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SESQUITERPENOIDS OF *WARBURGIA* SPECIES—I
WARBURGIN AND WARBURGIADIONE

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Abstract—Structures are assigned to two crystalline sesquiterpenoids of eremophilane type, warburgin and warburgiadione, isolated from the heartwood of *Warburgia ugandensis* Sprague (Canellaceae). Warburgin (II) has been correlated with furanoligularenone, and warburgiadione (III) has been obtained from petasin.

THE genus *Warburgia* is a class of the small and widely distributed family *Canellaceae*, which in turn is a division of the phytogenetically ancient group of woody plants of the order *Magnoliales*.[1] Our interest in *Warburgia ugandensis* Sprague was originally stimulated by the report[2] that this species of tree* afforded "a fragrant wood, also a resin". We found no record of any chemical investigations on *Warburgia* except for an early description[3] of the essential oil derived from the bark of *W. stuhlmannii* Engl. ("Karambusi").

Supplies of *Warburgia ugandensis* were obtained from Uganda through the agency of the Tropical Products Institute of London. It has not yet been possible to obtain satisfactory samples of resin. Two consignments of heartwood have been examined and found to differ quite markedly in physical appearance and in the chemical composition of their oils. The examination of the first consignment is described in this paper.† The wood somewhat resembled teak and showed a satin lustre: its fragrance, reminiscent of incense, persisted during 4 years' storage. Milling of the wood gave rise to a dust which combined an intense fragrance with sternutatory character. Solvent extraction of the powdered heartwood, and chromatography of the resulting oil, afforded three crystalline components, shown to comprise the known sesquiterpene alcohol drimenol (I)[4] and two new compounds which we have named warburgin and warburgiadione. The identity of drimenol was proved by mixed m.p. determination, comparison of optical rotation data, IR, mass spectra and chromatographic behaviour.

Warburgin (II)

Warburgin was isolated from fractions eluted by benzene in the chromatography of the heartwood extract, and crystallized as pale yellow prisms, m.p. 159–161°, $[\alpha]_D$ +120° ($CHCl_3$), principal λ_{max} (EtOH) 370 mμ (ε, 20,000) (unaltered in 0·05 N NaOH). Solutions of the compound darkened rapidly on standing, and a similar effect was observed during TLC. 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

* Also known as Muziga, East African Greenheart, Sok, or Musunui.

† Salient results have been briefly reported.[3,9]

structural clues. An IR band at 1679 cm^{-1} indicated a conjugated ketone, while a band at 1734 cm^{-1} suggested an ester function. An OMe group, evident from a peak at 6·10 τ in the NMR spectrum, was shown to be part of a methyl ester function when it was found that alkaline hydrolysis followed by methylation regenerated warburgin as the major product. Evidence that the remaining oxygen should be accommodated in a furan ring came from IR bands at 3156 and 1566 cm^{-1}, associated with furan ν_{CH} and ν_{ring} modes.[5] Confirmation of this structural feature, and further evidence of constitution, was derived from NMR data as follows. In a study of various 2- and 3-mono-substituted furan derivatives, Gronowitz *et al.*[6] reported ring proton τ values in the range 2·17 to 5·04 and coupling constants 0·7 to 3·55 c/s. Electron-donating substituents tend to shield the ring protons while electron-withdrawing groups have a deshielding effect. Of the four low-field protons in the 100 Mc/s NMR spectrum of warburgin (Fig. 1a), two were strongly coupled (doublets at 4·02 and 3·06 τ, J = 10·0 c/s) and could not be associated with the furan ring. They best fitted the ethylenic protons of an enone of type (IV).[7] The remaining protons were sharp singlets at 3·45 and 2·13 τ. By analogy with recorded examples[6] the low-field proton was tentatively assigned to a suitably deshielded furan α-position. The peak at 3·45 τ could then be due to the proton of a trisubstituted double bond.

The NMR spectrum also indicated a tertiary Me group (9·21 τ) and a secondary Me (8·80 τ, doublet J = 6·5 c/s) coupled with a deshielded methine (7·47, qu J = 6·5 c/s). Finally a coupling of 16·9 c/s between protons at 7·42 and 6·80 τ strongly suggested a deshielded methylene in a dissymmetric environment.

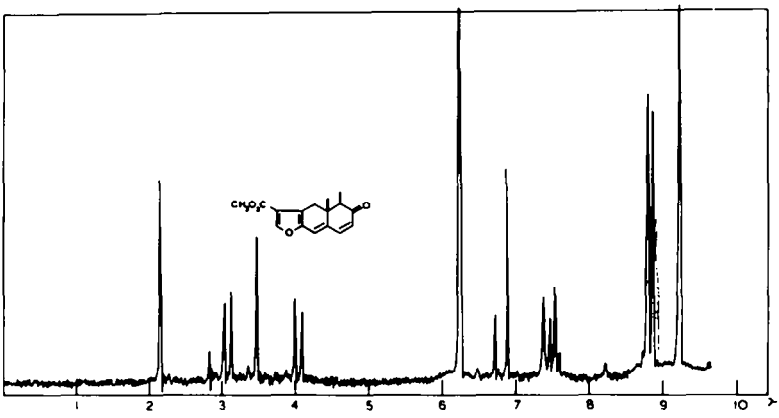
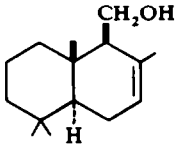


FIG. 1a. 100 Mc/s NMR spectrum of warburgin in $CDCl_3$.

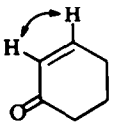
Before proposing a structure we considered the following further evidence. Catalytic hydrogenation of warburgin in ethyl acetate with 10% Pd-C as catalyst gave a single colourless crystalline product, tetrahydrowarburgin. Elemental analysis indicated the formula $C_{16}H_{20}O_4$ and the uptake of two moles of hydrogen. The IR spectrum showed the expected weak furan ν_{CH} frequency at 3155 cm^{-1} and carbonyl bands at 1730 (ester) and 1721 cm^{-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.

* NMR data refer to measurements at 60 Mc/s except as specified.

The spectrum also showed the expected peaks indicative of angular Me (9·33 τ), secondary Me (8·91 τ, J = 7 c/s) and OMe (6·16 τ) groups. The UV spectrum which had λ_{max} 255 mμ (ε, 2650) was as expected for a furan 3-carboxylic ester [compare (V) λ_{max} 259 mμ (ε, 3300)[8]]. 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" cleavage[9] promoted by the furan ring as in (VI–VII, R = CO_2Me).



I



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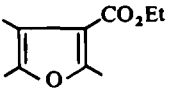
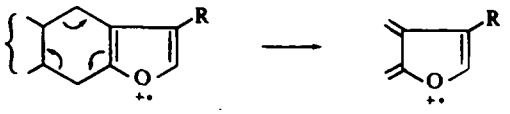


V



VI VII

The above physical and chemical data allowed few structural possibilities for warburgin. Structure II (excluding stereochemistry) was preferred since it fitted the eremophilane carbon skeleton VIII. The hydrogenation product, tetrahydrowarburgin, then has structure IX. "Retro-Diels-Alder" cleavage, where ions corresponding to the furan-derived fragments (VII) were the most abundant in the spectra, was later found to be characteristic in a series of degradation products from tetrahydrowarburgin.

Hydrolysis of warburgin in refluxing ethanolic potassium hydroxide gave two products as judged by TLC. These were assumed to be keto-acids from their TLC behaviour. The mixture was treated with ethereal diazomethane. Warburgin accounted for approximately 70% of the product and was identified by TLC and GLC. A second product was isolated by preparative TLC as a discoloured oil, highly unstable in air, ν_{max} 1734 and 1676 cm^{-1} in carbon tetrachloride and λ_{max} 372 mμ (ε, ca. 20,000) in ethanol. Difficulties in isolation and purification prevented complete identification. However, the mass spectrum included a molecular ion at *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.

We considered that the evidence detailed above strongly supported structure II (excluding stereochemistry) for warburgin. To confirm this we undertook its conversion to a furanohydrocarbon for comparison with the naturally occurring furanoeremophilane (X) of known absolute configuration.[10] It was hoped to prove the position of the ketone function by making the enol acetate in which the C-4 Me should give rise to a singlet at about 8 τ in the NMR spectrum. This was not achieved, but became unnecessary when the elucidation of the structure of furanoligularenone[11] permitted a simple correlation (see below).

Reduction of tetrahydrowarburgin with excess LAH in anhydrous THF at room temperature gave an unstable product XI of low m.p. The IR spectrum showed no CO absorption and at high dilution in carbon tetrachloride two OH bands of approximately equal intensity at 3624 and 3615 cm^{-1}. UV absorption at λ_{max} 221 mμ (ε, ca. 5000) was consistent with structure XI. [Compare menthofuran which has λ_{max} 222 mμ (ε, 6020)[12]]. A singlet in the NMR spectrum at 5·52 τ was assigned to the methylene protons of the primary alcohol and a multiplet at 6·15 τ to the methine proton of the secondary alcohol. The mass spectrum included the expected molecular ion, *m/e* 250 and the "retro-Diels–Alder" ion as the base peak, *m/e* 124 [(VII), R = CH_2OH].

Oxidation with chromium trioxide in pyridine gave the crystalline ketoaldehyde (XII). The IR 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 showed UV absorption at 271 mμ (ε, 2600) [cf. evodone (XIII), λ_{max} 265 mμ (ε, 3700)[13]]. In the NMR spectrum the furan proton appeared at 2·03 τ and the aldehyde proton at 0·01 τ.

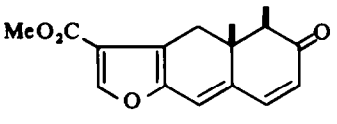
Huang–Minlon reduction of the ketoaldehyde XII (in refluxing ethylene glycol) gave furanohydrocarbon (XIV) as judged by TLC. This product resembled authentic *cis*-furanoeremophilane* (X) polarity (R_f 0·70 in benzene) and colour reactions, but was found to be distinguishable from X by GLC. An unresolved peak at 3·05 τ in the NMR spectrum was assigned to the furan α-proton coupling with the furan Me group at 8·13 τ (doublet, *J* ca. 1·5 c/s). Closely similar data are recorded for atractylon XV.[14] Weak bands in the liquid film IR spectrum of *cis*-furanoeremophilane X at 1576, 1660, 1776 and 1810 cm^{-1} have been assigned to the furan ring.[15] We observed only two corresponding bands (at 1565 and 1648 cm^{-1}) in the spectrum of XIV, and the spectra also showed other significant differences. However the closely similar mass spectra proved the two compounds to be isomeric. Conversion of tetrahydrowarburgin into an isomer of *cis*-furanoeremophilane suggested a possible *trans*-ring fusion in IX, XI, XII and XIV. Evidence given below indicates that the distinction between X and XIV is due to the 10α-H stereochemistry in XIV.

Hydrogenation of XIV in acetic acid with PtO_2 catalyst gave two major products in a ratio of 3:2 as judged by GLC. They were separable from an authentic sample of tetrahydrofuranoeremophilane (XVI)[10]† and were shown by GC–MS to be isomeric with XVI. 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 (VIII). Hydrogenation of *cis*-furanoeremophilane (X)[10] under the same conditions gave as the major product the known compound XVI. Analysis by GLC disclosed a minor peak attributable to an eremophilane, results consistent with those reported.[10, 15] 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 1.

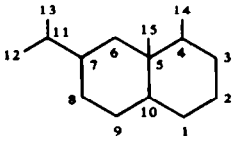
At this stage we were informed by Dr. K. H. Overton of the then unpublished

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

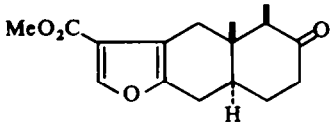
† This sample was kindly provided by Dr. L. Novotný.



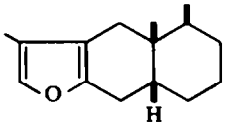
II



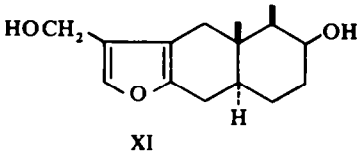
VIII



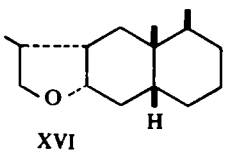
IX



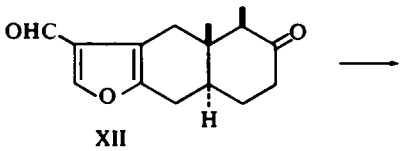
X



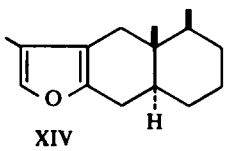
XI



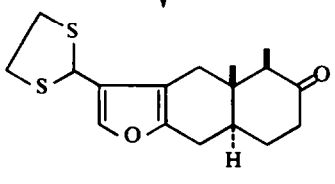
XVI



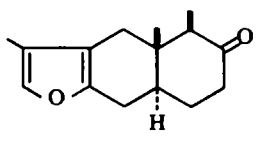
XII



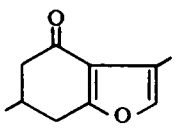
XIV



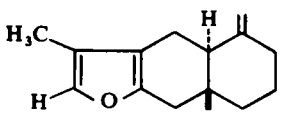
XVIII



XVII



XIII



XV

TABLE 1. HYDROGENATION PRODUCTS OF *trans*- AND *cis*-FURANOEREMOPHILANES (XIV AND X)

Retention times are for a 4' column of 1% SE-30 at 125°. Mass spectra were obtained by GC-MS[a]. 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 XIV	[b]3·7 (5%)	[c]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 X	[b]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			

[a] 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.

[b] Peaks incompletely resolved, evidently (from GC-MS) mixtures of eremophilane isomers.

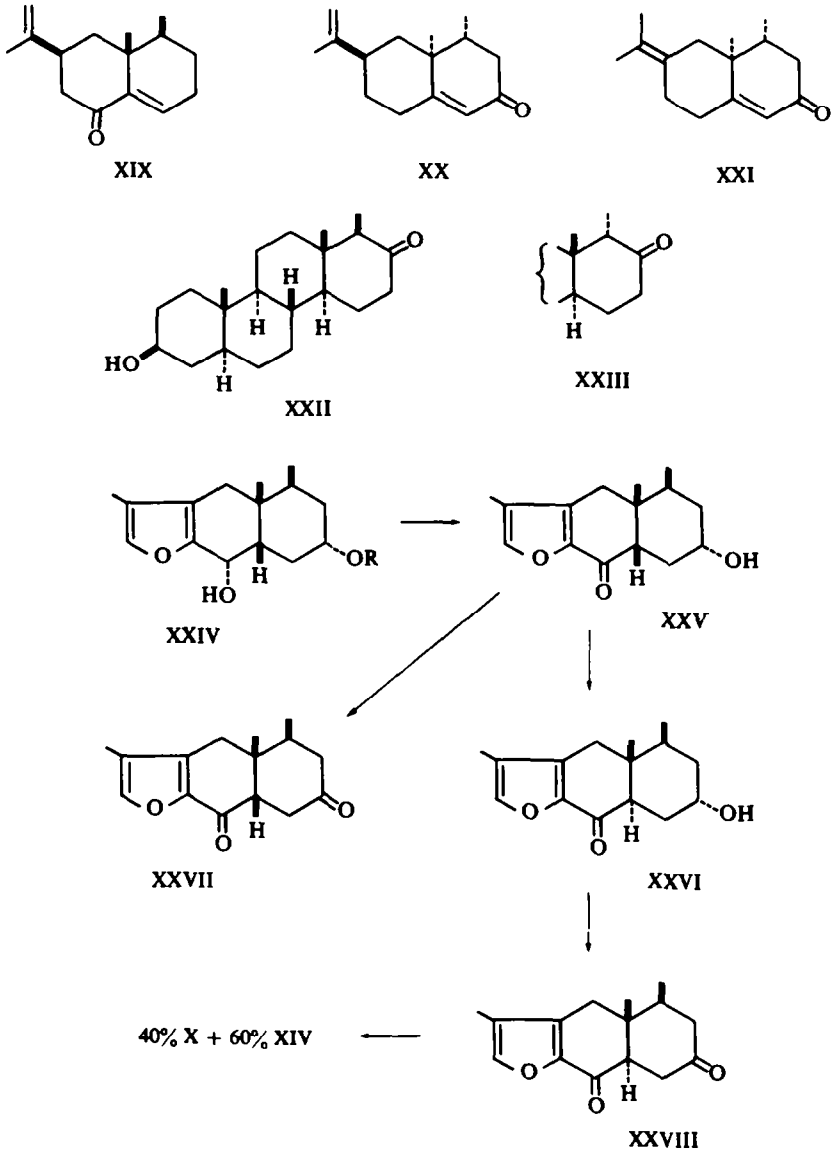
[c] Figures in parentheses refer to the estimated percentage compositions.

work of Professor G. Ourisson and collaborators on the constitution of furanoligularenone [isolated from the Chinese drug "San-shion" derived from a species of *Ligularia* (Compositae)],[11] and of its dihydro-derivative, furanoligularanone (XVII). A significant point in the structural proof was the base-catalysed exchange deuteration of furanoligularanone, when the C-4 doublet Me resonance in the NMR spectrum became a broad singlet: $(CH_3—CH—) - - - > (CH_3—CD—)$. This observation placed the CO function unambiguously at C-3.

We achieved a correlation of the ketoaldehyde (XII) with furanoligularanone (XVII) by making use of the relatively hindered nature of the ketone function in XII. Treatment with 1·1 moles of ethanedithiol in ether with boron trifluoride etherate as catalyst resulted in selective conversion to the thioacetal (XVIII). This compound was characterized by its IR absorption ($\nu_{C=O}$ 1717 cm^{-1} in CCl_4) 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, while singlets at 4·58 and 2·79 τ were assigned to the C-13 proton and furan proton respectively. 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 Ni. The product was purified by preparative TLC as an unstable oil and proved to be inseparable from an authentic sample of furanoligularanone (XVII)* on five GLC phases. The IR, NMR and MS 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 rotary dispersion data, to be discussed below.

* A sample of furanoligularanone was supplied by Professor G. Ourisson, Institut de Chimie, Strasbourg.

The above evidence proved the gross structure of warburgin as II. The stereochemistry of the C-4 and C-5 Me groups and the nature of the ring fusion (tentatively assumed to be *trans*) in tetrahydrowarburgin (IX) and its congeners remained in doubt.



Configuration at C-4 *and* C-5 *in warburgin*

Brief comment on absolute configurational assignments in the eremophilane series is necessary at this point. The earliest known members of this group have the stereochemistry implied in formula XIX for eremophilone.[16] A smaller group of

naturally-occurring sesquiterpenoids, typified by nootkatone (XX)[17] and α-vetivone (XXI)[18] is characterized by the opposite configuration at C-4 and C-5.

(a) *Optical rotary dispersion data.** The Cotton effect curves due to the carbonyl chromophore in compounds IX, XII and XVII had amplitudes in the range −76 to −108. Comparison of the data for the furanoketone from warburgin; $[\phi]_{303m\mu}$ −4400°, $[\phi]_{260m\mu}$ +6400°; $a = -108$, with that for authentic furanoligularanone (XVII): $[\phi]_{304m\mu}$ −4770°, $[\phi]_{267m\mu}$ +6680°; $a = -114{\cdot}5$, proved the stereochemical identity of these compounds. Data are summarised in Table 2.

TABLE 2. COTTON EFFECT EXTREMA (METHANOL SOLNS)

Compound	Molecular Rotation	Amplitude (a)
Tetrahydrowarburgin (IX)	$[\phi]_{304}$ mμ −4180; $[\phi]_{279}$ mμ +5300	−95
Ketoaldehyde (XII)	$[\phi]_{313}$ mμ ±0; $[\phi]_{275}$ mμ +7620	−76
Ketone (XVII)	$[\phi]_{303}$ mμ −4400; $[\phi]_{266}$ mμ +6400	−108

Assuming a cyclohexanone "chair", application of the octant rule indicates the absolute stereochemistry as represented in II with 4β-CH_3 (equatorial), 5β-CH_3 (axial) rather than the antipodal stereochemistry of the nootkatone (XX) series. Useful analogies are the 17-keto-D-homosteroids XXII and XXIII[19] (cf. also refs[20]). In example XXIII where the 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 II for warburgin itself.

(b) *Benzene induced shifts in* NMR *spectra.* Evidence supporting the above conclusions from the ORD data came from the benzene solvent shifts[21] in the NMR spectra of the ketones IX, XII and XVII. Results summarized in Table 3 are consistent with the stereochemistry shown in formula II. However, since benzene

TABLE 3. 60 Mc/s NMR SOLVENT SHIFTS OBSERVED FOR WARBURGIN DERIVATIVES

Compound	Δ values ($\delta_{CHCl_3}-\delta_{benzene}$)		
	4-CH_3	5-CH_3	Furan substituent
Tetrahydrowarburgin (IX)	+0·05	+0·27	+0·37 (OC$\underline{H}_3$)
Ketoaldehyde (XII)	+0·10	+0·34	+0·37 (C$\underline{H}$O)
Ketone (XVII)	−0·01[a]	+0·20[a]	+0·11[a] (C$\underline{H}_3$)

[a] These values refer to ($\delta_{CCl_4}-\delta_{benzene}$)

molecules are probably associated with the substituted furan ring in each case, the overall effect is of uncertain significance.

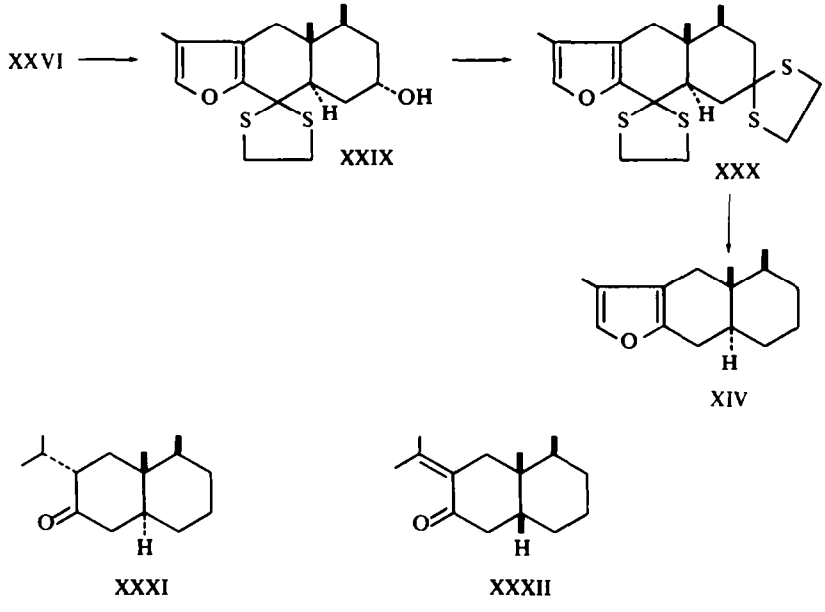
Configuration at C-10 *in tetrahydrowarburgin and its congeners*

We had assumed that the marked differences between the furanoeremophilane isomers (X and XIV) implied a *trans*-ring fusion in XIV.

* ORD curves were kindly recorded by Professor W. Klyne, Westfield College, London.

At this time Dr. L. Novotný generously provided a sample of furanopetasol (XXIV, R = H).[22] The absolute stereochemical assignments at C-4, C-5 and C-10 are established as in formula XXIV. We prepared the *cis*-fused ketoalcohol (XXV) as described:[22] equilibration 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 IR and UV data proved this product to be XXVI, the *trans*-fused isomer of XXV.

Jones' oxidation of keto-alcohols (XXV and XXVI) gave the respective diones (XXVII and XXVIII). The purity of compounds XXV–XXVIII could be established by TLC. Under GLC conditions, pure *cis*-isomers (XXV and XXVII) suffered partial epimerization to the *trans*-isomers as indicated by retention data and by GC–MS. Huang–Minlon reduction of *trans*-dione (XXVIII) in refluxing ethylene glycol gave a 60:40 mixture of the 10α-H and 10β-H furanoeremophilanes XIV and X 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 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 (XXIX), obtained from XXVI in poor yield by prolonged heating in ether-ethanedithiol with boron trifluoride etherate as catalyst under scrupulously oxygen-free conditions. The mass spectrum showed the molecular ion at *m/e* 324 corresponding to $C_{17}H_{24}O_2S_2$. Controlled oxidation of XXIX with chromium trioxide in pyridine under nitrogen gave a crystalline thioketal ketone characterized by analysis, IR and mass spectra. Further treatment with ethanedithiol in ether at room temperature again under nitrogen and with boron trifluoride etherate as catalyst gave the *bis*-thioketal (XXX) as a colourless

gum, characterized by GLC and GC–MS. [The chief difficulty in the preparation of the thioketals was the ready oxidation of the furan ring to a butenolide as judged by the IR absorption of crude products. A similar oxidation of furanoeremophilane has been reported[23]]. Raney nickel reduction of XXX in dioxan gave a product which was shown by GLC and GC–MS to contain approximately 80% of *trans*-furanoeremophilane (XIV). This was not further purified. However it was found to be indistinguishable from the furanohydrocarbon (XIV) from tetrahydrowarburgin on four GLC phases. The two products had identical mass spectra (GC–MS). We consider these results to be a demonstration of the 10α-H *trans*-stereochemistry in tetrahydrowarburgin and the compounds derived from it. Professor Ourisson kindly informed us before publication of the confirmation of this point of stereochemistry in furanoligularanone (XVII) and its congeners by correlation with 5β,10β-dimethyl-3α-isopropyl(9α-H)-2-decalone (XXXI) prepared from fukinone (XXXII).[24]

Warburgiadione (III)

Warburgiadione was eluted after warburgin in the benzene fractions from chromatography of the heartwood extract. In contrast to warburgin, warburgiadione proved to be stable to air and light and could be crystallized or sublimed without serious loss. Elemental analysis indicated the molecular formula $C_{15}H_{18}O_2$. This was confirmed by the mass spectrum which showed a molecular ion at *m/e* 230. The IR spectrum of a carbon tetrachloride solution showed two bands of approximately equal intensity at 1686 and 1659 cm^{-1}. Both were assigned to conjugated CO functions. A weaker band at 1614 cm^{-1} was assigned to the $\nu_{C=C}$ frequency of a double bond. UV absorption at 292 mμ (ε, 21,500) indicated an extended chromophoric system.

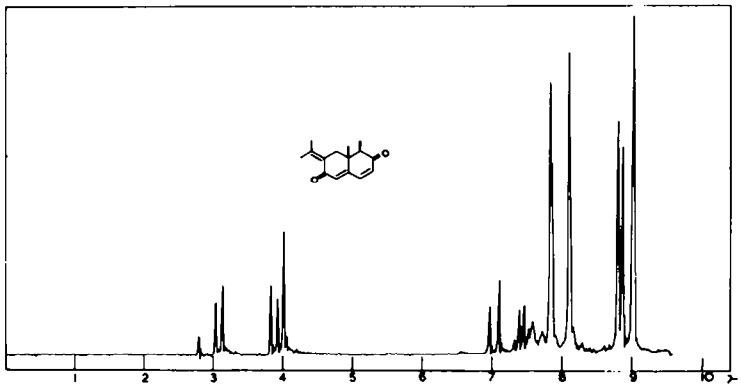


FIG. 1b. 100 Mc/s NMR spectrum of warburgiadione in $CDCl_3$.

Comparison of the NMR spectrum of warburgiadione (Fig. 1b) with that of warburgin (Fig. 1a) revealed several points of structural similarity. The spectroscopic data, together with an assumed biogenetic relationship with warburgin, allowed deduction of structure III (excluding stereochemistry) for warburgiadione. Double irradiation experiments, carried out subsequently, confirmed all the couplings

assigned. The spectrum of warburgiadione (Fig. 1b) had a singlet at 9·03 τ and a doublet at 8·85 τ (J = 6·8 c/s) which showed the presence of tertiary and secondary Me groups respectively. As in the spectrum of warburgin the secondary Me was coupled with a deshielded methine, which afforded a quartet at 7·44 τ. A large coupling (13·6 c/s) 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 (J = 10·0 c/s) 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 indication of a probable isopropylidene function in warburgiadione (Me group signals at 7·85 and 8·12 τ). We assigned the peak at lower field to the C-12 methyl group adjacent to the C-8 CO. Both the C-12 and C-13 Me signals were split and coupled to the higher field (7·65 τ) C-6 proton. Stereochemical information derived from the NMR data is discussed below.

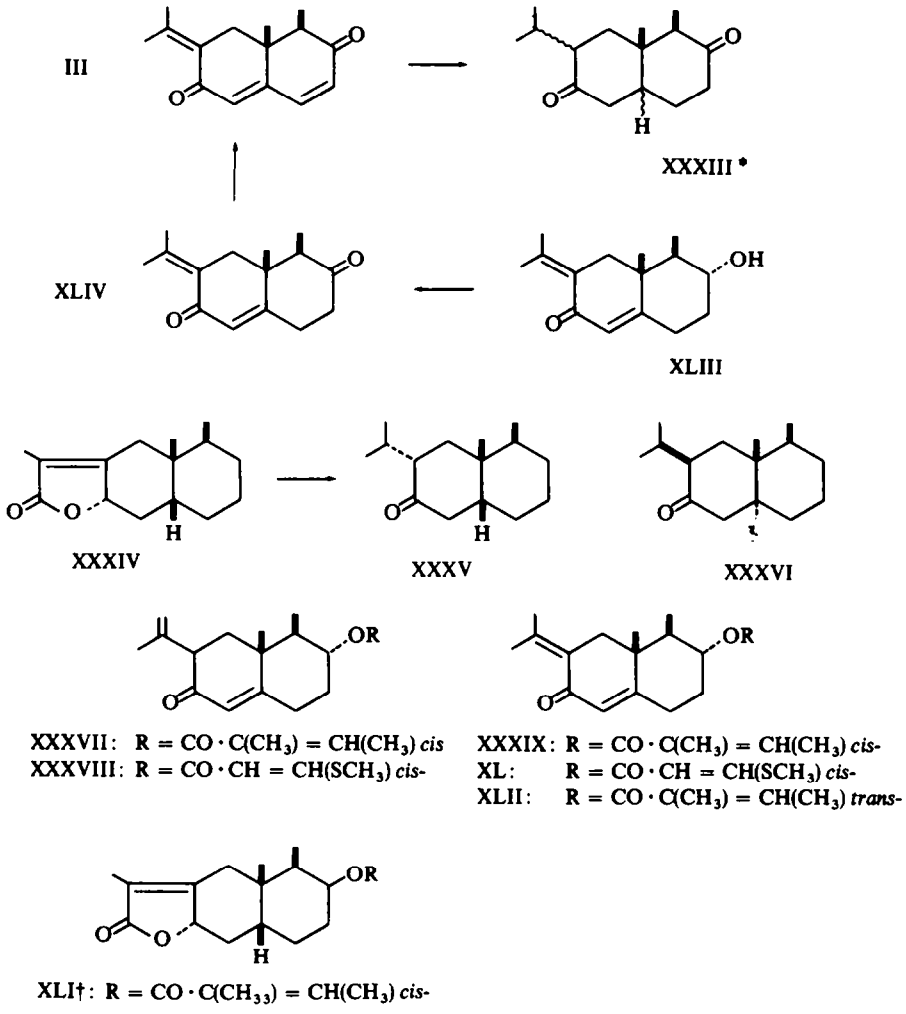
The conjugated chromophore based on the Δ^1-3-one has a calculated[25] λ_{max} value of 286 mμ, in reasonable agreement with the observed λ_{max} 292 mμ.

Catalytic hydrogenation of warburgiadione with 10% Pd–C in ethyl acetate gave a colourless oil (XXXIII) for which elemental analysis indicated the formula $C_{15}H_{24}O_2$, corresponding to the uptake of three moles of hydrogen. The IR spectrum in carbon tetrachloride showed only one CO band at 1716 cm^{-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 indicated two main components in a ratio (estimated from GLC) of 3:2. When examined by GC–MS, both products showed the molecular ion *m/e* 236 (corresponding to $C_{15}H_{24}O_2$) and thus appeared to be isomeric hexahydrodiketones.

8-Ketoeremophilanes have been the subject of considerable interest. The base-labile isomers (XXXV and XXXVI) have been obtained by degradation of eremophilenolide (XXXIV[10]) and by synthesis respectively.[16] The preferred conformations of the thermodynamically stable 8-ketoeremophilanes are considered to be those in which the isopropyl and C-4 Me groups are equatorial.[26] In view of these results the observations of at least two isomeric hexahydrowarburgiadiones, and the lability of one of these to base, are not unexpected. The presence of the C-3 keto group may cause the thermodynamic stabilities of 3,8-diketo systems to be different from those of simpler 8-keto eremophilanes.

Huang-Minlon reduction of the crude diketone mixture (XXXIII) gave in poor yield a hydrocarbon fraction. This product, though apparently homogeneous to GLC on the phase SE-30, was probably a mixture of isomers. The mass spectrum showed a molecular ion at *m/e* 208 ($C_{15}H_{28}$), and was similar to that obtained from the mixture of eremophilane isomers from the catalytic hydrogenation of *trans*-furanoeremophilane (XIV). The conversion of warburgiadione (III) through (XXXIII) to hydrocarbons $C_{15}H_{28}$ confirms that the seven double bond equivalents consist of three double bonds, two ketone functions and two rings.

It appeared that eremophilane sesquiterpenoids of the petasin type could be used as starting materials in a partial synthesis of warburgiadione. Petasin (XXXVII)



and S-petasin (XXXVIII) were originally isolated[27] from *Petasites hybridus* L (Compositae). The structures were established by Aebi, *et al.*[28] Absolute configurational assignments as XXXVII and XXXVIII were later made by Aebi and Djerassi[29] and by Herbst and Djerassi.[30] The isopropylidene analogues isopetasin (XXXIX) and iso-S-petasin (XL) were also isolated. However, these may be artefacts of the separation procedure, since the ready isomerisation of XXXVII to XXXIX has been demonstrated.[28]

Varieties of *Petasites hybridus* of Czechoslovakian provenance were found by Šorm *et al.* to contain, instead of petasin and its congeners, furanoeremophilane derivatives and analogous butenolides as typified by furanopetasin (XXIV: R = angeloyl)[22] and petasitolide A (XLI).[23]

* The wavy lines denote mixtures of stereoisomers.

† The 3-configuration is homogeneous but unspecified.

In a later study,[31] *P. hybridus* plants from several locations in Europe were found to show an approximately equal distribution of the furanoid and isopropenyl (petasin) types but the two did not co-occur.

P. hybridus was collected from a site near Motherwell (Scotland). An 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. 2 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 original extract. Mass spectral scans of peaks A–E were made and the results are summarised in Table 4. The mass spectra of components B and C which were inferred to be petasin and isopetasin respectively, both showed losses of 100 mass units as expected for the elimination of angelic acid moieties from the molecular ions. The base peaks of the two spectra were different, *m/e* 148 for petasin and *m/e* 161 for isopetasin. Component D appeared to be a third isomer: the base peak of the spectrum was at *m/e* 161 as for isopetasin. [D may be the tiglate analogue (XLII): a tiglate isomer of petasitolide A has been isolated and is considered to be an artefact of the isolation procedure.[23]] Peak E in later extracts was resolved into two components, probably *S*- and iso-*S*-petasin (XXXVIII) and (XL). In these cases the initial ester loss was 118 mass units (elimination of β-methylthioacrylic acid).

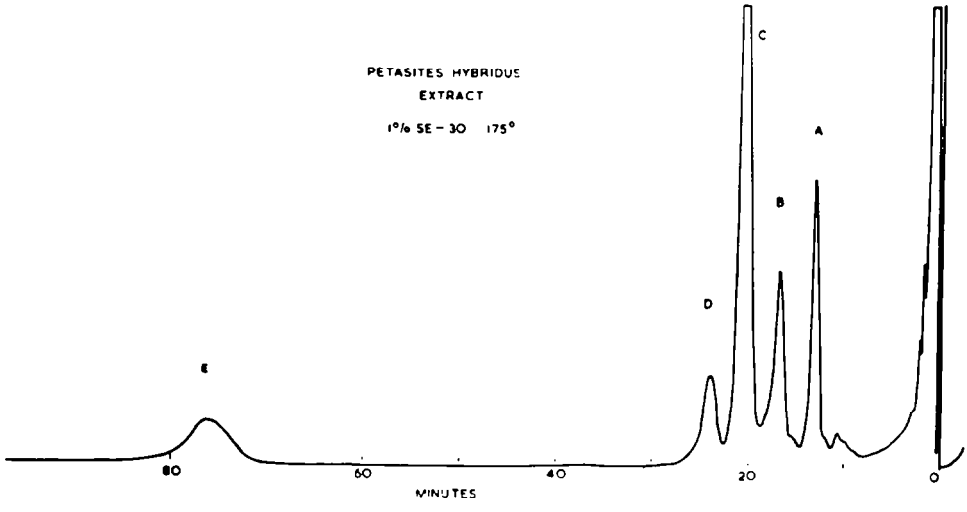


FIG. 2. GLC of an extract of *Petasites hybridus* after vacuum sublimation: 1% SE-30 on silanised Gas-Chrom P, 4-ft column, 175°, Pye Argon Chromatograph, gas flow 40 ml/min.

The GLC and GC–MS evidence confirmed the presence of the petasin type in our sample of *P. hybridus*, and a large scale extraction was carried out. Chromatography on silica gel resulted in the isolation of petasin containing some isopetasin. Advantage was then taken of the ready isomerization of petasin to isopetasin on alumina, described by Aebi and Waaler.[28]

Alkaline hydrolysis of isopetasin gave isopetasol (XLIII), the common hydrolysis product of the petasin-type esters.[28] Oxidation of isopetasol with chromium trioxide in acetone gave isopetasone (XLIV). Our physical data for these compounds were consistent with those reported.[28]

TABLE 4. GC–MS OF *Petasites hybridus* EXTRACT

Peak	t_R (min) SE-30	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 of B and C
E	76·0	334	161	Iso-S-petasin

Retention times are for a 9 ft column on 1% SE-30 at 175°. Mass spectra were obtained by GC-MS.

Isopetasone was dehydrogenated to warburgiadione using 2,3-dichloro-5,6-dicyanobenzoquinone (D.D.Q.)[32] in refluxing dioxan. The reaction was carried out with 1·1 moles of reagent under nitrogen for 10 hr. The product was proved to be identical with the naturally-occurring warburgiadione by the undepressed mixed melting point and by comparison of optical rotation data, UV, IR and mass spectra and GLC behaviour. Provided that no epimerization has taken place at C-4 in the transformation of isopetasol through isopetasone to warburgiadione, the absolute configuration of warburgiadione is as represented in formula III.

Assignment of the NMR signals due to the C-6 *protons of warburgiadione and warburgin*

In the 100 Mc/s NMR spectrum of warburgiadione (Fig. 1b) 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 Me groups. As expected, the transoid 12-H–6H coupling (2·0 c/s) was greater than the cisoid 13-H–6H coupling (1·4 c/s).[33,34] The magnitude of such a coupling also depends on the angle between the planes of the 7–11 double bond and the C-6 to H-bond in formula III. The coupling will be greatest when this angle is close to 90° and a minimum when it is close to zero.[33,34] From models the preferred confirmation of III 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 (II). In the spectrum of warburgin (Fig. 1a) the geminal C-6 protons appear at 6·80 and 7·42 τ as doublets with J = 17·0 c/s. 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 Me 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°.[35]

EXPERIMENTAL

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 TLC on 0·25 mm

layers. Spots were detected by charring with ceric sulphate-sulphuric acid reagent. Preparative TLC was carried out on 0·5 mm layers of "Kieselgel H" or "HF_{254}". Bands have been detected by suppression of the fluorescence of "Kieselgel HF_{254}" or by locating material in separate "lanes" with a destructive reagent. In specific cases non-destructive fluorescent dyes have been used.

Analytical GLC, unless otherwise stated, was performed on 4′ × 4 mm I.D. packed columns in Pye Argon chromatographs. Stationary phases denoted by the following abbreviations have been used: SE-30 (methylsiloxane polymer); Apiezon L: QF-1 (trifluoropropylmethyl silicone); PEGA (polyethyleneglycol adipate); Carbowax 20M (polyalkyleneglycol, M.W. 20,000); CHDMS (Cyclohexanedimethanol succinate); PVP (polyvinylpyrrolidone); JXR (methyl siloxane polymer); F-60 (chlorophenyl/methylsiloxane polymer); EGSP-Z (ethyleneglycol succinate-phenylsiloxane copolymer); DC 710 (methyl/phenylsiloxane).

M.ps 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. UV spectra were measured on an automatic-recording instrument (Unicam SP 800). Routine IR spectra were measured on a Unicam SP 200 model and high resolution spectra on an 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 a modified Atlas CH_4 instrument (Institute for Lipid Research, Houston) and with an LKB 9000 instrument (Glasgow). Nuclear magnetic resonance (NMR) spectra* were determined on a Perkin–Elmer 60 Mc/s instrument or a Varian HA 100 model equipped with a spin decoupler. Optical rotations, $[\alpha]_D$, were measured in $CHCl_3$ solution unless otherwise stated.

Thanks are due to Mr. J. M. L. Cameron and his assistants for microanalyses, Mrs. F. Lawrie and Miss A. M Robertson (IR), Mr J Gall (NMR), Miss H. Humphrys and Miss J. Malcolm (GC-MS) and Dr. J. Martin (mass spectra, MS-9).

Technical assistance in the extraction of *Petasites hybridus* rhizomes was provided by Mr. G. Milmine and his staff.

Extraction of Warburgia ugandensis heartwood: Chromatography of extracts

Dried powdered heartwood of *Warburgia ugandensis* (2·1 kg) was extracted at room temp for 3 days with pet ether (8 l.). 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. 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–pet ether (1:1), drimenol (I) crystallized (1·2 g) and was purified by crystallization from pet ether and vacuum sublimation to m.p. 96–97°, $[\alpha]_D$ −17°. This material was identical by mixed m.p. determination and comparison of IR (KCl disc) and MS with an authentic sample of drimenol.[4]

Crystalline warburgin (350 mg; m.p. 150–159°) separated from the first benzene fractions (25–28). Washing with pet ether, crystallization from MeOH and vacuum sublimation gave pale yellow prisms (m.p. 159–161°, $[\alpha]_D$ +120°. (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 pet ether afforded warburgiadione (320 mg; m.p. 110–130°) recrystallized from MeOH to yield yellow prisms, m.p. 127–128°, $[\alpha]_D$ +25°. (Found: C, 77·96; H, 7·73. $C_{15}H_{18}O_2$ requires: C, 78·23; H, 7·88%).

Extraction of the wood was completed by treatment with 15 l. of EtOH at room temp 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 $CHCl_3$, leaving a brown solid residue (44 g), which was not further examined. The $CHCl_3$ extract was washed with water, dried and 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. The mother liquors from chromatography of A concentrated in warburgin and warburgiadione, were combined with extract B for 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. Warburgin (2·50 g, m.p. 145–160° after washing with pet ether) crystallized from the benzene–pet ether (1:1) fractions. Elution with increasing concentrations of benzene gave semi-crystalline material (12 g) rich in warburgin and warburgiadione.

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

Rechromatography 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 soln of warburgin (40 mg) in EtOH (5 ml) was added 10% KOH aq (5 ml) and the mixture refluxed for 50 min. The solution was made *slightly* acid with dil HCl aq and extracted with ether. The ethereal soln was washed, dried and evaporated, giving non-crystalline material (32 mg). TLC in benzene-dioxan-AcOH (90:25:4) indicated two products [R_f 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 1675 cm^{-1}. Methylation with ethereal diazomethane regenerated warburgin (identified by 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 by TLC (EtOAc–pet ether 3:7). Preparative TLC on a 0·5 mm thick layer of silica gel using a 50 cm development achieved partial separation and allowed isolation of a small amount of the new product (3 mg; II, 4α-CH_3) which was highly unstable to light and air: ν_{max} (CCl_4) 1676 cm^{-1}; λ_{max} (EtOH) 372 mμ (ε, ca. 20,000); MS:M^+ ion *m/e* 272 corresponding to $C_{16}H_{16}O_4$.

Hydrogenation of warburgin

Warburgin (1·05 g) in EtOAc was hydrogenated at room temp and atm press in the presence of 10% Pd-C catalyst (250 mg). After 35 min H_2 uptake had ceased. The colourless solid product tetrahydrowarburgin (IX; 980 mg) was recrystallized from EtOAc to m.p. 172–173·5°; $[\alpha]_D$ +50°; ν_{max} (CCl_4) 1730, 1721 cm^{-1}; λ_{max} (EtOH) 255 mμ (ε, 2650). 60 Mc/s NMR ($CDCl_3$) τ 9·35 (3H), 8·91 (3H, d J = 7 c/s), 6·16 (3H), 2·07 (1H). MS M^+ 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%).

Ketoaldehyde (XII)

A soln of IX (860 mg) in dry THF (20 ml) was added dropwise to a stirred suspension of LAH (150 mg) in THF (30 ml). After 8 hr stirring at room temp the mixture was decomposed with water (100 ml), made just acid with dil HCl aq and extracted with ether (6 × 50 ml). The extract was washed, dried and evaporated to give the semi-solid diol XI (675 mg), which became discoloured in air. TLC indicated a major product (90%) having R_f 0·40 in the solvent system EtOAc-benzene (1:1); minor polar impurities were evident. A sample was purified by preparative TLC as a colourless gum: $[\alpha]_D$ +62°; ν_{max} (CCl_4) 3624, 3615 cm^{-1}; ν_{max} (liquid film) 1570 cm^{-1} (furan). 60 Mc/s NMR ($CDCl_3$) τ 9·12 (3H), 8·90 (3H, d, J = 7 c/s) 6·15 (1H, m), 5·52 (2H), 2·7 (furan C<u>H</u> obscured by C<u>H</u>Cl_3). MS M^+ ion *m/e* 250 (32%), base peak *m/e* 124. A satisfactory analysis was not obtained.

To a soln of the crude diol XI (500 mg) in pyridine (10 ml) was added slowly a suspension prepared from CrO_3 (1·30 g) and pyridine (12 ml). The mixture was stirred for 18 hr at room temp and then filtered. The residue was washed with pyridine. The combined pyridine solns were added to water (100 ml) and extracted with ether. The extract was washed carefully with dil HCl aq and with water, dried, and evaporated to give colourless XII (380 mg) m.p. 123–133°. Crystallization from EtOAc gave material, m.p. 133–134°; $[\alpha]_D$ +58°; ν_{max} (CCl_4) 2724, 1718, 1690 cm^{-1}; λ_{max} (EtOH) 272 mμ (ε, 2600): 60 Mc/s NMR ($CDCl_3$) τ 9·33 (3H), 8·91 (3H, d, J = 7 c/s) 2·03 (1H), 0·01 (1H). MS M^+ 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 (XIV)

Ketoaldehyde XII (140 mg) was heated for 1 hr at 100° in ethylene glycol (8 ml) with 90% hydrazine hydrate (1 ml). KOH (1·6 g) was dissolved by warming in ethylene glycol (8 ml) and added to the ketoaldehyde hydrazone soln. The mixture was heated under reflux for 3 hr at bath temp 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 temp) for 8 hr. The reaction mixture and distillate 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 0·70, and a more polar component of R_f 0·40. Preparative TLC gave XIV (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 (X). It was however distinguished by GLC. [10% Apiezon L at 175°; gas flow 40 ml/min: (X), t_R 23·0 min; (XIV), 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 (XIV) 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/s NMR (CCl_4) τ, 9·32 (3H), 9·07 (3H, distorted d), 8·13 (3H, d J = 1·5 c/s) 3·05 (1H, m). MS (Atlas CH_4, admission via 6' 1% SE-30 at 125°) M^+ ion ($C_{15}H_{22}O$) *m/e* 218 (40%); 122 (3%), 108 (base peak).

The IR and MS of *cis*-furanoeremophilane (X) were recorded under the same conditions as for the *trans*-isomer. 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). MS M^+ ion *m/e* 218 (23%); 122 (36%), 108 (base peak).

Hydrogenation of furanoeremophilanes

Hydrogenation of XIV (10 mg) in AcOH for 12 hr in the presence of PtO_2 catalyst (15 mg) afforded a mixture of products as judged by GLC. Examination by GC-MS (Table 1) showed the two major products to be isomeric tetrahydrofuranoeremophilanes, distinguishable from an authentic sample of XVI both by GLC and GC-MS. At least two minor products proved to be eremophilanes (GC-MS).

cis-Furanoeremophilane (X) was hydrogenated similarly. As previously reported, the major product (96%) was the tetrahydrofuranoeremophilane (XVI), the minor component comprising a mixture of eremophilanes. In addition we detected approximately 2% of a component which appeared to be a further isomer of XVI.

Furanoketone (XVII)

Treatment of XII (31 mg) with approximately 1·1 moles of ethanedithiol (13 mg) and BF_3-etherate (4 drops) in dry ether for 2 hr at room temp gave a quantitative conversion to a less polar product. NaOH aq (1%; 10 ml) was added to the reaction mixture which was then extracted with ether and the extract washed (water) and dried. Evaporation gave an oil (35 mg) which was purified by preparative TLC (EtOAc–benzene 1:1) and obtained as a colourless gum [(XVIII); 32 mg]: ν_{max} (CCl_4), 1718 cm^{-1}, 60 Mc/s NMR (CCl_4) τ·9·35 (3H), 8·99 (3H, d, J = 7 c/s), 6·72 (4H), 4·58 (1H), 2·79 (1H). MS M^+ ion ($C_{17}H_{22}O_2S_2$) *m/e* 322 (base peak) This thioacetal XVIII (27 mg) was dissolved in dioxan (5 ml); a suspension of freshly prepared Raney Ni was added in dioxan (0·5 ml) and the mixture refluxed for 30 min. The Raney Ni was filtered off and washed with dioxan. The combined solution was evaporated to dryness. TLC in EtOAc–benzene (1:19) indicated a major product (R_f 0·30) with traces of starting material (R_f 0·20). Preparative TLC gave XVII (8 mg) as an oil, which decomposed in air on standing: ν_{max} (KCl) 1711, 1650, 1563 (w) cm^{-1}. 60 Mc/s NMR (CCl_4) τ 9·36 (3H), 9·01 (3H, d, J = 7 c/s), 8·12 (3H, d, J = 1·5 c/s), 3·03 (1H, m). MS M^+ ion ($C_{15}H_{20}O_2$) *m/e* 232 (39%), 108 (base peak).

Comparison of these data and of GLC behaviour on the phases 1% SE-30, 1% QF-1, 10% Apiezon L, 7% F-60–1% EGSP-Z and 2% Carbowax 20M proved the identity of our product with furanoligularanone.[24] "Seeding" with the authentic material and trituration with pet ether gave a solid. Vacuum sublimation gave material, m.p. 84–88°, undepressed when mixed with furanoligularanone.

ORD data provided further proof of identity (Table 2).

Furanoketoalcohols (XXV and XXVI)

Furanopetasol (XXIV R = H) (1·01 g) in $CHCl_3$ (150 ml) was shaken for 24 hr at room temp with MnO_2 (10·0 g). The MnO_2 was filtered off and washed with $CHCl_3$.[36] Evaporation of the solvent gave XXV (960 mg). No starting material was recovered as judged by TLC. Crystallization from EtOAc failed to produce a sharp m.p. (180–188°); ν_{max} (CCl_4) 3621 and 1680 cm^{-1}; ν_{max} (Nujol) 3200 (w) and 1535 cm^{-1} (furan) λ_{max} (EtOH) 281 mμ (ε, 16,000); MS M^+ ion *m/e* 248 (12%), base peak 163. (Found: C, 72·55; H, 7·86. Calc. for $C_{15}H_{20}O_3$: C, 72·55; H, 8·12%).

Furanoketoalcohol XXV (500 mg) was refluxed for 1 hr in a soln of NaOH (2 g) in EtOH (50 ml). The soln was concentrated by evaporation, added to water (50 ml) and extracted with ether. The ether extract was washed, dried and evaporated to give crystalline epimeric XXVI (410 mg); recrystallized from EtOAc to m.p. 170–171°; ν_{max} (CCl_4) 3621 and 1689 cm^{-1}; λ_{max} (EtOH) 281 mμ (ε, 16,000); MS M^+ 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%).

Satisfactory separation of the epimeric furanoketoalcohol was achieved by TLC using multiple development. GLC analysis of mixtures was complicated by a degree of *cis- trans*-equilibration: for example, on 5% Apiezon L at 200°, injection of pure *cis*-isomer afforded an approximately 1:1 mixture of *cis*- and *trans*-

isomers (confirmed by GC-MS): also, the peak observed after injection of pure *trans*-isomer showed a shoulder corresponding to the retention time of the *cis*-isomer.

It was also observed that melted *cis*-isomer on a soda glass slide on the Kofler hot stage, left at 200° for 2 hr, then recovered and examined by GLC and TLC, showed substantial epimerisation. The formation of the *trans*-isomer was confirmed by GC-MS.

Furanodiketones (XXVII *and* XXVIII)

Furanoketoalcohol XXVI (100 mg) was dissolved in acetone (10 ml) and treated with a slight excess of CrO_3 in H_2SO_4 (Jones' reagent)[37] at 0° for 10 min. 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 XXVIII (96 mg, m.p. 100–112°) recrystallized from EtOAc to m.p. 111–112°; ν_{max} (Nujol) 1710, 1675 and 1540 cm^{-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 XXVII was prepared in a similar manner from XXV and had m.p. 145–149° on recrystallization from EtOAc. 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 Heating on the Kofler hot stage produced enhancement of proportion of the *trans*-isomer as judged by GLC.

Huang–Minlon reduction of furanodiketone (XXVIII)

Furanodiketone XXVIII (12 mg) was heated at 80° for 1 hr in ethylene glycol (2 ml) with 90% hydrazine hydrate (0·3 ml). KOH (150 mg) was added in ethylene glycol (1·5 ml), the mixture refluxed at 180° for 3 hr, concentrated by distillation to b.p. 195° and refluxed for a further 8 hr. On working up as previously described (in the prep of XIV) an oil (10 mg) was recovered. Preparative TLC in EtOAc–pet ether (1:19) gave 6 mg of a mixture of XIV and X. 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 ft, 2% JXR column at 175° when, despite incomplete resolution, scanning through the distorted peak gave mass spectra which indicated XIV and X as the only products.

Furanoeremophilane (XIV) *via thioketal* (XXIX)

Furanoketoalcohol XXVI (180 mg) was dissolved in dry ether (10 ml) together with ethanedithiol (2 ml). Redistilled BF_3-etherate (0·3 ml) was added and the mixture refluxed under N_2 for 36 hr. (Traces of O_2 present in the N_2 supply were removed using Fieser's soln.[38] The reaction mixture was diluted with ether (20 ml); 4% NaOH aq (30 ml) was added and the mixture allowed to stand for 3 hr, after which the layers were separated. The ether layer was washed twice with 4% NaOH aq and with water to neutrality, dried and evaporated to give a colourless oil (150 mg). Preparative TLC (MeOH–$CHCl_3$ 3:97) gave XXIX as a colourless solid m.p. 66–72° (45 mg). Impure starting material (70 mg) was also recovered. The product (XXIX) had ν_{max} (Nujol), 3500 (broad band), 1570 cm^{-1} (furan); λ_{max} (EtOH) 239 mμ (ε, 8,600); 60 Mc/s NMR ($CDCl_3$) τ 9·19 (3H), 9·05 (3H, distorted d), 8·38 (O<u>H</u>), 8·14 (3H, d, *J* ca. 2 c/s), 6·2–6·8 (5H, m, superposition of S C<u>H</u>$_2$ C<u>H</u>$_2$–S– and C<u>H</u>–OH), 2·91 (1H, m). MS M^+ 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 development in three separate solvent systems and to GLC on the phases 1% SE-30 (200°), 1% QF-1 (200°), 1% Apiezon L (200°) and 1% CHDMS-2% PVP (225°).

Thioketal XXIX (35 mg) was dissolved in dry pyridine (2 ml) and a suspension prepared from CrO_3 (150 mg) in dry pyridine (1 ml) was added. The mixture was left at room temp under O_2-free N_2 for 14 hr, at which time TLC indicated complete reaction to the ketone. The chromium salts were filtered off and washed with ether–pet ether (1:1). The combined filtered soln was diluted with water (10 ml) and the organic layer separated. The aqueous pyridine layer was extracted with ether–pet ether (1:1). The extract was washed with water to remove all traces of pyridine (washing with dil acid caused decomposition) dried and the solvent evaporated. The semi-solid residue (29 mg) was purified further by preparative TLC (MeOH–$CHCl_3$ 1:99) and by crystallization from EtOAc–pet ether to m.p. 145–150° (10 mg); ν_{max} (Nujol) 1710 cm^{-1}; λ_{max} (EtOH) 239 mμ (ε, 8300); MS M^+ ion *m/e* 322 (60%) 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%).

The thioketalketone (7 mg) was dissolved in dry ether (2 ml) together with ethanedithiol (0·1 ml) and freshly distilled BF_3-etherate (0·1 ml). The soln was left for 14 hr at room temp under O_2-free N_2. The reaction mixture was diluted with ether, and excess ethanedithiol was removed by washing with 4% NaOH aq. 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 characterized by MS M^+ ion *m/e* 398 (38%) ($C_{19}H_{26}OS_4$) base peak *m/e* 61.

Bis-thioketal XXX (4 mg) was dissolved in dioxan (1 ml) and added to a suspension of Raney Ni in dioxan (0·5 ml). The mixture was refluxed for 30 min, cooled and filtered. The Raney Ni residue was washed with dioxan (1 ml). The dioxan soln was concentrated and the residue was taken up in benzene–water (2:1; 10 ml): the benzene layer was separated, washed, dried and evaporated, leaving an oil (3 mg). GLC and GC-MS led to the conclusion that XIV accounted for 80% of this product. GC-MS using 1% JXR at 125° and 10% Carbowax 20M at 150° confirmed this assignment and indicated that the remaining components comprised mainly dehydro-derivatives (M.W. 216) and dihydro-derivatives (M.W. 220).

Reduction of warburgiadione

Warburgiadione (55 mg) in EtOAc (10 ml) was hydrogenated for 30 min with 10% Pd-C catalyst (20 mg). A colourless, fragrant oil XXXIII (50 mg) was recovered. Micro-distillation at 110–120°/0·2 mm gave an oil (40 mg): ν_{max} (CCl_4) 1716 cm^{-1} (ε^e ca. 800). (Found: C, 76·16; H, 10·29. $C_{15}H_{24}O_2$ requires: C, 76·23; H, 10·24%). TLC with EtOAc–pet ether (1:1) and GLC (1% SE-30 and 5% QF-1) indicated two products in a ratio of 3:2. GC-MS via a 10 ft. 1% QF-1 column confirmed that these were isomeric: major component, M^+ ion 236 (77%), base peak 194; minor component M^+ ion *m/e* 236 (35%), base peak 122.

Without separation, diketone isomer mixture XXXIII (30 mg) was dissolved in ethylene glycol (3 ml) with 90% hydrazine hydrate (0·3 ml) and heated at 60° for 2 hr. KOH (300 mg) in ethylene glycol (3 ml) was added and the soln heated at 180° (bath temp) for 6 hr then concentrated by distillation at 195–200° during 3 hr. The distillate was combined with the bulk of the soln, diluted with pet ether (20 ml) and water (50 ml) and the layers separated. The organic extract was washed, dried and evaporated at room temp. This total material (25 mg) was chromatographed on alumina (grade II; 0·5 g) and two fractions were taken. A hydrocarbon fraction (6 mg, eluted with pet. ether) was separated from ketonic polar material (11 mg, eluted with ether). The hydrocarbon fraction, apparently homogeneous on GLC (1% SE-30) was most probably a mixture of isomeric eremophilanes. GC-MS of the unresolved peak: M^+ ion ($C_{15}H_{28}$), *m/e* 208 (70%), base peak 165.

Extraction of the rhizomes of Petasites hybridus L.

Isolation of isopetasin. Fresh rhizomes (6·5 kg) of *P. hybridus* L collected near Motherwell, Lanarkshire were milled in EtOH and allowed to stand for 6 days at room temp in EtOH (30 l.). The solvent was evaporated and the residue was shaken with $CHCl_3$ (2·0 l.) and water (500 ml). The organic layer was separated, washed with water (250 ml), dried and the solvent evaporated to give a brown oil (46 g). The total oil was chromatographed on commercial grade chromatographic silica (1800 g) and 34 fractions of 500 ml were taken using a gradient elution with pet ether–EtOAc. Chromatography was directed specifically at the isolation of XXXVII and XXXIX, 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 EtOAc–pet ether (1:4). A portion (5·0 g) of these combined fractions was taken up in pet ether and allowed to stand over alumina grade I; 100 g) on a column for 24 hr. Elution with ether afforded crude semi-solid isopetasin (3·1 g) recrystallized from MeOH to m.p. 90–94° (1·20 g). An analytical sample from MeOH had m.p. 95–98°; $[\alpha]_D$ +26°; ν_{max} (CCl_4) 1716, 1667, 1630 cm^{-1}; λ_{max} (EtOH) 242 mμ (ε, 15,500), 280 mμ (ε, 7900); λ_{max} (cyclohexane) 228 mμ (ε 17,900), 269 mμ (ε, 7,200). (Found: C, 75·97; H, 8·90. Calc. for $C_{20}H_{28}O_3$: C, 75·91; H, 8·92%). These data are in good agreement with those of Aebi and Waaler.[28]

Isopetasol (XLIII)

Isopetasin (1·0 g) was dissolved in EtOH (20 ml) and 3% KOH aq (20 ml) was added. The soln was refluxed for 2 hr, concentrated by evaporation, diluted with water (100 ml) and extracted with ether. The extract was washed, dried and evaporated to give a semi-solid (702 mg). This product was recrystallized from EtOAc–ether to give XLIII m.p. 126–127°; $[\alpha]_D$ +105°; ν_{max} (CCl_4) 3634, 1669, 1630 cm^{-1}; λ_{max} (EtOH) 246 mμ (ε, 12,900), 280 mμ (ε, 7700). (Found: C, 76·88; H, 9·52. Calc. for $C_{15}H_{22}O_2$: C, 76·88; H, 9·46%).

Isopetasone (XLIV)

Isopetasol (XLIII; 380 mg) was dissolved in acetone (20 ml). Jones' reagent[37] (1·0 ml) was added slowly

with stirring over 10 min at 0°. After 15 min the reaction mixture was diluted with water (50 ml) and extracted with ether. The extract was washed, dried and evaporated to give a solid product (340 mg). Recrystallization from MeOH gave XLIV: m.p. 113–115°; $[\alpha]_D$ +31°; ν_{max} (CCl_4) 1724, 1669, 1630 cm^{-1}; λ_{max} (EtOH) 244 mμ (ε, 12,300), 281 mμ (ε, 7000). (Found: C, 77·57; H, 8·55. Calc. for $C_{15}H_{20}O_2$: C, 77·55; H, 8·68%).

D.D.Q. dehydrogenation of isopetasone (XLIV) *to warburgiadione* (III)

Isopetasone (XLIV; 50 mg) was dissolved in dioxan (5 ml) with 2,3-dichloro-5,6-dicyanobenzoquinone (D.D.Q.) (ca. 1·1 mole, 55 mg) and heated under O_2-free N_2 at 110° bath temp for 10 hr. The reaction mixture was cooled, filtered and evaporated. 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 indicated a 3:2 ratio of III to XLIV. The two compounds were separated by preparative TLC with EtOAc–pet ether (3:7), and crude warburgiadione was obtained as a solid (15 mg) m.p. 105–120°. Recrystallization from MeOH gave 9 mg m.p. 122–126, $[\alpha]_D$ +29°. This material was proved to be identical with natural warburgiadione by mixed m.p.; also be comparison of IR (KCl disc), UV and mass spectra and of GLC data on the phases 1% QF-1, 1% SE-30 and 1% CHDMS–2% PVP.

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