to farnesyl pyrophosphate[76] and fig.(14) gives a possible pathway to petasin (8). [see also references (74 and (75] through the known compound eremoligenol (129)[77] a suggested biogenetic parent.[33] Markownikoff cyclisation of conformer (128) of the monocyclic diene (127) followed by methyl migration leads directly to a structure with the correct absolute configuration of eremoligenol (129). It is interesting to note in passing that valerianol (130)[79] a member of the nootkatone (48) group (see p. 22) can be derived in a similar manner either by cyclisation of (131), the antipode of (128), which necessitates epimerisation at C-7, or by cyclisation of the alternative conformer (132)[75] which from examination of a model is no more



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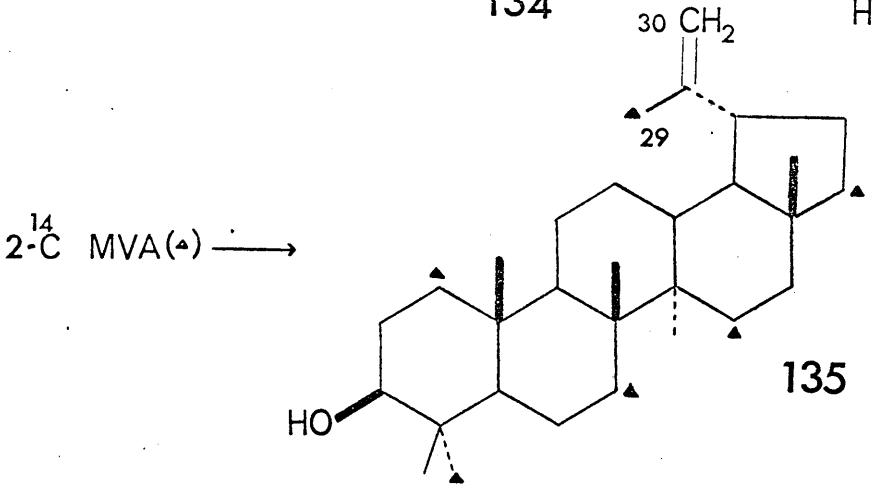

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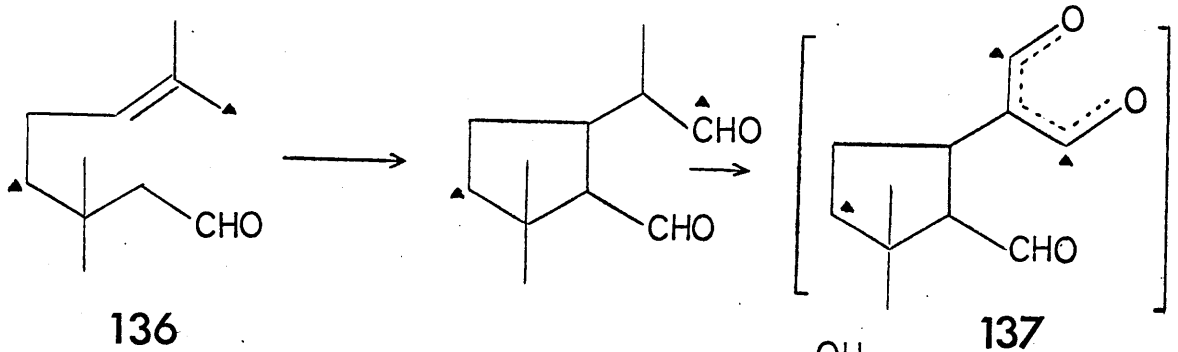

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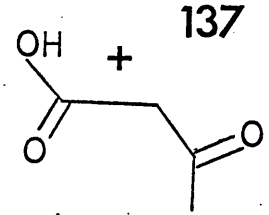

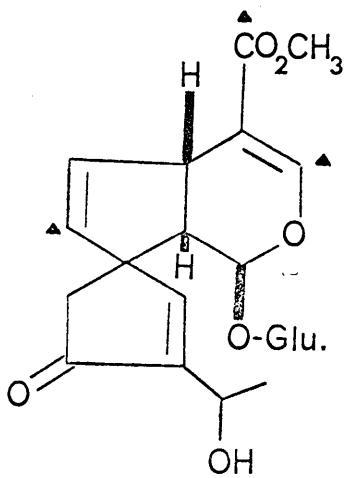

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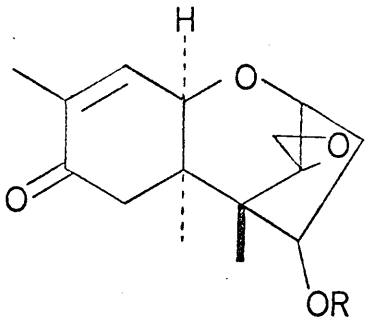
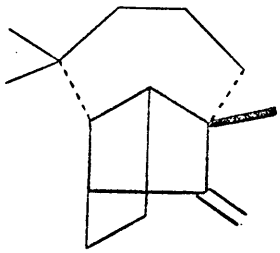
strained than (131).

Figs.(13) and (14) also show the anticipated labelling pattern in farnesol pyrophosphate and in the petasin esters derived from 2-^{14}C and from 3'-^{14}C mevalonic acid lactone (MVA) (133). The distinction made for terpenoid transformations in biological systems, between the C-2 and C-3' positions of MVA has been demonstrated in a number of cases.(80 For example this has been shown directly for the geraniol side chain of mycelianamide (134), where half of the label from 2-^{14}C MVA appears in the terminal trans-methyl group of the side chain.(81 Lupeol (135) has been shown to incorporate label from 2-^{14}C MVA exclusively at C-29 in the isopropenyl side chain.(82 In contrast to these and other observations, 2-^{14}C MVA gave the distribution shown in (138) for plumieride aglycone.(83 Randomisation of the isopropylidene methyl carbons of the presumed precursor citronellal (136) had occurred possibly via (137). If the transformations of fig.(14) are enzyme controlled and stereospecific, it should be possible to decide from which position of MVA (i.e. C-2 or C-3') the carbon atom of the exomethylene moiety of the isopropenyl unit in petasin (8) is derived (see p.133).

Confirmation of the theories of sesquiterpene biogenesis with a few exceptions has been limited to certain atypical



139 R = CO.CH=CH(CH_3) cis-



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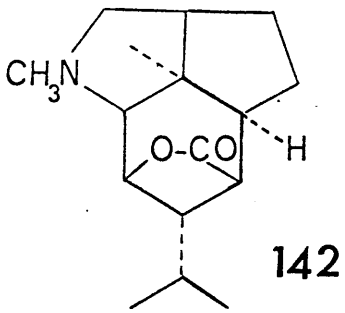

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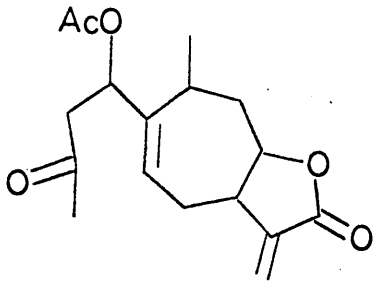

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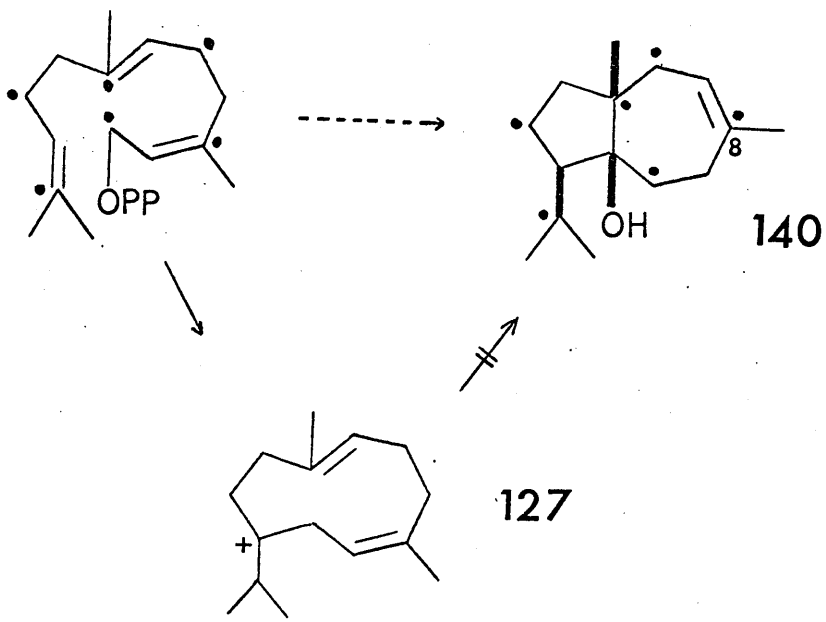
1-^{14}C acetic acid (•)



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fungal metabolites.‡ Trichothecin (139), where incorporation of 1-^{14}C acetate and 2-^{14}C mevalonate gave the predicted labelling pattern, provides an outstanding example.(84 Of the vast number of plant sesquiterpenoids only carotol (140(85,89 in Daucus carota L., longifolene (141) in Pinus longifolia Roxb.(86 the nitrogen containing dendrobine (142) in Dendrobium nobile(87 and xanthinin (143) in Xanthium strumarium L.(88 have received attention. Degradation of carotol labelled from 1-^{14}C acetate showed that one sixth of the activity was present in acetic acid derived from C-8 and its attached methyl group. This disproved the biogenetic scheme via the ten-membered diene (127) with methyl migration to C-8 in (140) and therefore supported the alternative route, via the direct cyclisation of farnesol.

The limited number of biosynthetic investigations with plant sesquiterpenoids is attributable to the difficulties generally encountered in precursor incorporation studies with higher plants.(90,91 Thus one consequence of the complexity of their metabolism is that synthesis of a specific product or intermediate may occur only in one plant organ at a particular time in the growth cycle. For example a preliminary study of Daucus carota showed that carotol (140) was formed in the seeds during a two week vegetative period.(85 This season was

‡ For leading references to the theories experimentally confirmed see ref. (75.

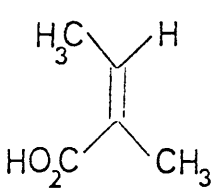
then chosen as the optimum, and perhaps the only effective time in which to feed the labelled acetate precursor. A second complicating feature is that the plant product may be transported from the original site of synthesis and stored elsewhere with or without further modification. Further problems arise, as mentioned below, in the accession of precursors to the sites of synthesis.

Methods commonly used for the introduction of precursors in solution to whole plants are application to the roots, spraying of the leaves, direct injection to the stem, or infusion achieved by piercing the stem with a cotton wick and allowing slow uptake into the transport system by capillary action. Of these, the last, "wick feeding" appears to be the most frequently used. One example from the many available is in the work of Battersby in studies of indole alkaloid biosynthesis where acetate, malonate, mevalonate and monoterpenoid units have been introduced in this way.(92

One difficulty is that material may be taken up into the transport system of the plant but may not reach the site of synthesis of the desired product, through its failure to pass through the cell membrane or because of alternative metabolic processes. An example of degradation of an administered

precursor, which is relevant to our work, is the observation by Arigoni that feeding 2-^{14}C-mevalonate to Eucalyptus globulus gave a 0.01% incorporation into the monoterpene cineole with complete randomisation of the label.(93 This he has ascribed to breakdown to $^{14}CO_2$ and re-incorporation, since activity was also found in glucose residues. A further consideration is the relative sizes of the metabolic "pools" of the introduced compound and of the expected product at the time of the experiment. If the latter "pool" has reached a maximum, the precursor may be diverted through other pathways.

Administration of precursors to separated plant parts is also commonly employed. Tissue slices may be incubated with solutions of the labelled precursor. Excised leaves or twigs may be dipped into solution. There are obviously many variables in such cases: among these are substrate concentration, temperature and pH. Moreover the effects of contamination by extraneous metabolically active systems are likely to be more serious than in a living plant.



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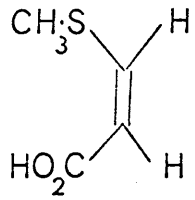

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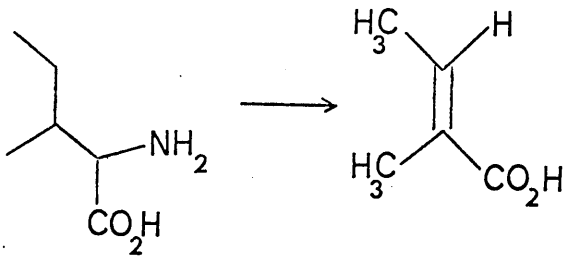

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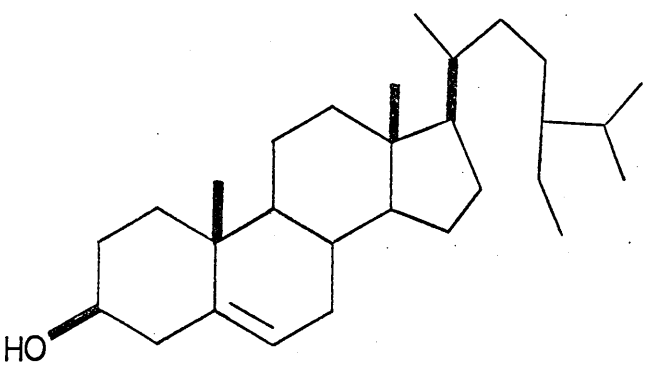

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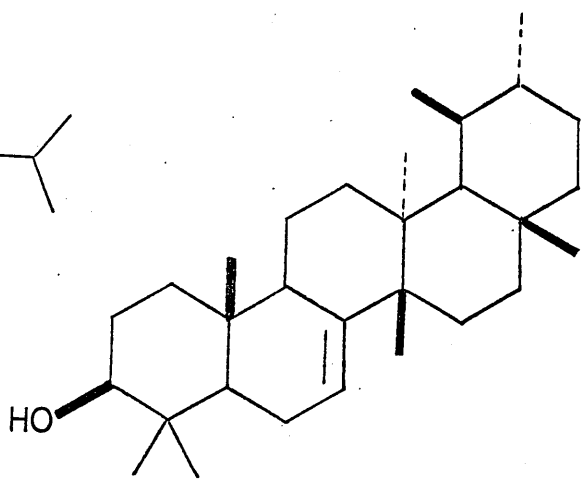

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Present Investigation: Objectives

The principal objective of this study is to achieve incorporation of 3′-^{14}C-MVA into petasin (8) and its analogues in the plant Petasites hybridus L. [see figs.(13) and (14)]. Degradation of the common hydrolysis product of the petasin-type esters, isopetasol (89)[56] and removal of each of the C-4 and C-5 methyl groups would then be required. Schemes of synthesis of the precursor DL-3′-^{14}C MVA and of possible degradation procedures are under consideration. Whether or not these are developed depends on the results from experiments now in progress with the commercially available DL-2-^{14}C MVA.

A further objective is to determine the fate of label from C-2 and C-3′ of MVA in the isopropenyl unit of petasin (8) itself and thus to gain some information concerning stereospecificity in the transformations of fig.(14).

No mention has so far been made of the possible biogenetic origin of the esterifying acids, angelic (144) from petasin and methylthioacrylic (145) from S-petasin (84). These may be formed from amino acid precursors by analogy with tiglic acid (146) and would not therefore incorporate label from MVA. Leete has demonstrated the incorporation of DL-isoleucine (147) into the tiglic acid fragment of the alkaloid meteloidine.[94]

Methods

The work described here was carried out in the spring of 1967. Young P.hybridus plants, rapidly growing but not in flower, were used. As a first step, to check on the presence of the petasin-type esters, two plants were separately dissected into the parts:- leaf, stem (petiole), bud rhizome and root. These were then extracted (see below) and examined by GLC (1% SE-30) and TLC. Our previous work had provided a pure sample of isopetasin, as well as GLC and mass spectrometric data for petasin (8), isopetasin (85), S-(84) and iso-S-petasin (86). We were able to establish their presence in all the tissues examined; the greatest relative concentration of these components appeared to be in the rhizomes. Complex mixtures of sterols (as inferred from GLC retention data) were also present in all tissues.‡ Leaf and petiole extracts were complicated by the presence of carotenoids and chlorophylls (inferred from their TLC behaviour).

A modified extraction and purification procedure, developed from that originally used [p.78 and ref.(56] was employed. This involved the homogenisation of fresh tissue in benzene, alkaline hydrolysis of the total benzene concentrate

‡ Novotný et al have reported the isolation of β-sitosterol (148) and the triterpenoid baurenol (149) from P.officinalis (syn.hybridus) of Czechoslovak origin.(95 For a further discussion of the work of this group with Petasites spp. see p.67.

and separation of isopetasol (89) by preparative TLC. The isopetasol was then acetylated and the product further purified by preparative TLC. The isopetasol acetate was assayed for mass by GLC and for radioactivity using a Packard Tri-Carb Liquid Scintillation Spectrometer.‡

Results

The first experiments were incubations of chopped fresh tissue (root, rhizome, bud, leaf and petiole) with 2-^{14}C sodium acetate. These were carried out under a variety of conditions: a representative example is described in detail in the experimental section (p.138). In all cases it was found that incorporation of radioactivity to ispetasol acetate was near zero. No attempt was made to determine the fate of the label in these experiments.

We then adopted the method of wick feeding of whole plants through the petiole. The first experiments were again with 2-^{14}C sodium acetate. Four plants were fed with 1.0 or 2.5 μc (0.026 or 0.066 μmole); two were harvested after four days, one after seven and one after fourteen days. Each was divided into leaf plus petiole and rhizome plus root; extracts were made and purification as isopetasol acetate was carried out. The results [table (17) facing p.139] were rather less consistent

‡ Kindly made available by Dr.A.F. Lever (MRC Blood Pressure Research Unit).

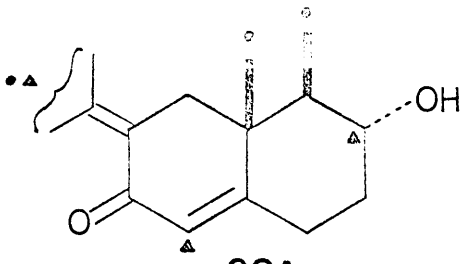
than we had hoped. They nevertheless showed in each case no significant incorporation to isopetasol acetate from rhizome and root tissue and 0.004-0.010% incorporation from leaf and petiole.

2-^{14}C MVA was then fed to four plants (20 μc; 4.0 μmole each) by the wick technique. Two plants were left for five days and two for ten days. In this case an aliquot of the total leaf extracts before hydrolysis was examined by autoradiography, i.e. X-ray film was placed over TLC plates of the extracts for ten days and then developed. The films revealed no significant differences between the five and ten day feeding results. In each case the most radioactive bands were those thought to be associated with sterol and carotenoid (or other non-polar terpenoid) components; the origin also showed intense darkening of the films. The composite petasin/isopetasin bands showed relatively little activity. All bands causing darkening of the films were removed and their activities measured. In this way an estimate of the activity incorporated into the leaf benzene extracts could be made [see table (18) facing p. 140]. This was of the order of 3% of the total activity fed.

Extracts of leaf plus petiole, and of rhizome plus root from the two five day 2-^{14}C MVA feeding experiments were

converted to isopetasol acetate, which was assayed for mass and radioactivity as already described. As with the acetate wick feedings there was no significant incorporation to the isopetasol acetate from rhizome and root tissue. Incorporations by leaf tissue were 0.007 and 0.010% (based on total administered activity); specific activities of the isopetasol acetate were 14.3×10^3 and 15.2×10^3 dpm/μM respectively [table (18)].

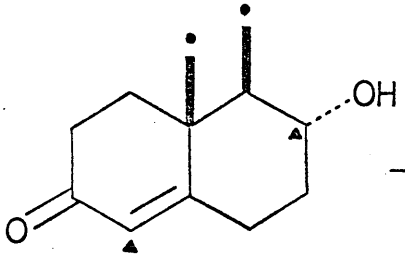
Seven further feedings of 2-^{14}C MVA have been carried out under a variety of conditions in attempts to increase the % incorporation to the petasin sesquiterpenoids. These have so far only been examined by autoradiography of aliquots of the total leaf extracts, and they await working up to yield isopetasol acetate. Three plants were fed with 10 μc and left for one, two and three days respectively. Autoradiography showed quite marked differences; new radioactive bands, absent in the extracts made after one day, appeared in the two and three day extracts. Trials with the sterol inhibitors SKF 7997A [tris-(2-diethylaminoethyl)-phosphate trihydrochloride] and SKF 525A (β-diethylaminoethyldiphenylpropyl acetate hydrochloride)[96] have been made. These were fed to four plants (two with each inhibitor), at concentrations of 20 ppm in water, four days prior to MVA feeding. Autoradiography suggests (subject to confirmation) that some decrease of sterol radioactivity relative to that associated with petasin and isopetasin may have been achieved.



89A

(•) label from 3′-^{14}C MVA

(▲) label from 2-^{14}C MVA



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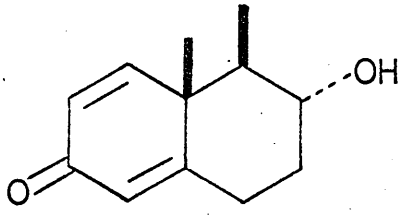
2 × CH_3CO_2H



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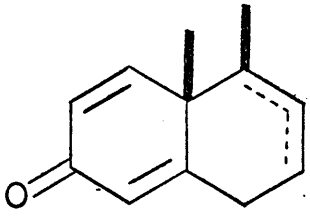

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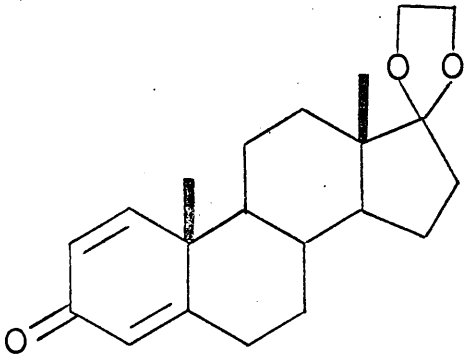

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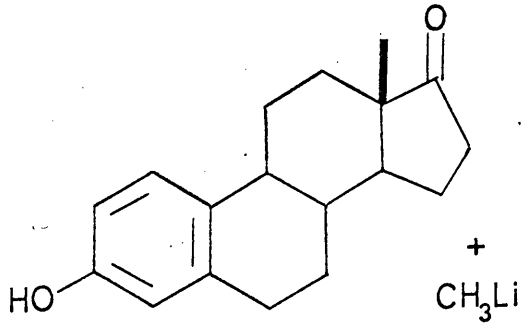

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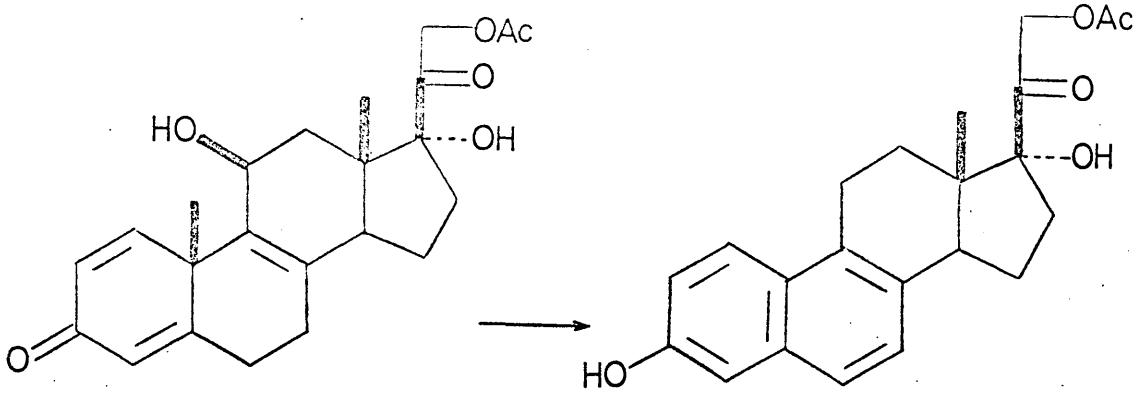
Discussion

The nine remaining extracts of leaf tissue made after feeding with 2-^{14}C MVA will, when time permits, be converted to and assayed as isopetasol acetate. If these results substantiate the two observations where isopetasol acetate of high specific activity was obtained, there will be justification for the continuance of the work towards the objectives already discussed. One obvious difficulty is the small mass of isopetasol acetate isolated from leaf tissue. Individual variation between plants harvested at different times has been observed but the concentration was normally of the order of 0.04 mg/g fresh weight. The relatively high specific activities so far obtained would however permit considerable dilution with "cold" carrier, making degradations feasible.

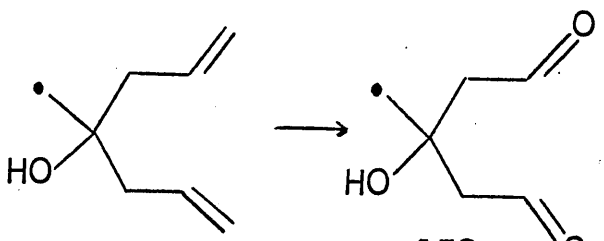
Degradations of isopetasol will be directed primarily at confirming the methyl shift mechanism of fig.(14) when label from 2-^{14}C MVA and 3'-^{14}C MVA should appear in the positions shown in formula (89A). With this aim a reasonable first step would be the removal of the isopropylidene function under vigorous basic conditions to give the known desisopropylidene-isopetasol (150)[56] with loss of one third of the activity, assuming that no randomisation has taken place. Kuhn-Roth[97] (chromium trioxide/sulphuric acid) treatment of (150) should



155 156

$^{14}CH_3CO_2C_2H_5$ (•)

157



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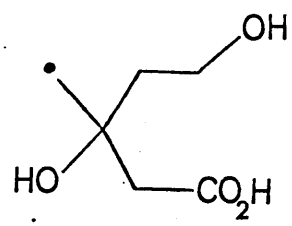


then effect removal of both methyl groups as acetic acid. With 3′-^{14}C MVA as precursor the acetic acid should contain all of the residual radioactivity and with 2-^{14}C MVA as precursor the acetic acid should be inactive.

Removal of the angular methyl group either of isopetasol or a degradation product such as (151) or (152) may be possible by analogy with the aromatisation of steroidal dienones. Thus the 17-ethylene ketal of androsta-1,4-diene-3,17-dione (153) with lithium/diphenyl in refluxing tetrahydrofuran is reduced to estrone (154).[98] The presence of a reagent to trap the lithium methyl formed and prevent its reaction with (153) was necessary for high yields of estrone. Diphenylmethane has been used for this purpose. Ionic aromatisation of dienones of type (155) has been reported in situations where at least two double bonds or potential double bonds are distributed over rings B and C.[99] Conditions in some cases were exceptionally mild; for example treatment of (155) in acetone with hydrochloric acid gave (156) as the major product after ten minutes at room temperature. The application of reactions of the above type to suitable transformation products of isopetasol would require extensive trials with "cold" material.

Degradation of petasin (8) from 2-^{14}C MVA and 3′-^{14}C MVA to determine the labelling pattern in the isopropenyl function would require isolation of the exomethylene carbon as

formaldehyde. This could be done either by ozonolysis or, as in the case of lupeol (135) (see above, p.123), by osmium tetroxide hydroxylation and oxidative cleavage. Both reactions would yield acetaldehyde from the angelate function and, unless the 9-10 double bond was first selectively removed, an unstable α-ketoaldehyde would be produced. Again a careful study of the reaction with "cold" material would be an essential first step.

Lastly an attractive synthesis of 3'-^{14}C MVA starting from 2-^{14}C ethyl acetate (157) should be mentioned. This involves a Grignard reaction with two moles of allyl magnesium bromide and ozonolysis to the dialdehyde (158). Aluminium isopropoxide treatment of (158) is then reported to give mevalonic acid.(100

Experimental

General

Petasites hybridus (L) plants were collected from a site near Motherwell, Lanarkshire, in December 1966. The rhizomes were separated and planted in pots. Those plants used in this study in the months of February to May 1967 were all rapidly growing, not in flower and less than 12" in height.

Radioactive materials used were 2-^{14}C sodium acetate of specific activity 464 μc/mg (38 mc/mM); DL-2-^{14}C mevalonic acid lactone (MVA) of specific activity 38.7 μc/mg (5.03 mc/mM) and a second batch of specific activity 37 μc/mg (4.82 mc/mM) obtained from The Radiochemical Centre, Amersham, Buckinghamshire.

Radioactivity assays were carried out with a Packard Tri-Carb Liquid Scintillation Spectrometer (Series 3000). Efficiency for ^{14}C counting was *ca.* 79%. Lipophilic substances or mixtures were counted using a solution of 0.10 g dimethyl POPOP [1,4-bis-2-(4-methyl-5-phenyloxazolyl)-benzene] and 3.0 g PPO (2,5-diphenyloxazole) in toluene (1 litre). For the counting of more polar substances, a mixture of the above solution (9 ml) with ethanol (1 ml) was used with a suitable quenching correction factor for the ethanol.

GLC was carried out using a Pye 104 instrument with 1% SE-30 columns of lengths 3', 7' and 9' x 1/4" (I.D.)

For detection of bands in preparative TLC the fluorescent spray reagent 3-hydroxypyrene-5,8,10-trisulphonic acid sodium salt was used and visualised at 250 mμ.

Extraction and Isolation Procedure

Initially the method of extraction used was similar to that already described (p. 78) i.e. an ethanol extract of the fresh tissue was made, followed by benzene or chloroform-water partitioning. In the work discussed below extraction of fresh tissue was carried out directly with benzene, and isolation of the petasin esters as isopetasol acetate was then as described in the example below.

Fresh leaf tissue (5.0 g) was homogenised (Silverson homogeniser) in benzene (50 ml) and left at room temperature for 48 hours. The solution was filtered and evaporated to near dryness (in vacuo). Ethanol (5 ml) and ethanolic potassium hydroxide (5 ml of a stock solution containing 0.5 g potassium hydroxide in 18 ml ethanol and 2 ml water) were added. The mixture was refluxed for 2 hours, then concentrated to 1/5 volume (in vacuo), diluted with water (5 ml) and extracted with ether. The ether extract was washed (water), dried and evaporated yielding a dark oil (7 mg). Preparative TLC (using ethyl acetate-petroleum ether, 1:1) allowed isolation of the isopetasol band (R_f 0.25).

The isopetasol was then acetylated using pyridine (0.1 ml) and acetic anhydride (0.2 ml) at room temperature for 12 hours. Addition of benzene followed by evaporation (ca. 60° in vacuo) removed most of the residual acetic anhydride/pyridine. Preparative TLC allowed purification of isopetasol acetate [R_f 0.60 in the solvent system ethyl acetate-petroleum ether (1:1)]. The isopetasol acetate was then dissolved in a known volume of benzene and aliquots were removed for mass estimation by GLC. A pure sample of the acetate was prepared from isopetasol as previously described[56] and a curve drawn of mass against detector response (peak height) under standard GLC conditions (9' 1% SE-30; 222°C).

From leaf extracts made from separate plants during the course of this work, quantities of isopetasol acetate isolated proved to vary quite markedly in the range 0.013 to 0.111 mg/g of fresh tissue. Root and rhizome extracts were treated in the same way. The highest concentration of the petasin esters, determined as isopetasol acetate, was in the rhizomes and quantities of the acetate isolated were of the order of 0.10 to as high as 1.26 mg/g of fresh tissue.

Example of the Procedure used in Incubations ‡

Separate solutions of 0.1, 0.01 and 0.001 M sodium acetate (10 ml) and standard phosphate buffer(101 solutions were combined to give nine mixtures of pH ca. 6.5, 7.0 and 7.5. Sliced fresh rhizome tissue (2.0 g) and 1 μc 2-^{14}C sodium acetate (in 0.10ml water) were added to each mixture. Incubations were carried out at 25° with gentle shaking in an atmosphere of nitrogen (this to prevent the darkening of the tissue observed in previous runs carried out in air). After 18 hours the solutions were filtered and the tissue rinsed with water, homogenised and extracted with benzene. Isopetasol acetate was separated; mass and radioactivity were measured as described above. In each case the counts were not significantly above background and incorporation to isopetasol acetate was therefore near zero. No attempt was made to determine the fate of radioactivity fed in this case.

Previous experiments of this type, carried out in air, with chopped leaf, petiole, bud, rhizome and root tissue had been observed to give near zero incorporation of activity into the total benzene extracts of the tissues.

‡ Incubations were carried out with the assistance of Dr.A.M.M. Berrie whose expert advice in this and other aspects of the investigation is gratefully acknowledged.

TABLE (17)[a

2-^{14}C Sodium Acetate Wick Feedings

Duration of feeding (days)	Counts fed - residue (dpm x 10^6)	Isopetasol acetate mg/g fresh wt. leaf	root + rhiz.	dpm/µM	% incorpn.
4	2.195	0.033		425	0.009
			0.460	18.4	0.003
4	5.487	0.040		200	0.004
			[b 0.020	0	0
7	2.195	0.111		95.9	0.010
			0.760	0	0
14	5.478	0.037		190	0.007
			1.26	?	

(a Data compiled by Dr. J.A. Zabkiewicz

(b Root tissue only

Wick Feeding Procedure

(a) 2-^{14}C sodium acetate.

Cotton wicks were threaded through the stems (petiole) of four separate plants, about 2" above the rhizome, and dipped into 2 ml vials containing 2-^{14}C sodium acetate (1.0 or 2.5 μc; 0.026 or 0.066 μM) in water (0.5 ml). When uptake of the solutions was complete (normally after 24 hours) a further 0.25 ml water was added. Two plants were left for 4 days, one for 7 and one for 14 days in a high intensity light growth chamber (12 hour "day"; 21°). They were then each separated into leaf plus petiole and root plus rhizome tissue (fresh growth only), extracted and the isopetasol acetate isolated as previously described. The vials and cotton wicks were rinsed with aqueous ethanol, this solution carefully evaporated and the residual activity measured. This figure varied slightly but did not exceed 3% of the total activity administered. Table (17) details the results of these experiments.

(b) 2-^{14}C MVA.

2-^{14}C MVA (20 μc; 4.0 μM) was fed to each of four plants in a manner similar to that described for the wick feeding of 2-^{14}C sodium acetate. In this case two plants were left for 5 days and two for 10 days. The results of the 5 day experiments are given in table (18).

TABLE (18)[a

2-^{14}C MVA Wick Feedings

Duration of feeding (days)	Counts fed - residue (dpm x 10^{-6})	% incorporation to total leaf extract	Isopetasol acetate mg/g fresh wt. leaf	root	dpm/μM	% incorpn
(b 5	47.510	2.8	0.018		14.3 x 10^3	0.010
				0.092	0	0
(c 5	47.515	2.97	0.013		15.2 x 10^3	0.007
				0.026	0	0
(b 10	46.718	2.6				
(c 10	47.454	3.67				

(a Data compiled by Dr. J.A. Zabkiewicz

(b 2-^{14}C MVA in buffered (pH 8) aqueous solution

(c 2-^{14}C MVA in neutral aqueous solution.

10% aliquots of the total leaf plus petiole extracts in all four cases were examined by TLC (double elution in the solvent ethyl acetate-petroleum ether, 15:85). Ilford X-ray film was placed over the plates, left for 10 days, and then developed. Apart from small variations in the relative intensity of the bands, the results were similar. Darkening of the film was most intense over the bands of R_f 0.00 to 0.05, 0.35 and 0.95 to 1.00. An earlier examination of leaf extracts by TLC had suggested that the band of R_f 0.35 was due chiefly to free sterol (similar R_f and staining characteristics to β- sitosterol). Temperature programmed GLC on the phase SE-30 revealed a complex mixture from this band with t_R in the range expected for molecular weights of the same order as β-sitosterol. (The presence of β-sitosterol itself has not been established conclusively by us). The bands at R_f 0.95-1.00 were yellow in colour and were considered to be chiefly non-polar pigment, possibly carotenoid. Only slight darkening of the films was associated with the composite petasin/isopetasin band (R_f 0.50-0.55) or with the green chlorophyll bands also observed.

Every band which showed darkening of the film was removed and the radioactivity measured. This enabled estimation of the % incorporation into the total leaf benzene extracts [table (18)].

Further wick feedings with 2-^{14}C MVA are briefly discussed on p.131

Errors

Counting of radioactive samples was carried out for times sufficient to give a standard deviation of less than 2%. Estimation of mass by GLC was done by interpolation from a previously obtained curve of mass against detector response (peak height) for isopetasol acetate. Replicate injections yielded peak heights which did not vary by more than 1% in the majority of cases.

APPENDIX

Mass spectra:

warburgin (16A)

warburgiadione (74)

ugandensolide (104)

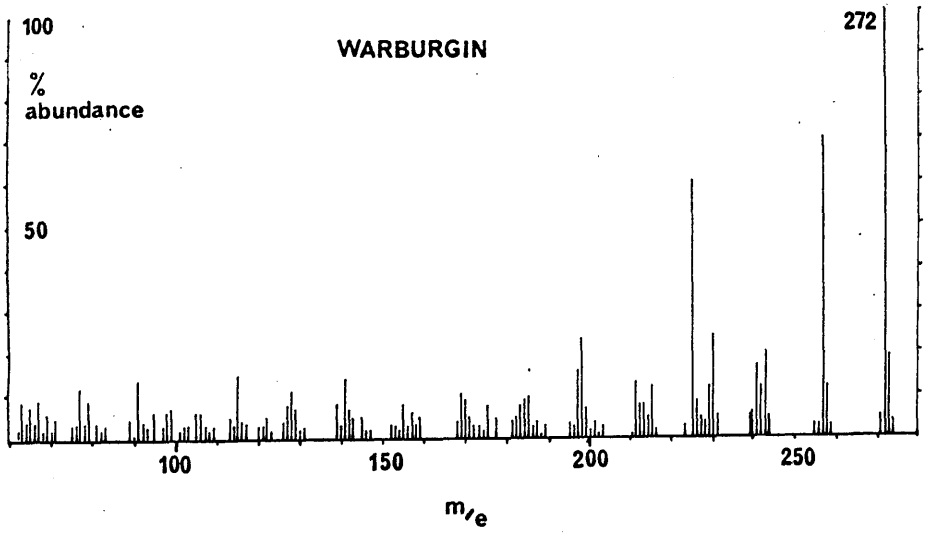
ugandensidial (119)

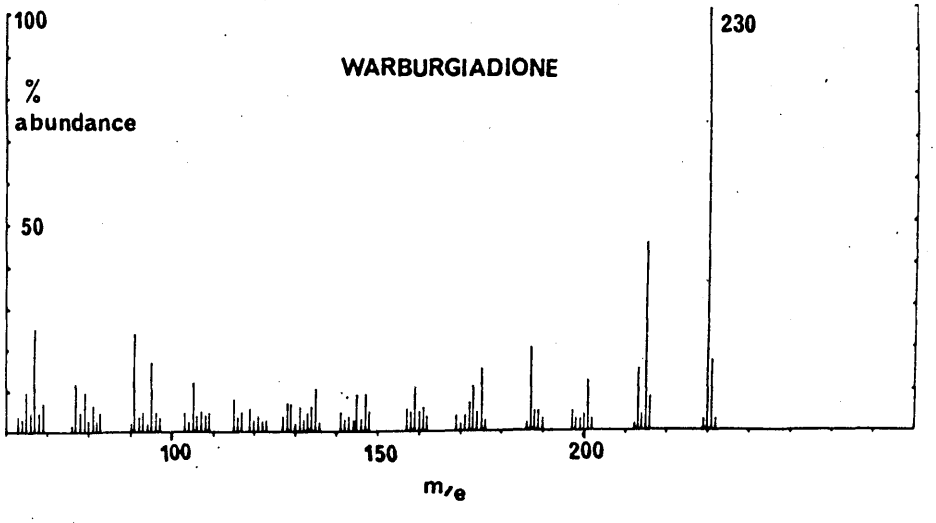
petasin (8)

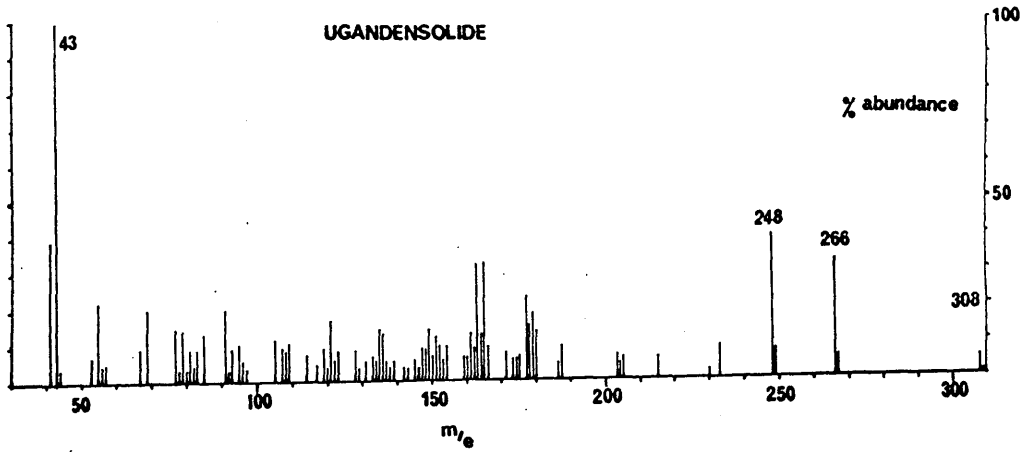
isopetasin (85)

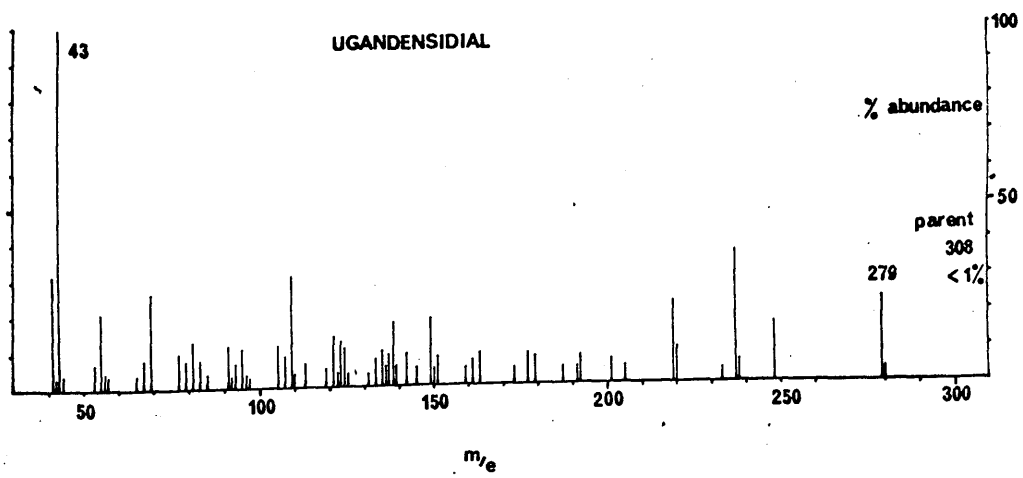
trans-(10α-) furanoeremophilane (26A)

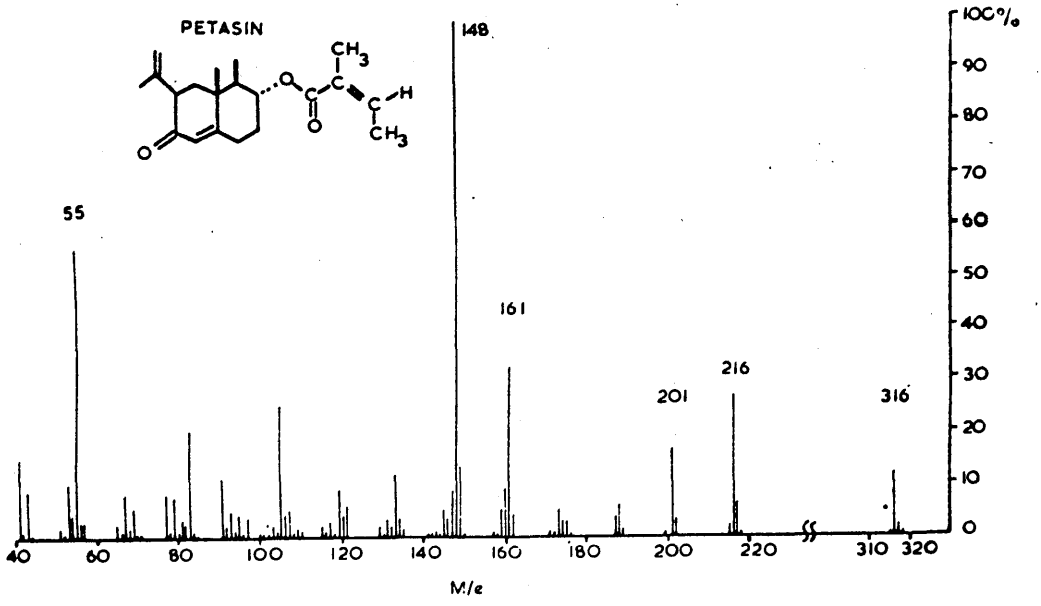
cis-(10β-) furanoeremophilane (23)

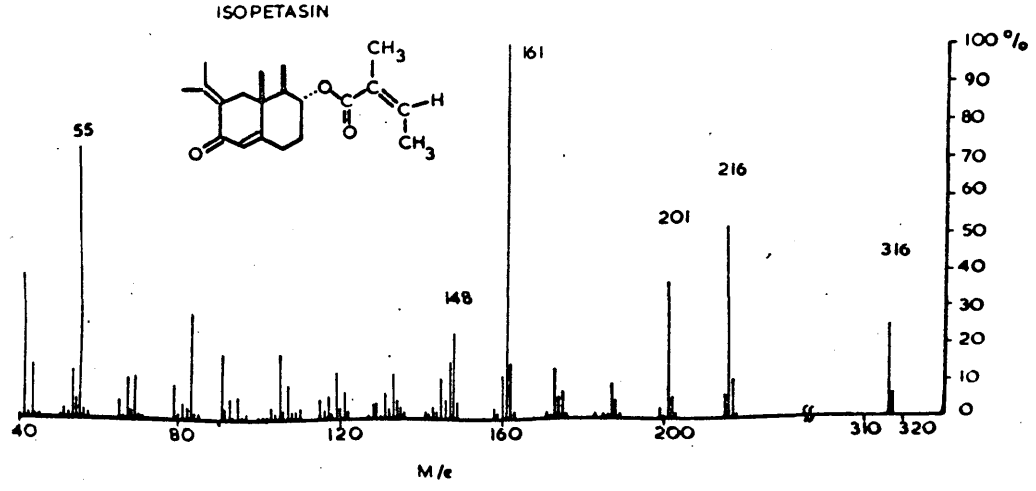

100
WARBURGIN
272
%
abundance
50
100
150
200
250
m/e

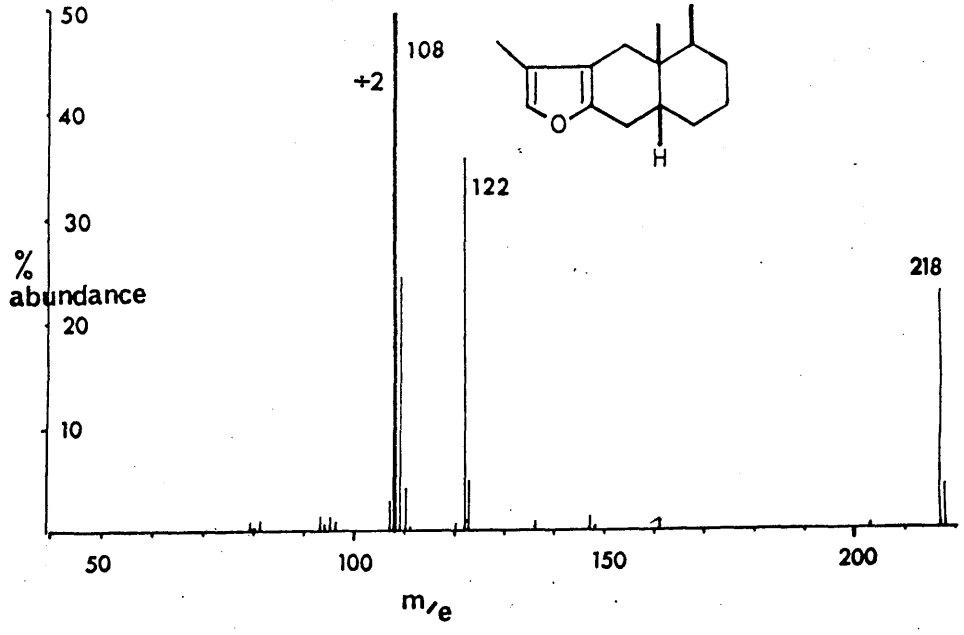

100
WARBURGIADIONE
230
%
abundance
50
100
150
200
m/e

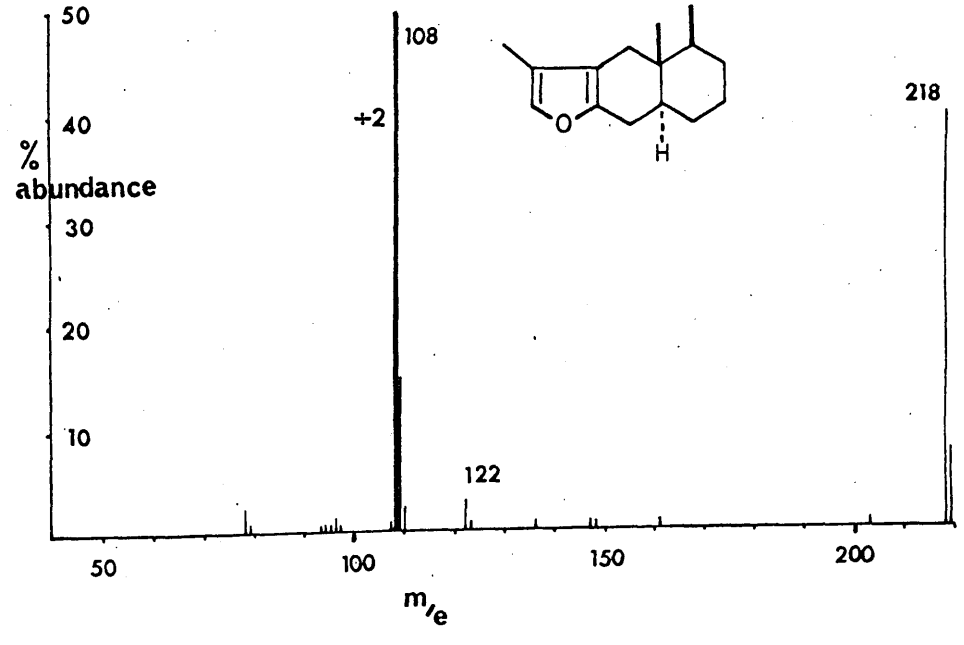

UGANDENSOLIDE
43
% abundance
100
50
248
266
308
50
100
150
200
250
300
m/e


UGANDENSIDIAL
43
% abundance
100
50
parent
308
279
< 1%
50
100
150
200
250
300
m/e


PETASIN
148
55
161
201
216
316
100%
90
80
70
60
50
40
30
20
10
0
40
60
80
100
120
140
160
180
200
220
310
320
M/e


ISOPETASIN
161
55
216
201
316
148
100 %
90
80
70
60
50
40
30
20
10
0
40
80
120
160
200
310
320
M/e


50
40
30
% abundance
20
10
108
+2
122
218
O
H
50
100
150
200
m/e


50
40
% abundance
30
20
10
108
+2
122
218
O
H
50
100
150
200
m/e

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PHYSICO-CHEMICAL TECHNIQUES APPLIED TO ORGANIC NATURAL PRODUCTS

Studies of Sesquiterpenoids from Warburgia ugandensis (Sprague)

by G.H. DRAFFAN

SUMMARY

The major part of the thesis is concerned with the isolation and structural elucidation of four new crystalline sesquiterpenoids, constituents of the heartwood of Warburgia ugandensis (Sprague) Canellaceae, a tree native to East Africa. The principal method used in the assignment of structure and absolute configuration has been chemical degradation to products for comparison with known compounds. Extensive use has been made of chromatographic and spectroscopic techniques.

Two of the components isolated have been shown to be members of the eremophilane group of sesquiterpenoids. A brief description of the proof of constitution of these compounds has been made in two preliminary communications: "Warburgin, a New Sesquiterpenoid of the Eremophilane Group", Chem.Comm., 1966, 393 and "The Constitution of Warburgiadione", ibid 1966, 701, both by C.J.W.Brooks and G.H. Draffan. The known sesquiterpene

alcohol drimenol (H.H. Appel, C.J.W.Brooks and K.H. Overton, J.Chem.Soc., 1959, 3322) was also proved to be a heartwood constituent of Warburgia ugandensis.

Further work with a second consignment of the heartwood has resulted in the isolation and identification of two sesquiterpenoids of the bicyclofarnesane group, a butenolide hydroxyacetate named ugandensolide and a dialdehyde (ugandensidial). Ugandensidial has been proved to be identical with cinnamodial, a constituent of Cinnamosma fragrans (Baillon) Canellaceae (L. Canonica, A. Corbella, G. Jommi, J. Křepinský, G. Ferrari and C. Casagrande, Tetrahedron Letters, 1967, 2137).

The final section of the thesis describes biosynthetic studies with the eremophilane sesquiterpenoids of the plant Petasites hybridus (L.) Compositae. These are compounds of the petasin type (D.H. Herbst and C.Djerassi, J.Amer.Chem.Soc., 1960, 82, 4337). Preliminary results in this long-term project, which involves the feeding of ^{14}C-labelled precursors to the plant, are briefly reported and proposals for further research are discussed.