Some Aspects of Natural Product Chemistry

Thesis

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by

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Part 3. *In vitro* synthesis of colchicine

Summary

The chemistry of some natural products has been investigated with particular reference to their biogenesis.

Part 1(a) - Various theories on the stereochemistry of diterpene biogenesis are reviewed and the theory is advanced that all diterpenes arise from the *trans-anti* cyclisation of a geranyl-geraniol precursor. A number of diterpenes whose stereochemistry does not comply with this theory are listed and the evidence for the assignment of the respective stereochemistries is critically appraised. The structure and stereochemistry of two of the apparent exceptions to the rule, gibberellic acid and cafestol, is unambiguously defined by X-ray structural analysis and circular dichroism studies. This is the first use of circular dichroism in structural organic chemistry. The new stereochemical assignments are in full agreement with biogenesis by *trans-anti* cyclisation of a geranyl-geraniol precursor.

Part 1(b) - The mould *Trichothecium Roseum* was grown and four new metabolites were isolated from it. Two of the metabolites were liquid esters with molecular weights of 258 and 342 respectively and a third metabolite was an amide $C_{32}H_{46}O_{10}N_4$, m.pt. = 271-274°C, $[\alpha]_D = +5.56^{\circ}$. The fourth metabolite was an acid, $C_{20}H_{30}O_3$, and a working structure is suggested for this compound.

The structure and absolute stereochemistry of the metabolite rosololactone is defined by chemical and X-ray studies.

Part 2 - The biogenesis of cholesterol and methods for functionalising inert methyl groups are reviewed, and an attempt to remove the 14α-methyl group from lanosterol is described. The removal of this methyl group was not achieved, but a series of interesting compounds was obtained. Evidence for the structures of these compounds is presented.

Part 3 - Hypotheses for the biogenesis of colchicine are reviewed and a synthesis of colchicine is based on one of these. The bicyclic precursor, 1-(3, 4, 5 trihydroxyphenyl)-3-(β-tropolonyl)-propane, was prepared and ring closure to the tricyclic system by phenolic oxidative coupling was attempted. The ring closure was successful but over-oxidation of the pyrogallol moiety to a pyrone resulted.

The tricyclic compound which would be expected from the oxidative coupling reaction was prepared by degradation of colchicine, and this was successfully converted to desacetylamidocolchiceine from which colchicine had been synthesised by previous workers. The total synthesis of the alkaloid is, therefore, but one step from completion and the oxidation of the bicyclic precursor to a tricyclic pyrone has provided an *in vitro* analogy for the biogenetic hypothesis.

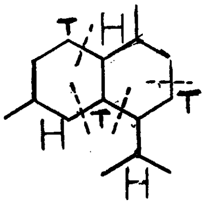
Part 1

Studies in Diterpene Chemistry

(a) Stereochemical implications of diterpene biogenesis

(b) Metabolites of the mould Trichothecium Roseum

Nomenclature: Steroid Numbering is used throughout the present work unless otherwise indicated.



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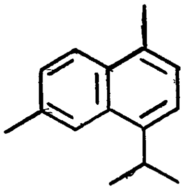

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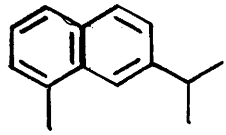

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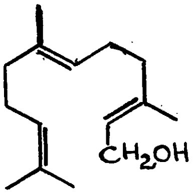

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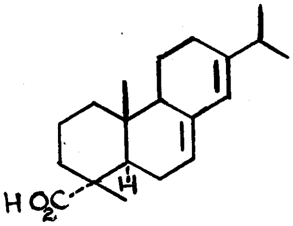

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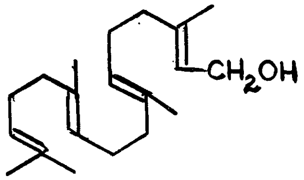

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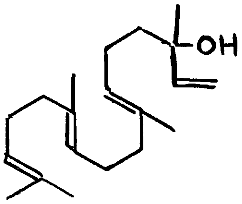

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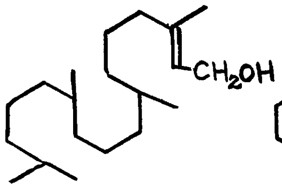

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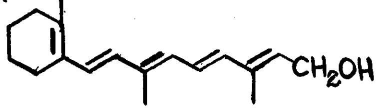

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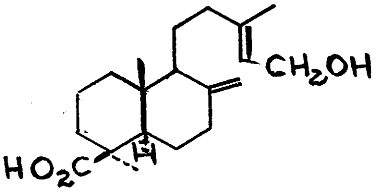

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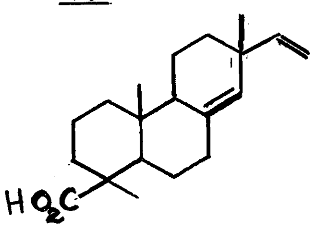

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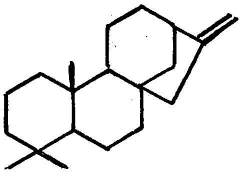

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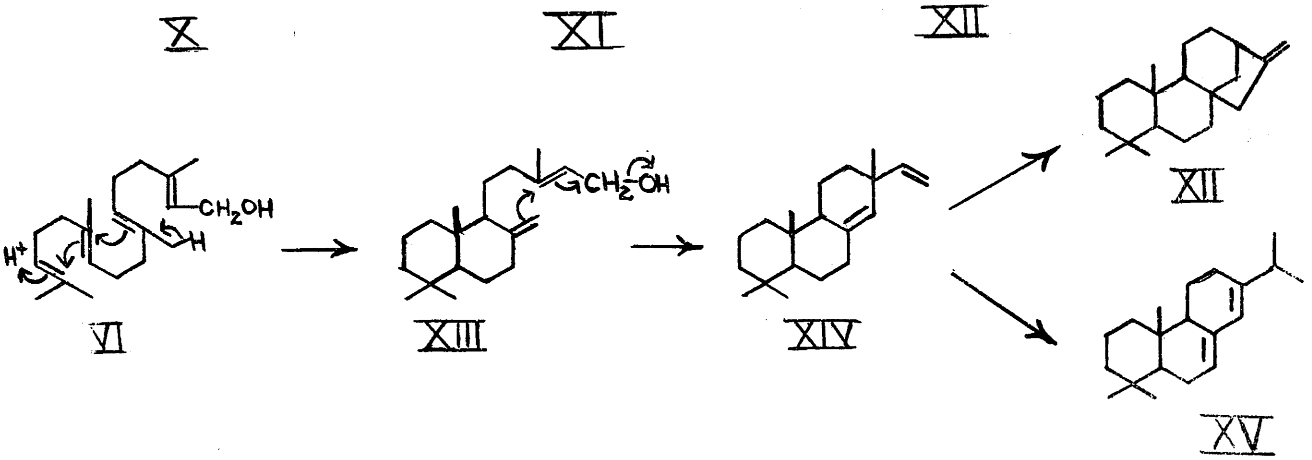


The Stereochemical Implications of Diterpene Biosynthesis

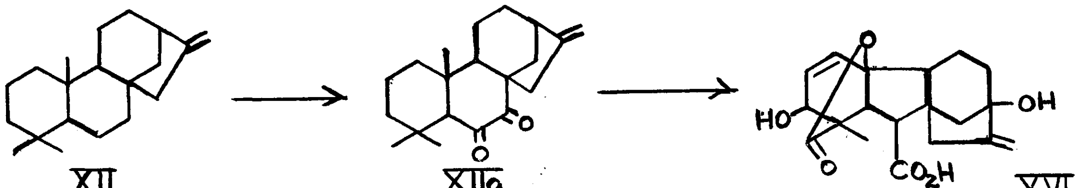
Historical

The first observation of any structural similarity in terpenes was made by Ruzicka who, having found monoterpenes which were not related to para-cymene(1), proposed the classical isoprene rule. This stated that terpenes were built up from isoprene units joined head to tail as in I. By using this hypothesis he was able to propose structures for cadalene (II) and eudalene (III), the hydrocarbons derived from sulphur dehydrogenation of some sesquiterpenes(2). He then verified these structures by synthesis(3)(4). In time the isoprene rule became the more sophisticated biogenetic isoprene rule, and it was held that sesquiterpenes were derived from farnesol(IV) by cyclisations and rearrangements(5)(6).

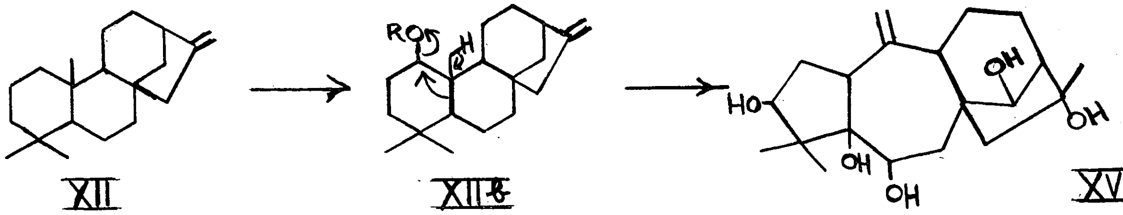
Concurrent with the work on sesquiterpenes was the elucidation of the structure of the diterpene abietic acid (V). Using a similar approach(7) the complete structure and configuration of the acid followed(8)(9). With the help of the isoprene rule, the structures of a large number of diterpenes have been found, and the simplest of these are classed as acyclic, mono-, di-, tri-, and tetracyclic diterpenes, derived directly from a geranyl-geraniol (VI) or a geranyl-linalool (VII) precursor. Examples of these groups are phytol (VIII)(10), vitamin A (IX)(11), agathic acid (X)(12), pimaric acid (XI)(13)(14), and phyllocladene(XII)(15) respectively. Geranyl-linalool has been found in nature(16), and both it and geranyl-geraniol have been synthesised(17).

The cyclisation of the geranyl-geraniol precursor may be written as VI → XIII → XIV → XII and XV as shown opposite.

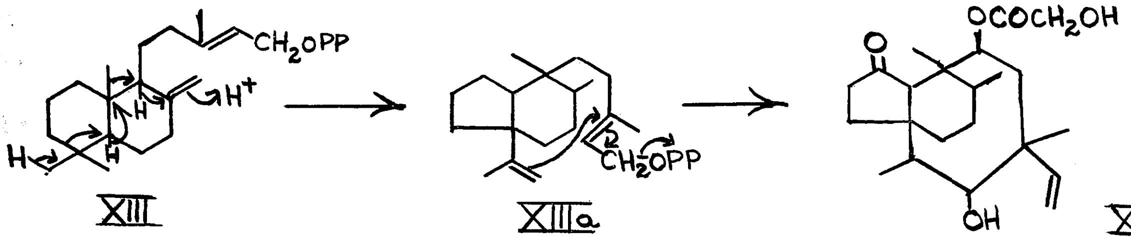
Several diterpenes are not derived directly from this sequence,



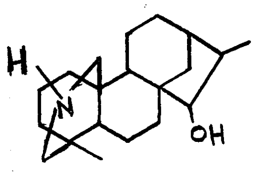
XII XIIa XVI



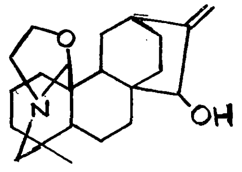
XII XIIb XVII



XIII XIIIa XVIII



XIX



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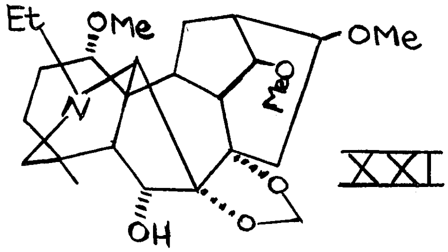
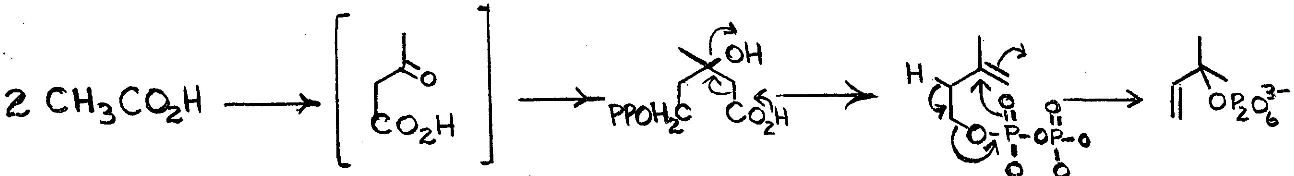
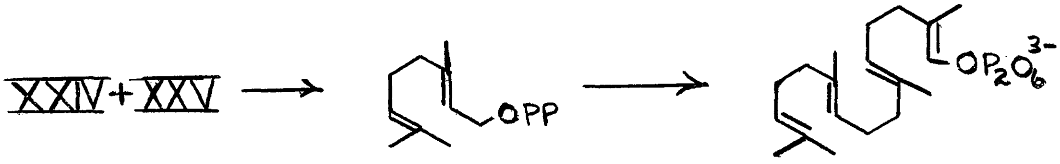


XXI



XXII XXIIa XXIII XXIV XXV



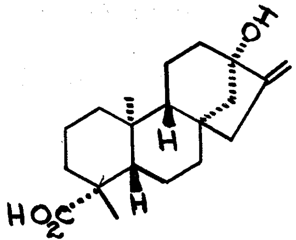
XXVI VI

but these can be explained very readily by a series of rearrangements. Gibberellic acid (XVI)(18), grayanotoxins (XVII)(19), and the recently discovered pleuromutilin (XVIII)(20)(21) are examples of this type of diterpene, and they can all be derived from the precursors XII or XIII by the rearrangements XII → XIIa → XVI, XII → XIIb → XVII, and XIII → XIIIa → XVIII respectively.

Many alkaloids with a diterpene skeleton are known(22), and a model synthesis from the diterpene skeleton has been achieved by azide photolysis(23). The garrya alkaloids such as XIX can be derived directly from a precursor in the Ruzicka scheme, while others such as atisine (XX)(24)(25), and delphinine (XXI)(26) require rearrangement of these precursors.

The wide applicability of Ruzicka's scheme is evident, and work on the biosynthesis of steroids (see p. 62) and mould metabolites(27)(28) has put the isoprene rule on a firm footing. This work, some of which will be detailed elsewhere in the present thesis, has translated Ruzicka's empirical predictions into the scheme XXII → XXIII → XXIV → XXV → XXVI → VI etc.

Acceptance of the simple structural biogenetic isoprene rule was followed by speculation on the biogenetic stereochemical relationships of diterpenes. At first it was thought that all diterpenes had the same trans A/B ring junction as was present in triterpenes and steroids(5), as many of these compounds had been interrelated by degradation products which retained the asymmetric centres at C_5 and C_{10}. It was soon evident that this was not a general rule from the increasing



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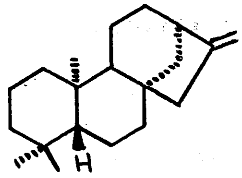

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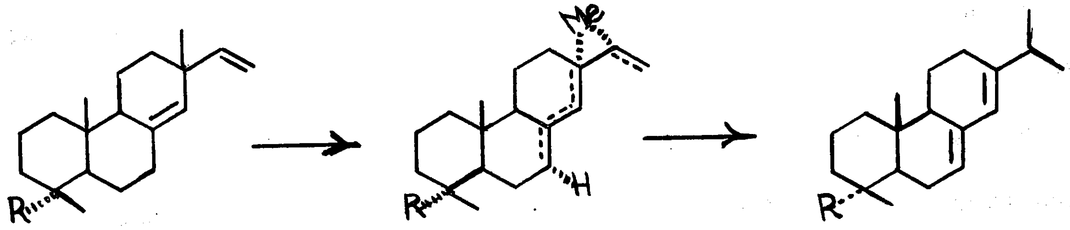
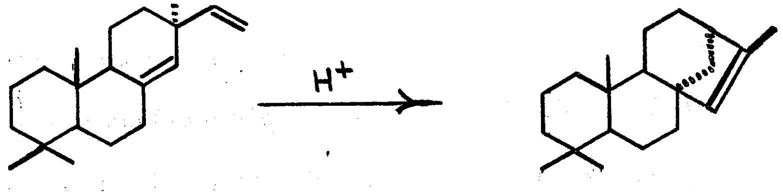


XXIX XXX V

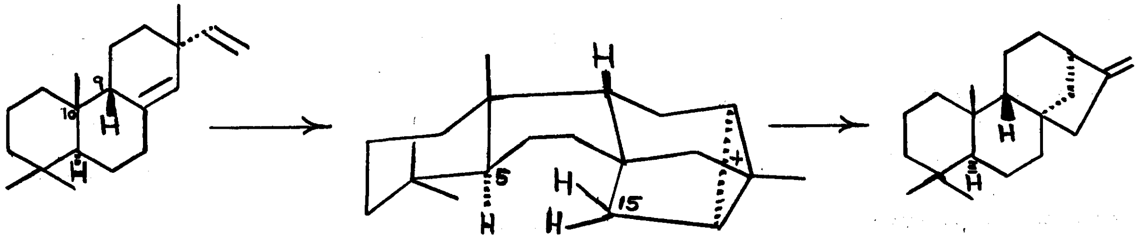


XXXI XXXII

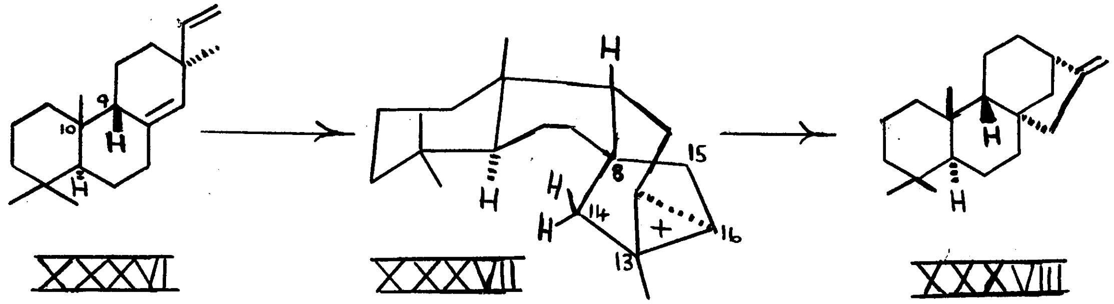
number of diterpenes such as steviol (XXVII)(29)(30) and kaurene XXXVII(30)(31) which had a trans A/B ring junction which was antipodal to that of most steroids and triterpenes.

Djerassi(32) examined those sesqui- and diterpenes with the rare C_3 hydroxyl function, and observed that the antipodal A/B ring junction was always present in such compounds. He predicted that, on this basis, gibberellic acid would have an antipodal A/B ring junction, and suggested that the enzyme system responsible for hydroxyl-cation-initiated ring closure would cause cyclisation to result in antipodal A/B ring junctions.

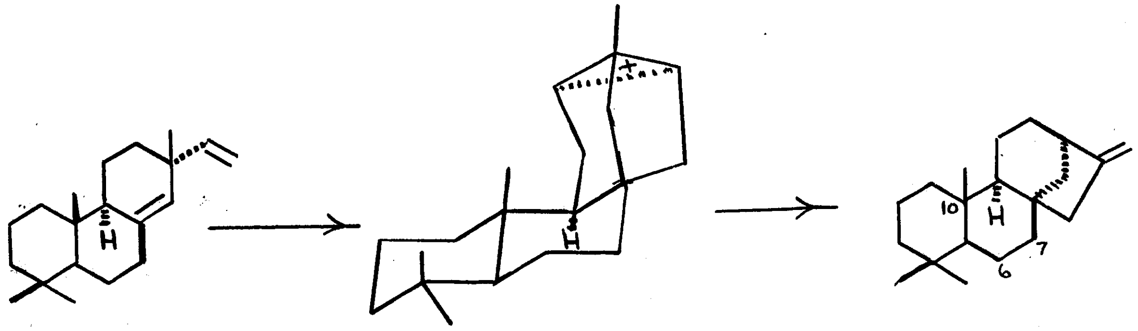
Ruzicka's theories have been developed by Wenkert(33) who suggested a pimaradiene precursor (XXIX) for the tetracyclic diterpenes, and made a series of stereochemical predictions on this basis. To obtain abietic acid (V) from a pimaradiene (XXIX), the methyl group at C_{13} must migrate to the adjacent position on the vinyl grouping. This is facilitated if the transition state XXX can be written and, if ring C is considered rigid, then only that conformation with an axial methyl and an equatorial vinyl group at C_{13} can give rise to such a transition state. Wenkert concluded from this that, since the abietic acid rearrangement was so facile, the only naturally occurring pimaradienes would be those with an equatorial methyl and an axial vinyl group at C_{13}. The axial vinyl group is well positioned for rearrangement of the pimaradienes to tetracyclic diterpenes, and rimuene (XXXI)(31)(34)(35), for example, yields isophyllocladene (XXXII) on treatment with formic acid(31)(33)(35). From these arguments, Wenkert concluded that the stereochemistry of the tetracyclic diterpenes would be as in XXXII.



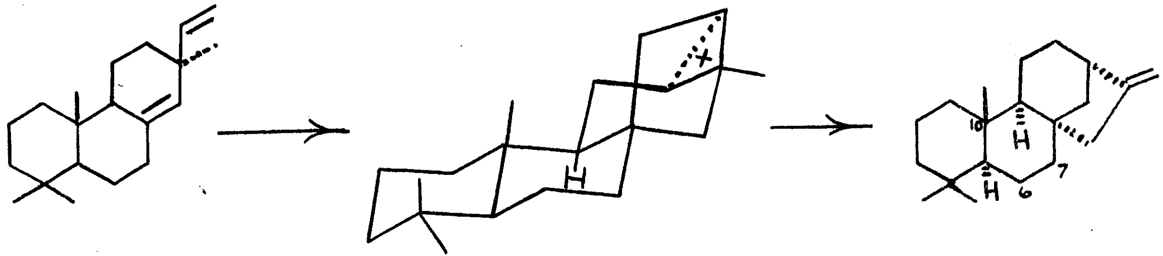
XXXIII XXXIV XXXV



XXXVI XXXVII XXXVIII



XXXIX XL XLI



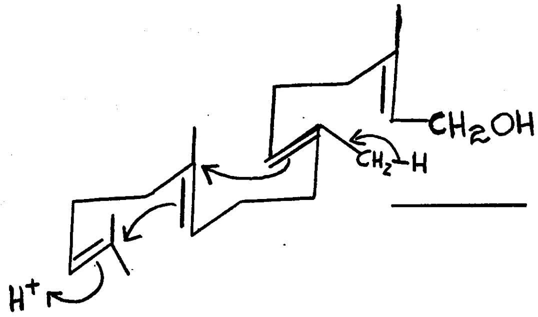
XLII XLIII XLIV

Wenkert(36) has now retracted his theory of axial methyl migration, and allows that the transition state XXX can arise from either C_{13} epimer by virtue of the flexibility of ring C. He and others(36)(37)(38) have shown that both C_{13} epimers of the pimaradienes can exist in nature, and the pimaradiene → abietic rearrangement has been achieved *in vitro* with both epimers(36). These results have invalidated the stereochemical implications of Wenkert's hypothesis.

Whalley(39) has recently proposed a theory to account for the stereochemistry of tetracyclic diterpenes and diterpene alkaloids in terms of their biogenesis from a pimaradiene precursor. He indicated that four pimaradienes were possible(37), two with a C_9-C_{10} *syn* backbone, and two with a C_9-C_{10} *anti* backbone, and considered the rearrangements which would be involved in transforming each of these to the respective tetracyclic compound.

The two *syn* pimaradienes, XXXIII and XXXVI, would give rise to the cations XXXIV and XXXVII respectively. Cation XXXIV is unfavoured due to the strong C_5-C_{15} hydrogen interaction, while cation XXXVII is relatively unstrained since the five membered ring D causes the C_8-C_{14} bond to be bent away from C_5. The tetracyclic diterpenes with a *syn* backbone would thus be expected to have the stereochemistry XXXVIII.

The two *anti* pimaradienes, XXXIX and XLII, give rise to the cations XL and XLIII respectively. Due to relief of strain through buttressing, tetracyclic deterpenes with an *anti* backbone can have the stereochemistry XLI or XLIV. The ring system of diterpene alkaloids does not allow buttressing of the C_{10} methyl group and so cation XL will be the more strained and XLIV will be the expected stereochemistry. Strain in the diterpene alkaloids can be removed when ring B is in the boat conformation, and this is the case in the various diterpene alkaloids which have a C_{10} methyl to C_7 nitrogen or oxygen bridge or carbon-carbon bond.



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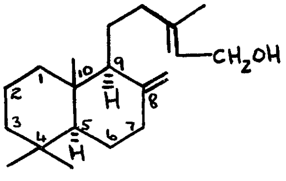

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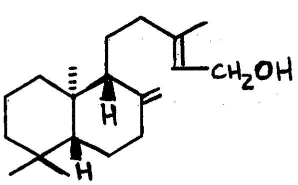

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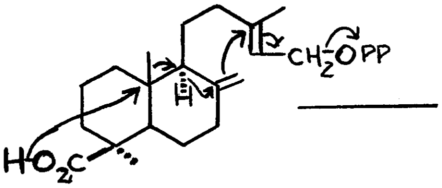

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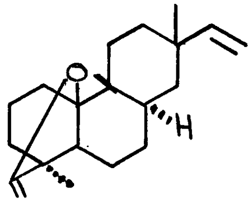

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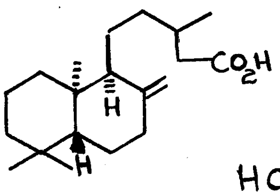
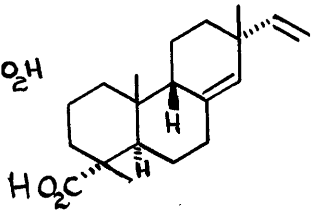


XLVI



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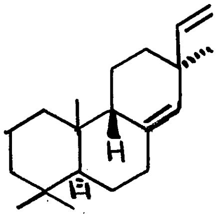

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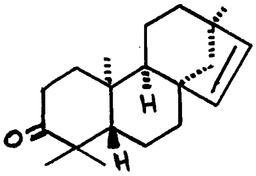

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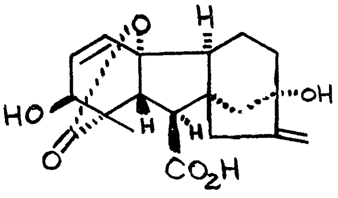

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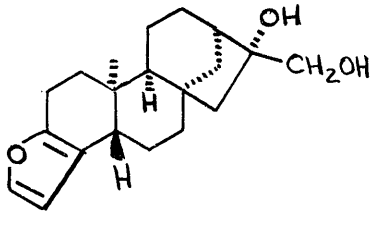
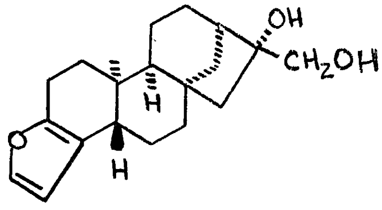


XLIX

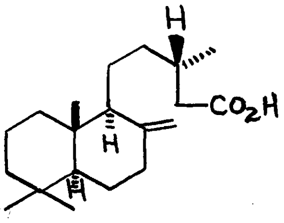


L

Theoretical

In Whalley's theory of diterpene stereochemistry provision was made for a group of diterpenes having a *syn* C_9-C_{10} backbone. We have observed that, apart from a very small group of compounds apparently having such a *syn* backbone, the diterpenes whose absolute configurations are known can be explained on the basis of a synchronous *trans*-*anti* cyclisation of the geranyl-geraniol precursor as shown opposite (VIa→XIII). We now propose that all tri- and tetracyclic diterpenes arise from such a cyclisation and will thus have the C_9-C_{10} stereochemistry present in XIIIa or its mirror image XIIIb. It follows that the stereochemistry of any diterpene with a postulated C_9-C_{10} *syn* backbone has been assigned wrongly, unless this backbone can result from a simple rearrangement of the C_9-C_{10} *anti* precursor XIII. This theory follows the suggestion(40) by Birch that rosenonolactone would have the stereochemistry XLV if it were formed as a result of rearrangement of the *trans*-*anti* precursor XIIIa as shown opposite. This suggestion was made before the finding of several compounds which apparently could not derive from a *trans*-*anti* precursor.

The group of diterpenes containing a C_9-C_{10} *syn* backbone which cannot be explained in terms of the simple skeletal rearrangement of a C_9-C_{10} *anti* precursor includes eperuic acid (XLVI)(41)(42), isopimaric acid (XLVII)(37)(43), rimuene (XXXI)(43), stachenone (XLVIII) and its relatives(44), gibberellic acid (XVIa)(45)(46), cafestol (XLIX)(32)(47), and kahweol (L)(32). We are of the opinion that the stereochemistry of these "exceptions" to a general "*trans*-*anti* precursor rule" has been wrongly assigned, and we have reinvestigated the evidence for



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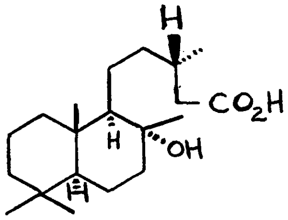

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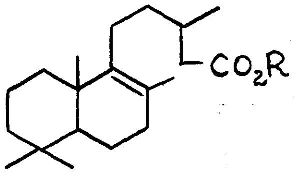

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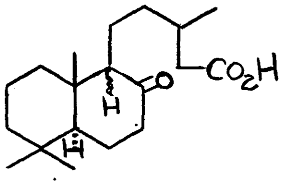
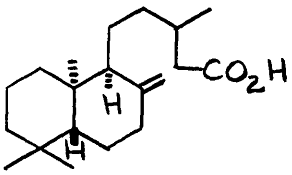
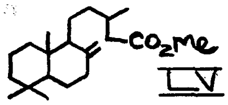
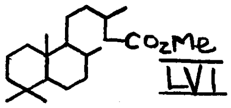
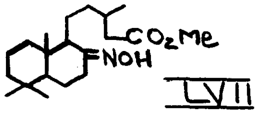
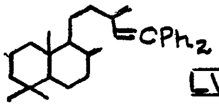
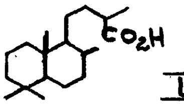
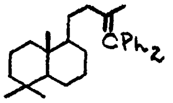
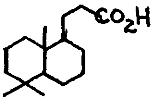


LIV



XLVIa

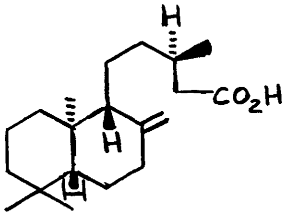
TABLE 1.

	Eperuic Series		Labdanolic Series	
	α_D	m.pt. or n_D	α_D	m.pt. or n_D
 LV	-28.2°	1.4982	+27°	1.4980
 LVI	-26.3°	1.4902	+28°	1.4902
 LVII	-79.4°	223°C	+ 74.5°	188-190°C
 LVIII	-	1.5520	+ 116°	1.5520
 LIX	-	1.5010	+49°	92-93°C
 LX	-24.4°	116°C	+36°	118-120°C
 LXI	-29.9°	134-5°C	+40.5°	141-143°C

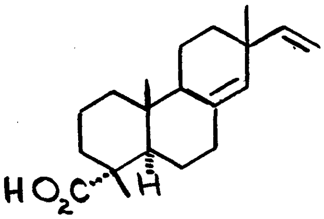
each of these formulations in turn. There were inconsistencies in the stereochemistry of each compound, and we present new and unambiguous evidence for the structure and stereochemistry of gibberellic acid and cafestol.

(1) Eperuic Acid is known to have the same gross structure as the product of dehydration (LI) of labdanolic acid (LII)$^{(42)}$. The compounds LIII derived from each in turn had identical infra-red spectra and rotations of equal magnitude but opposite sign, and so it was possible to state that, with the exception of C_9, eperuic acid was the complete mirror image of the dehydration product of labdanolic acid (LI). From O.R.D. studies of the ketones LIV derived from the eperuic and labdanolic series, Djerassi$^{(41)}$ pointed out that the O.R.D. curves were almost the mirror images which would be expected from complete antipodes. He attributed the very small deviation from antipody to the C_9 asymmetric centre and proposed structure XLVIa for eperuic acid. The divergence from complete antipody was substantiated, in his view, by the difference in melting points of the oximes LVII derived from the two series.

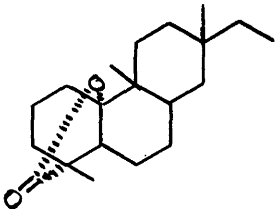
We have observed that the divergence in the two O.R.D. curves as published is not really great enough to warrant the assignment of the C_9 stereochemistry as in XLVIa. Furthermore we have observed that there are too many derivatives of labdanolic and eperuic acids which have nearly the same melting point or refractive index and equal but opposite rotation to be accounted for by mere coincidence. These data are listed in Table 1 opposite$^{(48)}$ and indicate that eperuic acid is the complete mirror image of the dehydration product (LI) of labdanolic acid. Eperuic acid is thus represented by XLVIb and may now be con-



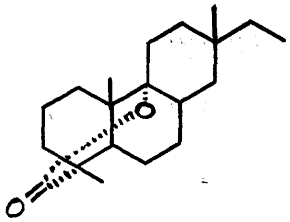
XLVIb



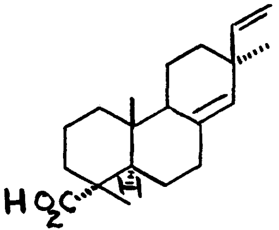
LXII



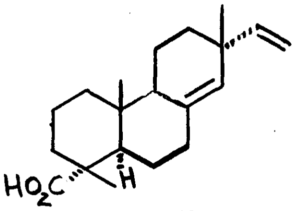
LXIII



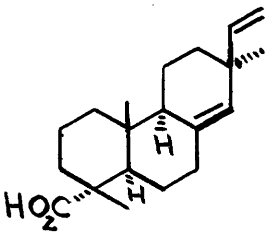
LXIV



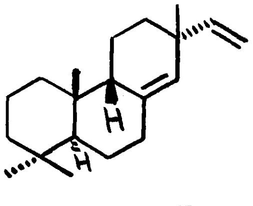
LXIIa



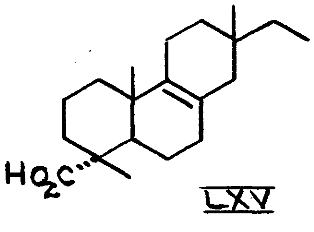
LXIIb



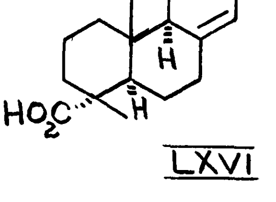
LXIIc



LXVII



LXV

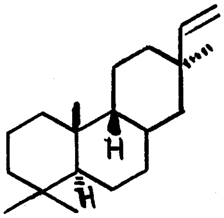


LXVI

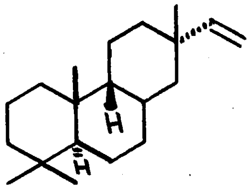
sidered as being derived from a <u>trans-anti</u> precursor.

(2) <u>Isopimaric Acid</u>. Three naturally occurring isomeric pimaric acids are known, pimaric acid, isopimaric acid and sandaracopimaric acid. The earlier workers gave the structure LXII to pimaric and isopimaric acids, with no provision for the stereochemistry at C_9 or C_{13}(13)(14). Wenkert(36) rearranged the dihydropimaric acids to five membered (LXIII) and six membered (LXIV) lactones. Since all of the asymmetric centres in the molecule were involved in the rearrangement except C_1, C_5 and C_{13}, and each acid gave a different set of lactones, the two pimaric acids must be epimeric at C_{13}. From the equilibrium data from these rearrangements, Wenkert suggested LXIIa for pimaric acid and LXIIb for isopimaric acid which was in agreement with surface tension studies(49). Whalley(37) used a similar argument for the C_{13} stereochemistry but suggested that the acids were also epimeric at C_9 since he could rearrange dihydroisopimaric acid to the $\Delta^{8(9)}$ene (LXV) but pimaric acid had not rearranged(50) under similar conditions. He reasoned from this that isopimaric acid would have the strained <u>syn</u> backbone in which strain might be relieved by rearrangement to the $\Delta^{8(9)}$ene, and concluded that LXIIc and XLVII represented pimaric acid and isopimaric acid respectively. Edwards(38) succeeded in isomerising pimaric acid to the Δ^{89} ene (LXV) and so Whalley's arguments have no basis in fact.

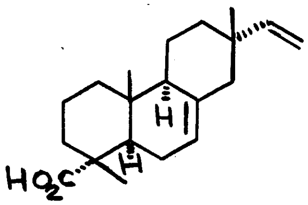
Recently the structure LXVI was assigned to the third acid sandaracopimaric acid(51) and this has been proved by partial synthesis from steroids(52), and by stereospecific synthesis(53). The stereochemistry of pimaric acid has been shown to be LXIIc by stereospecific synthesis(53).



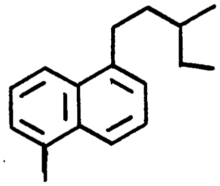
LXVII



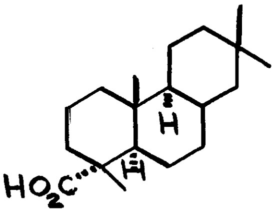
LXVIII



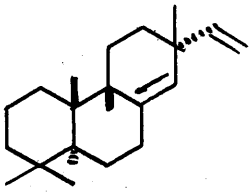
LXIX



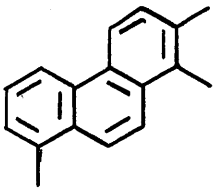
LXX



LXXI



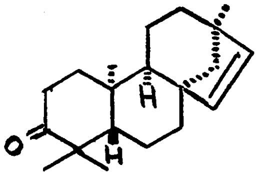
XXXI



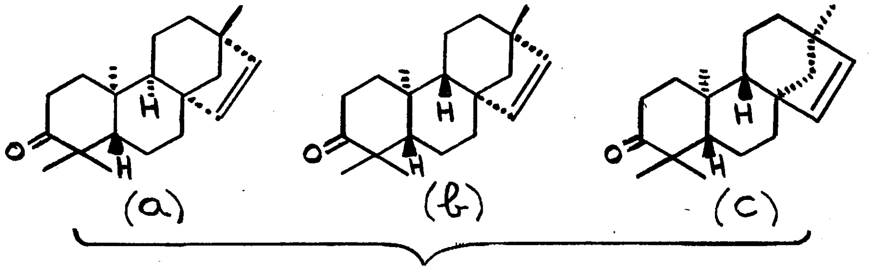
LXXII

In a recent paper, Wenkert(43) accepted Whalley's suggested stereochemistry for isopimaric acid, and advanced some rather tenuous n.m.r. evidence in support of this. Ireland(54) has since completed a stereospecific synthesis of the two C_{13} epimeric pimaradienes LXVII and LXVIII, but neither could be identified with the diene derived from isopimaric acid, and so the suggested structure XLVII is incorrect. In the n.m.r. of isopimaric acid(43), the non-vinyl olefinic proton occurred as a doublet and allylic protons were present. The structure LXIX accounts for this n.m.r. spectrum, and also allows for acid isomerisation to the $\Delta^{8(9)}$ ene (LXV). The compound must undergo olefinic migration, $\Delta^{7} \rightarrow \Delta^{8(14)}$, in one of its degradations to account for the misleading degradation product LXX after rupture of the double bond(14). The C_9 – orientation, and hence the <u>anti</u> backbone has been shown by relation of isopimaric acid to sandaracopimaric acid through the compound LXXI(55).

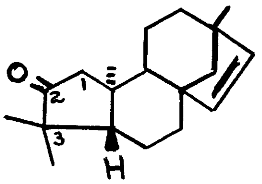
(3) <u>Rimuene</u>. The structure XXXI was assigned to rimuene on rather dubious n.m.r. evidence combined with the fact that it was found impossible to relate the hydrocarbon to any of the pimaradienes derived from the pimaric acids(43). That XXXI is not in fact a representation of rimuene was shown by Ireland's stereospecific synthesis(54) of the two C_{13} epimers of this structure, neither of which corresponded to rimuene in properties. It might be tempting to suggest that rimuene is a double bond isomer of XXXI, in view of the highly split olefinic proton in the n.m.r.(43), but the evidence for a $\Delta^{8(14)}$ double bond is compelling since dihydrorimuene on epoxidation, Grignard treatment and selenium dehydrogenation gave the phenanthrene LXXII(34). This inconsistency might be explained by double bond migration on hydrogenation



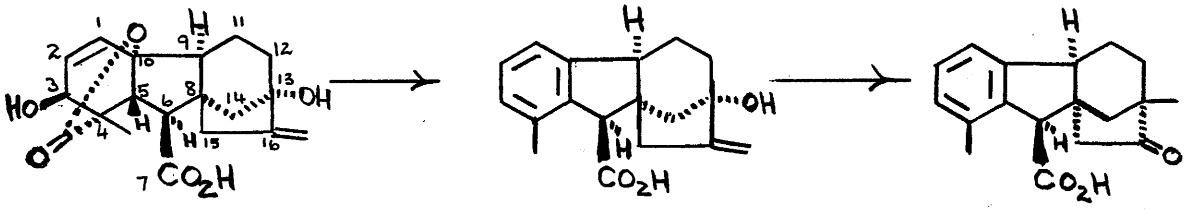
XLVIII



LXXIII



LXXIV



XVIa

LXXV

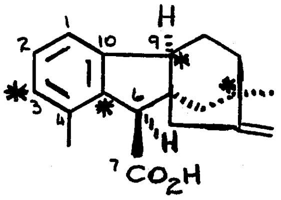
LXXVI

of rimuene and this could be proved by n.m.r. studies. An alternative explanation could be that samples of rimuene may not be homogeneous, as was the case with the diterpene hydrocarbon mirene(56).

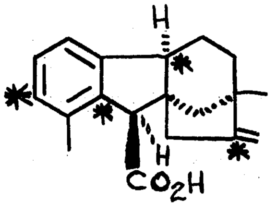
(4) Stachenone and other related compounds have recently been found in Tambooti Wood, and the structure XLVII was assigned to the parent compound(44). Molecular rotation difference comparison has shown the antipodal A/B ring junction, and four possible structures XLVIII, and LXXIIIa to c, were proposed. Since it had been found impossible to oxidise the C_1 position in the *nor*ketone LXXIV, it was considered that C_1 was a very hindered position. The *syn* backbone in XLVIII has considerable hindrance of C_1 from the C_{11} position, and so this structure was preferred. Although it is obvious that XLVIII involves considerable steric hindrance at C_1, this is far from convincing evidence that XLVIII is the correct structure, and we feel that further work on this compound will show that one of the two *trans-anti* isomers LXXIII b or c is the correct structure. Comparative optical rotation studies of a ring D ketone, and *nor*phyllocladenone would be extremely useful in this respect.

(5) Gibberellic Acid has been assigned the gross structure XVI as a result of the studies of the I.C.I. group of Grove, Cross et al.(18), and the stereochemistry XVIa has been presented for this compound(45)(46).

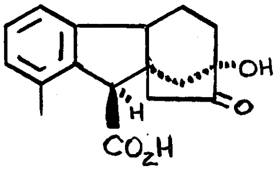
When treated with mineral acid, gibberellic acid yielded allogibberic acid (LXXV) which could be rearranged to gibberic acid (LXXVI). Birch(57) has shown that the C_{12}-C_{13} bond migrates in this rearrangement by feeding $[4\text{-}C^{14}]$-mevalonic acid to *G.fujikuroi* and



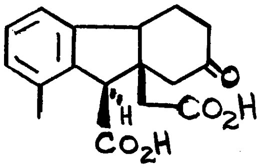
LXXV



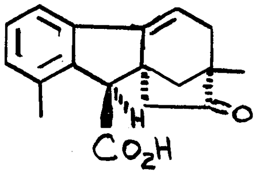
LXXVI



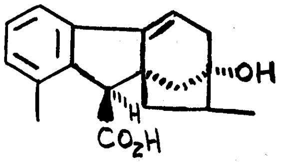
LXXVII



LXXVIII



LXXIX

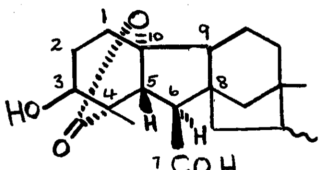


LXXX

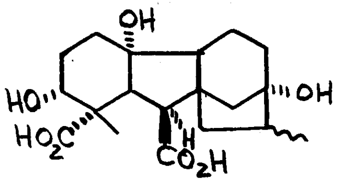
obtaining the compounds labelled as in LXXV and LXXVI opposite. Allogibberic acid (LXXV) ozonised to the norketone (LXXVII) which was treated with sodium bismuthate to yield the keto-acid LXXVIII. This was converted to an anhydride, and so the C_6 carboxylic acid group and the two carbon bridge were in a cis relationship. The mechanism of the allogibberic acid (LXXV) to gibberic acid (LXXVI) rearrangement requires that the two-carbon bridge be directed in an opposite sense in each compound, and this was verified by the observation that the O.R.D. curves of the norketone LXXVII and gibberic acid (LXXVI) were mirror images.

The stereochemistry at C_9 is unaffected in the allogibberic to gibberic acid conversion, and so the B/C ring junction will be cis in one compound and trans in the other. Palladium dehydrogenation of gibberic acid and dihydroallogibberic acid yielded styrenes LXXIX and LXXX which regenerated the parent compounds on catalytic reduction. Hydrogen had thus added trans to the two-carbon bridge in one compound and cis in the other. Trans addition would be favoured when the C_6 carboxyl group and the two carbon bridge were on the same side of the plane of the molecule and so stereochemistries LXXV and LXXVI could be written for allogibberic acid and gibberic acid respectively. These were shown to be the absolute stereochemistries by comparison of the O.R.D. curve of the keto-diacid LXXVIII with that of cholestanone.

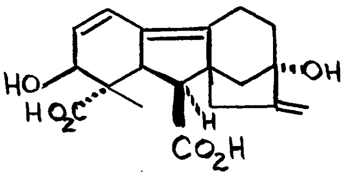
Stork has argued that the C_5 hydrogen in gibberellic acid would be β-oriented, since, if it were α, C_6 would be axial to ring A and the C_6 carboxyl group which is known to be β would have the least stable of the two possible orientations. The fact that the methyl ester



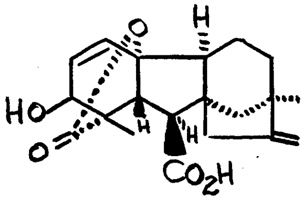
LXXXI



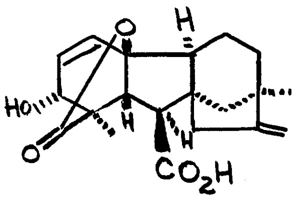
LXXXII



LXXXIII



XVIa



LXXXIV

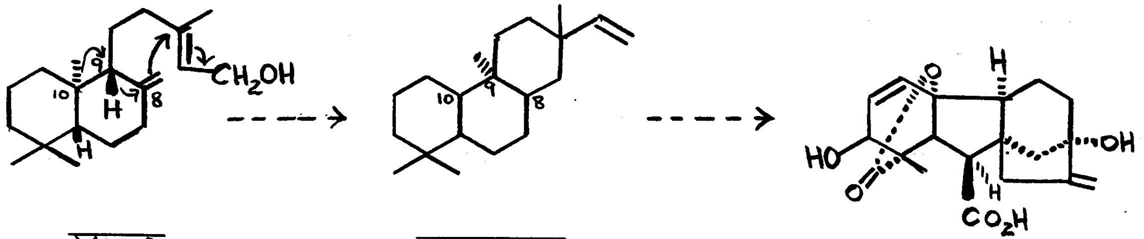
did not epimerise at C_6 was taken as evidence in favour of a C_5 β-hydrogen. The trans nature of the C_5 and C_6 hydrogen atoms has been verified by n.m.r. studies(58).

Base treatment of tetrahydrogibberellic acid LXXXI yielded the diacid LXXXII which could be relactionised to 3 epi-tetrahydrogibberellic acid. The rotation difference between the 3 epi-lactone and the 3 epi-acid was large and positive, and so the lactone was considered to be α on the basis of a modification of the Klyne-Hudson rule(59). The epimerisation of the C_3 alcohol, probably via a dealdolisation-realdolisation mechanism, was held to indicate the axial (3β) nature of this group.

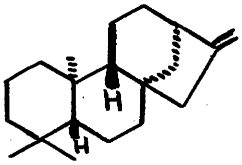
The C_9 α-hydrogen of allogibberic acid (LXXV) is not the same as the original C_9 hydrogen in gibberellic acid, as was shown by the considerable deuterium exchange when the rearrangement was carried out in deuterium oxide, and the aromatisation involves the intermediate gibberellenic acid (LXXXIII). The C_9 stereochemistry in gibberellic acid was concluded to be the same as that in allogibberic acid, due to the close correspondence in the O.R.D. curves of the keto-esters derived from the oxidation of ring D in methylallogibberate, and methyl-dihydrogibberellate. The stereochemistry XVIa could now be assigned to gibberellic acid.

Edwards(60) has suggested the formulation LXXXIV on the basis of lactone/acid rotation difference comparison with the pimaric acids.

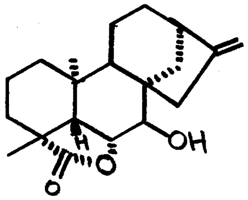
Using the arguments of Birch(40), we have considered that,



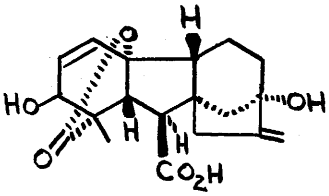
XIIIb LXXXV XVIa



LXXXVI



LXXXVII

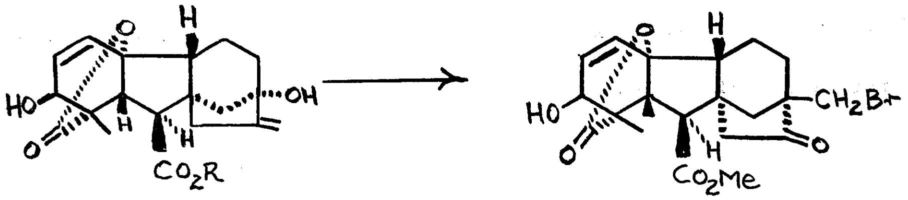


XVIb

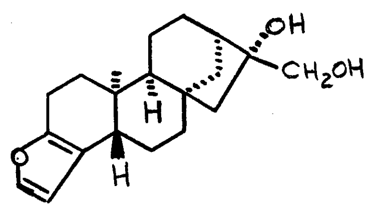
although structure XVIa has a trans-syn-trans backbone, it could arise from an antipodal trans-anti precursor XIIIb, if methyl migration, as encountered in rosenonolactone biogenesis (see p. 25), had occurred before oxidative removal of the methyl group. Such a migration, however, involves migration of the hydrogen at C_9 to C_8, and C_8 would be in the wrong oxidation level for entry to the tetracyclic group of diterpenes. The migration is, therefore, unlikely and we would suggest that the methyl group is oxidised without prior migration from C_{10}. The recent finding of kaurene (LXXXVI) and some of its relatives such as LXXVII,(61) which is oxidised in positions C_6 and C_7 as would be expected of a gibberellic acid precursor, in the mould Gibberella fujikuroi with the gibberellins has strengthened this view. Labelled kaurene has now been converted to gibberellic acid in vivo(62), showing that kaurene is, in fact, the precursor of gibberellic acid. The stereochemistry of kaurene (LXXXVI), substantiates our hypothesis that the stereochemistry of gibberellic acid is represented as XVIb opposite.

The fact that kaurene contains no C_3-hydroxyl group indicates that such a group must be added by subsequent biological hydroxylation, and Djerassi's reasoning(32) that the antipodal nature of gibberellic acid was a result of the cyclisation of the geranyl-geraniol precursor by an enzyme system which used the $HO^{\oplus}$ cation, can have no basis in fact.

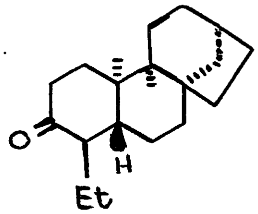
To verify our proposed stereochemistry XVIb, we have completed an X-ray structural analysis of a heavy atom derivative of gibberellic acid. Treatment of methyl gibberellate with pyridine perbromide gave a compound $C_{20}H_{23}O_6Br$ as prisms m.pt.= 215–217°C from



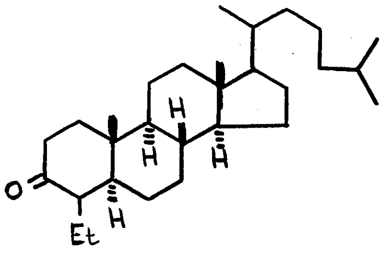
XVIb LXXXVIII



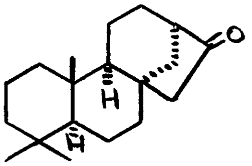
XLIX



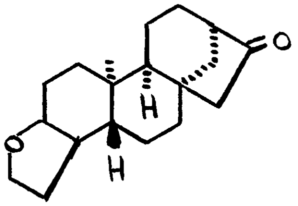
LXXXIX



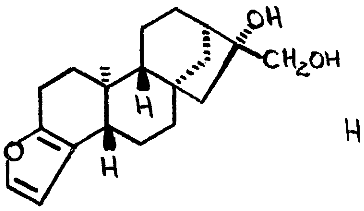
XC



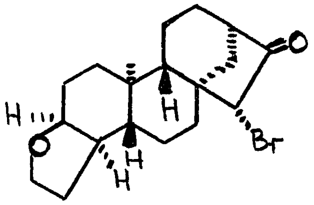
XCI



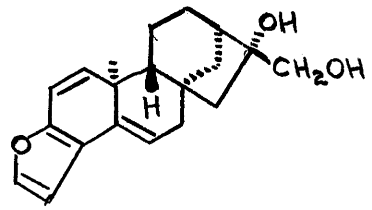
XCII



XCIII



XCIV



XCV

ethanol, ν_{max} = 1758 (lactone), 1745 (five membered ring ketone) and 1730 cm^{-1} (ester). The 1745 cm^{-1} band indicated that bromination had been accompanied by rearrangement, and the bromo-ketone was expected to have structure LXXXVIII. Three dimensional intensity data were recorded on equi-inclination Weissenberg photographs, and from the 1,716 data we have estimated visually, our colleagues were able to deduce that structure LXXXVIII was in fact a representation of the bromo-derivative, and so gibberellic acid is unambiguously defined as XVIb.

(6) <u>Cafestol</u> has been assigned the structure and stereochemistry XLIX by Djerassi(32)(47). The antipodal nature of the A/B ring junction was concluded from a comparison of the O.R.D. curves of the cafestol degradation product LXXXIX and 4α- ethyl cholestan-3-one (XC) which were mirror images. The stereochemistry about the B/C and C/D ring junctions was implied by a comparison of the O.R.D. curves of norphyllocladenone (XCl) and epoxynorcafestanone XCll. These curves were considered to be identical and so the B/C/D ring junction stereochemistry was assigned, since the stereochemistry of phyllocladene was known(63). The stereochemistry of the glycol grouping was assigned by partial stereospecific synthesis(64), and so cafestol was considered to have the stereochemistry XLIX.

This stereochemistry could not be derived from a <u>trans</u>-<u>anti</u> precursor, and so we concluded that some error had been made in its assignment. Stereochemical assignments made on the basis of O.R.D. measurements of relatively simple cyclohexanone types have invariably proved to be correct(65), and so we felt that the error had been made in the assignment of the B/C/D ring junction stereochemistry. The fact that a similar ring system had been present in gibberellic acid whose C_9 stereochemistry had been wrongly assigned on the basis of O.R.D. measurements gave fuller grounds for our doubts, and we suggested that XC111 might be a better expression for the stereochemistry of cafestol. We have vindicated this suggestion by X-ray structural determination.

In our hands, bromoepoxynorcafestanone XCIV crystallised from ethanol as needles m.pt.= 197 to 198.5°C (lit.(32) m.pt. = 183.5°C) and had a positive Beilstein halogen test. Equi-inclination Weissenberg photographs were taken and the data were estimated visually. From these data our colleagues have given structure XCIV for bromoepoxynorcafestanone, and the structure XCIII follows for cafestol.

(7) <u>Kahweol</u> has been given stereochemistry L on the basis of the stereochemistry of cafestol. The new formulation XCV follows from the above results.

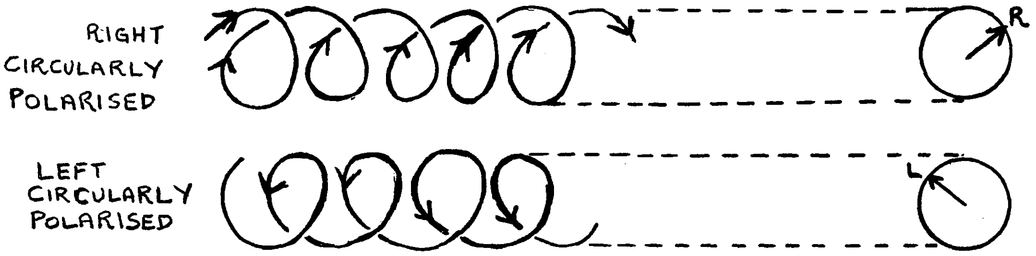


FIGURE 1

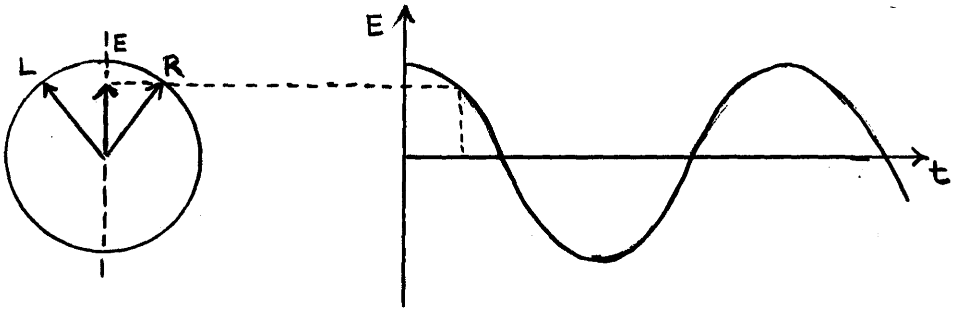


FIGURE 2

FIGURE 3

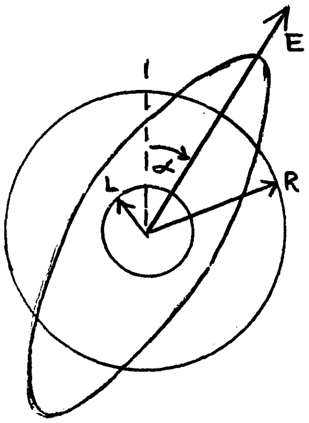


FIGURE 4

Rotary Dispersion(65)(66)

Since the incorrect stereochemistry had been assigned to gibberellic acid and cafestol on the basis of optical rotatory dispersion results, we decided to investigate the reasons for this tool breaking down in these cases, and, if possible, provide some improvement of the method of more general applicability.

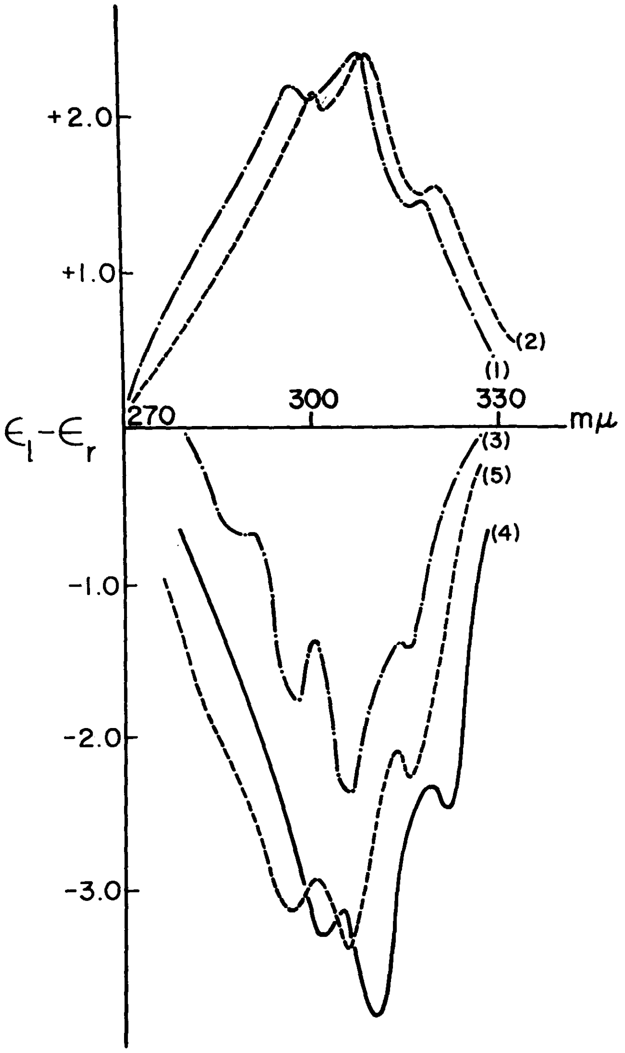
Light has electric and magnetic fields associated with it, and it is the interaction of these with various media which gives rise to the phenomena of absorption and rotation. Plane polarised light is regarded as the resultant of two vectors, right and left circularly polarised light, which describe right and left handed helixes as a function of time as shown in figure 1. The two components normally have the same speed, and the resultant vector will be two dimensional with time, describing a sine curve as shown in figure 2.

If the light passes through a transparent optically active medium, the refractive indices for right and left circularly polarised light will differ. Since the speed of light is dependent on the refractive index of the medium through which it is travelling, the right and left circularly polarised components will travel at different speeds, but with the same energy, through an optically active medium, so that the resultant vector, i.e. the resultant plane polarised light, will be at an angle to the incident light. This situation is shown in figure 3, and the angle between the incident and the resultant plane polarised light is the optical rotation.

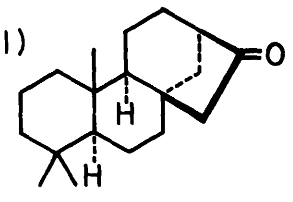
When a molecule absorbs light, the energy given to the absorbing molecule is dependent on the speed of light. Hence, if we have an optically active absorbing medium, the two components of plane polarised light will have different speeds, and so they will absorb differently. The two vectors at any one time can have different amplitudes as well as different directions so that the resultant describes an ellipse as in figure 4 (opp. p.18) and the emergent light is said to be <u>elliptically polarised</u>.

The rotation is measured here as the angle between the incident plane polarised light and the major axis of the elliptically polarised light. The rotation is wavelength dependent and a plot of the rotation against the wavelength is the optical rotatory dispersion curve. As we have seen, a compound need not absorb to have a rotation so that the rotation of a molecule is not merely a function of the absorbing chromophore and its environment, but contains a factor dependent on the asymmetry of the molecule as a whole. If other chromophores are present, it has been shown that the optical rotatory dispersion in the transparent region is a function of these chromophores, and the O.R.D. curve in the region of each chromophore will contain contributions from the other chromophores in the molecule.

To obtain a more precise measurement of the optical properties of a specific absorbing chromophore and its environment, we should measure the difference in absorption between the right and left circularly polarised components. Since this value is zero when there is no absorption, we would be measuring only the optical properties of the chromophore under consideration and graphs of this absorption difference with wavelength (i.e. circular dichroism curves) would give more

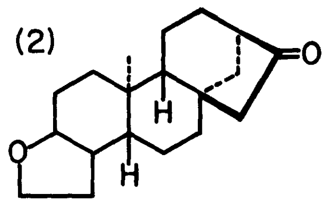


(1)



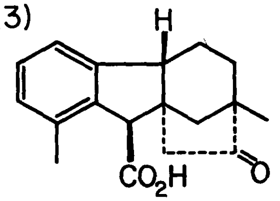
XCI

(2)



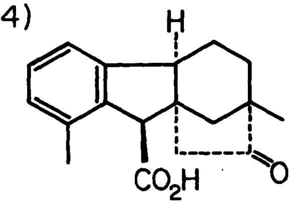
XCVI

(3)



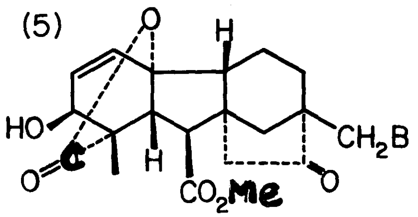
LXXVI a

(4)



LXXVI

(5)



LXXXVIII

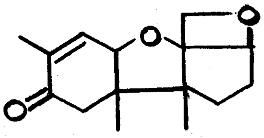
Compound	λ_{max}	$\epsilon_l - \epsilon_r$	λ_{max}	$\epsilon_l - \epsilon_r$	λ_{max}	$\epsilon_l - \epsilon_r$
LXXXVIII	296	−3.2	305	−3.3	315	−2.4
LXXVI a	297	−1.9	306	−2.4	316	−1.5
LXXVI	300	−3.5	309	−3.9	321	−2.5
XCI	297	+2.4	306	+2.4	317	+1.52
XCVI	299	+2.25	308	+2.4	319	+1.45

Figure 5. Circular Dichroism ($\epsilon_l - \epsilon_r$) of Tetracyclic Diterpenoid Ketones. Dioxane Solution.

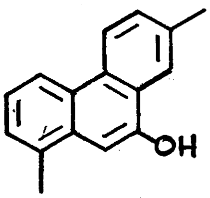
meaningful stereochemical correlations. We have made use of circular dichroism studies in the gibberellic acid and cafestol problems and this has been the first successful use of the new tool in structural organic chemistry(67)(68).

Gibberellic acid, on treatment with mineral acid, gave gibberic acid (LXXVI), and the C_9 epimer of this, epigibberic acid (LXXVIa) was obtained by heating gibberic acid with 10% palladium charcoal. The circular dichroism curves of methyl bromogibberellate (LXXXVIII), gibberic acid (LXXVI) and epigibberic acid (LXXVIa) were estimated, and are represented in figure 5 opposite. Epigibberic acid and bromomethylgibberellate had identical curves, while gibberic acid showed a marked shift of all three maxima to the red. These results would indicate that the stereochemistry of gibberic acid and epigibberic acid had been assigned correctly by the previous workers, but that the aromatisation of gibberellic acid to allogibberic acid had been accompanied by inversion of the C_9 stereochemistry.

The circular dichroism spectrum of epoxynorcafestanone (XCVI) showed a red shift from that of norphyllocladenone (XCI)(69), and, by analogy to the shift observed in the gibberellic acid case, structures XCVI and XCI were assigned to these compounds (see figure 5). This assignment was later verified by our X-ray investigation which we have already discussed (p.17). The bathochromic shift shown in going from C/D trans to C/D cis compounds may be useful in assigning the stereochemistries of other tetracyclic diterpenes.



XCVII



XCVIII

1(b) Metabolites of the mould Trichothecium Roseum Link

Historical

The metabolites of the mould T. roseum were examined by Freeman and Morrison(70)(72)(73) and by Michael(71), the first isolated compound being trichothecin, whose structure was shown to be XCVII(74). The other metabolites of the mould were a related series of diterpenes which were termed rosein I, rosein II, and rosein III(73). Rosein III, $C_{20}H_{28}O_4$, m.pt. = 221°C, $[\alpha]_D = -124^{\circ}$, was a ketone which isomerised in base in much the same way as rosein I, but no further work has been published on this compound.

Rosein I and rosein II were termed rosenonolactone and rosololactone respectively by the Liverpool group, who deduced a structure for rosenonolactone, and showed that rosololactone had one of two possible structures(75)(76)(77).

Rosenonolactone(75)(76), m.pt. = 214°C, $[\alpha]_D = -107.5^{\circ}$, $C_{20}H_{28}O_3$ showed the characteristic carbonyl absorptions of γ-lactone (1786 cm^{-1}) and six-membered ring ketone (1720 cm^{-1}) in the infra-red, and the ultra-violet absorption of 289 $m\mu$ (1·6) was in agreement with the latter assignment. The compound absorbed one mole of hydrogen on catalytic reduction, and ozonolysis gave formaldehyde and a nor-acid $C_{19}H_{26}O_4$. This evidence was indicative of a tricyclic lactone with a vinyl double bond.

Selenium dehydrogenation of the lactone yielded 1.7-dimethyl phenanthrene* and 9-hydroxy-1.7-dimethyl-phenanthrene* (XCVIII). This

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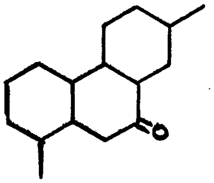
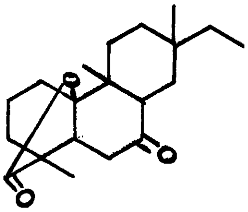
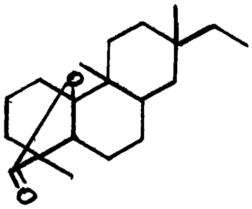
*phenanthrene numbering.



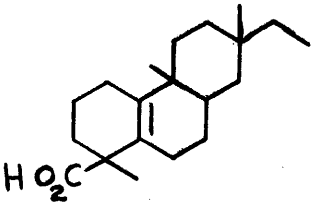
CVII



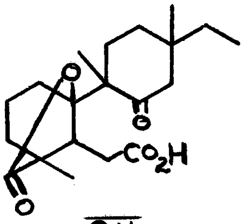
XCIX



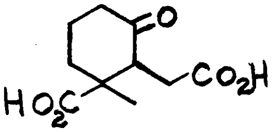
C



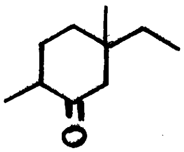
CI



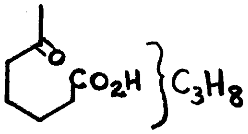
CII



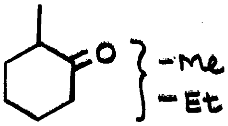
CIII



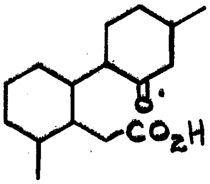
CIV



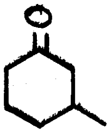
CV



CVI



CIIa



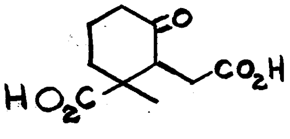
CVIa

indicated the partial skeleton (CVII), and showed that the ketone group was in the C_7 position. Dihydrorosenonolactone (XCIX) was converted to the desoxylactone (C), $C_{20}H_{30}O_2$, by Raney nickel treatment of the mercaptal. Treatment of C with ethanolic hydrochloric acid gave an acid (CI), $C_{20}H_{30}O_2$ which lost carbon dioxide at its melting point, and was, therefore a $\beta\gamma$-unsaturated acid.

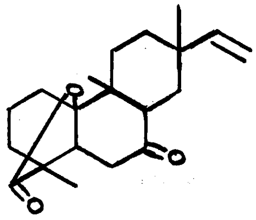
Oxidation of dihydrorosenonolactone (XCIX) gave a keto-acid $C_{20}H_{30}O_5$ (Cll) from which a keto-diacid $C_{10}H_{14}O_5$ (Clll) and a six-membered ring ketone, $C_{10}H_{18}O$ (CIV) were obtained on treatment with base.

The six-membered ketone, $C_{10}H_{18}O$ (CIV) was oxidised with potassium permanganate to a keto-acid, $C_{10}H_{18}O_3$, which gave a positive iodoform test. The partial structure CV could now be written for this keto-acid, and CVI for the six-membered ring ketone. Nitric acid oxidation of this ketone gave α-methyl α-ethyl succinic acid. The ketone CIV was derived from the keto-diacid CII by what could only have been a retro-aldol reaction. The selenium dehydrogenation of rosenonolactone had indicated a partial structure CVII, and so the partial structure of the keto-acid would be CIIa. A partial structure CVIa followed for the six-membered ring ketone which could now only have structure CIV. This has been proved by synthesis(78).

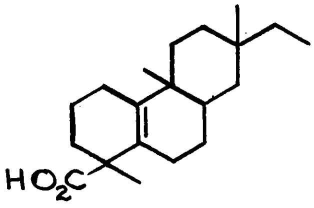
The lactone carbonyl of rosenonolactone has been assigned a quaternary position since when this group was reduced to methyl and the resultant product was dehydrogenated with selenium, the resultant phenanthrene was still 1.7 dimethyl phenanthrene. The lactone was assigned to the C_4 position in the partial structure CVII,



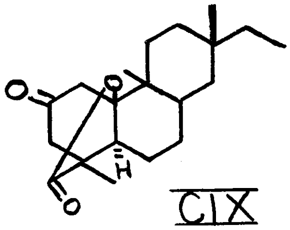
CIII



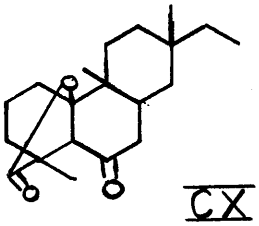
CVIII



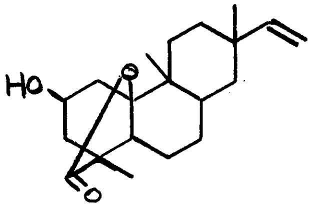
CI



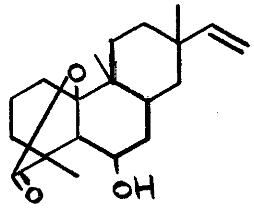
CIX



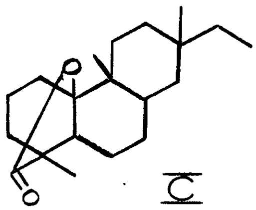
CX



CXI



CXII



C

and the keto-diacid (C111) was known to have its two carboxyl groups less than four carbon atoms apart since acetic anhydride treatment yielded an anhydride. Structure C111 was assigned to this compound, and this has been proved by synthesis(78).

The structure CVIII for rosenonolactone follows from this work.

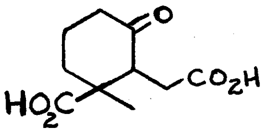
Rosololactone, m.pt. = 186°C, $[\alpha]_D = +6.3^\circ$, $C_{20}H_{30}O_3$, was shown to have lactone and alcohol groups from the infra-red spectrum, and selenium dehydrogenation gave 1.7 dimethyl phenanthrene. Ozonolysis gave a nor-acid, $C_{19}H_{28}O_5$ and formaldehyde. Rosololactone has been related to rosenonolactone as follows.

Treatment of dihydrorosololactone with naphthalene-2-sulphonic acid gave a diene-acid of $\lambda_{max} = 238\ m\mu$. Reduction of the diene-acid gave an acid which could be lactonised to allo- and neo- hydroxyrosanoic lactones which had been obtained by lactonisation of the acid Cl (see p. 22) derived from rosenonolactone.

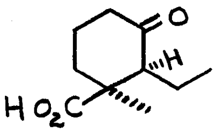
Oxidation of the alcoholic group in dihydrorosololactone gave a ketone, the lactone ring of which opened in acid or base to yield an enone $C_{20}H_{30}O_3$, $\lambda_{max} = 248\ m\mu\ (4.05)$ and $314\ m\mu\ (1.83)$. This suggested that the (-C-O-) group of the lactone was β to the ketonic carbonyl, and structures CIX and CX were proposed for the ketone and CXI and CXII for rosololactone.

Desoxyrosenonolactone, m.pt. = 115-116°C, $[\alpha]_D = +57^\circ$, $C_{20}H_{30}O_2$, was found in T. roseum by Arigoni(79), who proved its structure by identification of the dihydro compound with compound C (see p.22) derived from rosenonolactone.

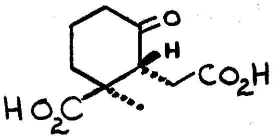
The stereochemistry of rosenonolactone(79)(80) and its relatives



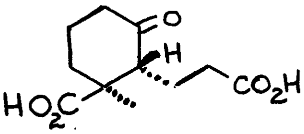
CIII



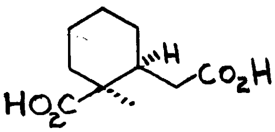
CIIIa



CIIIb

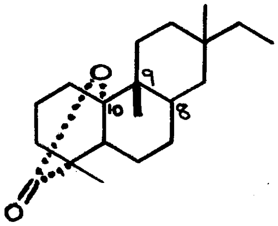


CXIII

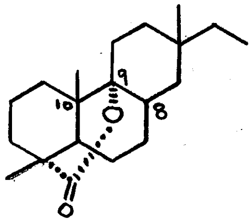


CXIV

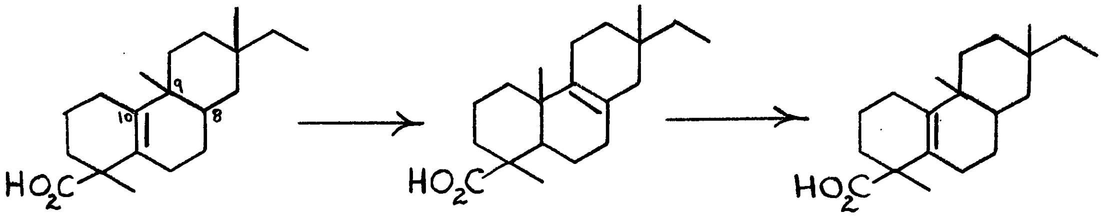
CXV



LXIII



LXIV



CI

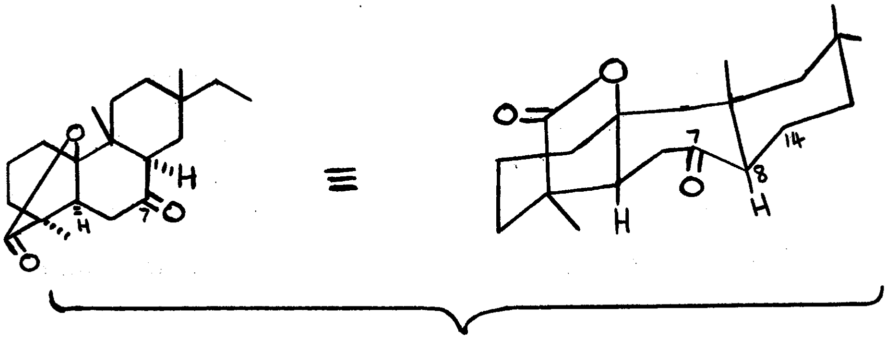
CXVI

CIa

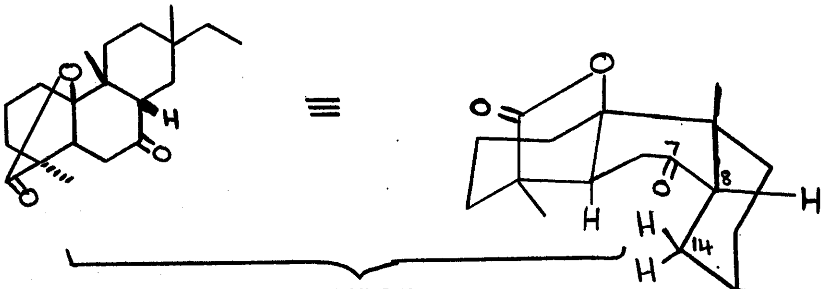
is of paramount importance in defining the biological precursor of the series (see p1a). The keto-diacid CIII (see p.22) from rosenonolactone epimerised in alkali and was thus assigned the *cis* arrangement CIIIa, going to the more stable *trans* arrangement CIIIb in alkali. The absolute stereochemistry of Clll has been assigned CIIIa since the O.R.D. curve of the epimer CIIIb was comparable to that of (-) *trans* 2-methyl-2carboxy-6-ketocyclohexyl-propionic acid (CXlll) whose absolute stereochemistry was known(81). Clemmenson reduction of the epimer CIIIa yielded (-) *cis*-2-methyl-2-carboxycyclohexyl-acetic acid (CXIV) of known configuration(82). The partial structure CXV may be written for rosenonolactone.

Whalley(79) concluded that the lactone ring and the methyl group at C_9 were *syn*, due to the fact that neither *neo*-rosanoic lactone, *allo*-rosanoic lactone, nor *desoxy*rosenonolactone corresponded to the γ-lactone LXIII derived from pimaric acid (see p.22) which had the *trans* backbone. He considered that the C_8 asymmetric centre was inverted in the formation of *neo*- and *allo*-rosanoic lactones from the acid CI. The only mechanism for this inversion would be the double methyl migration as in CI → CXVI → CIa opposite. This is improbable. We feel that the fact that the compounds CI did not lactonise to a more stable six-membered lactone such as LXIV, as had been the case with the pimaric acids, is more indicative of the *syn* C_9-C_{10} backbone, as it would be sterically impossible for such a compound to exist if the lactone carbonyl and the C_9 methyl group were *cis*.

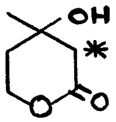
Rosenonolactone isomerises to its C_8 epimer, *iso*rosenonolactone, in base. Lithium aluminium hydride treatment of rosenonolactone



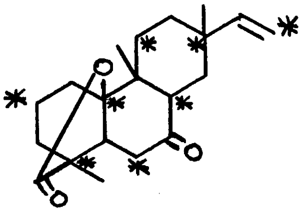
CXVII



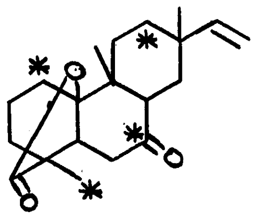
CXVIII



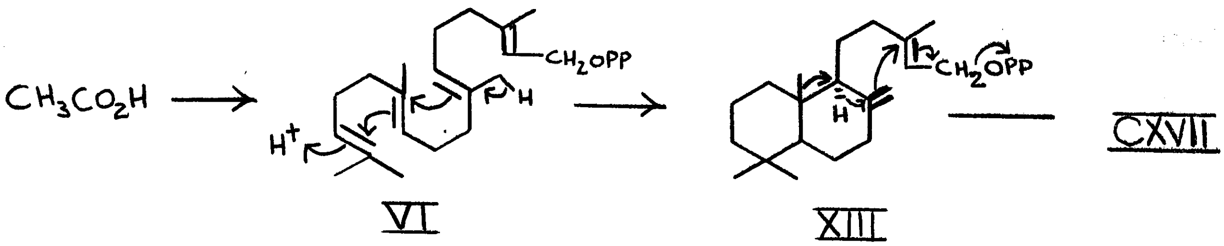
CXIX



CXX



CXXI



yielded a triol with a relatively unhindered C_7 position, while similar treatment of the C_8 epimer gave a mixture of triols epimeric at C_7. The hindered carbonyl group of isorosenonolactone can be explained by postulating the cis B/C ring junction of CXVIII opposite, in which the C_{14} hydrogens shield the C_7 position. The stereochemistry of rosenonolactone may thus be written as CXVII.

The Biosynthesis of rosenonolactone has been shown to occur by the scheme VI → XIII → CXVII opposite by tracer studies(40)(78)(83). When $[1C^{14}]$-acetic acid and $[2C^{14}]$- mevalonate (CXIX) were fed to T. roseum, rosenonolactone, labelled as in CXX and CXXI respectively, was obtained. The C_4 α-methyl group was shown to be derived specifically from C_2 of mevalonate, in agreement with similar results for the triterpenes(27), and gibberellic acid(28)(40)(84). The methyl group derived from $C_{3'}$ of mevalonate has always been the one to be oxidised in the cases investigated, and $C_{3'}$ and C_2 of mevalonate never become equivalent at any stage in the biosynthesis.

Theoretical

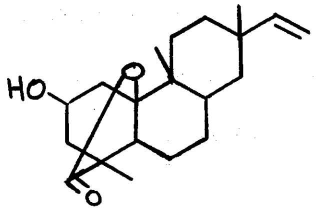
The foregoing review has shown that the stereochemistry of the C_{13} centre in the rosenonolactone group of compounds, and the position and stereochemistry of the hydroxyl group in rosololactone have yet to be defined. The mould T. roseum was, therefore, grown to isolate these metabolites for further study, and to see if other metabolites were present. Other moulds in the Trichothecium family have also been grown for study, but growth was not very prolific and no metabolites were isolated.

The mycelium from T. roseum was extracted in a soxhlet extractor with chloroform, and the gummy extracts were triturated with petrol to give a solid from which rosenonolactone m.pt. = 212°C, and rosololactone m.pt. = 186°C could be separated by virtue of their different solubilities in methanol. The petrol solubles from the mycelium were chromatographed on grade III alumina and desoxyrosenonolactone, $C_{20}H_{30}O_2$, m.pt. = 116-7°C, $[\alpha]_D = +60.7^\circ$, a liquid product which we have termed rosein V, and trichothecin, m.pt. = 115-7°C, were obtained.

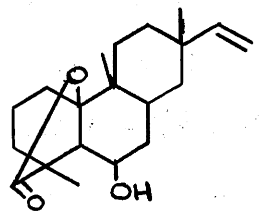
The broth from the T. roseum cultures was extracted with chloroform and separated into bicarbonate soluble and non-bicarbonate soluble portions. The neutral portion was chromatographed on grade III alumina to yield the liquid rosein V and trichothecin, and the acidic portion was chromatographed on silica gel to give a new solid metabolite which we have designated rosein VI. At no time was any of the metabolite rosein III(73) isolated and the mould appeared to have stopped producing this metabolite.

In two of the batches of T. roseum which were grown, an infinitessimal amount of a neutral, nitrogen-containing metabolite was isolated from both mycelium and broth. We have termed this metabolite rosein VII, and it crystallised from ethyl acetate, m.pt. = 271-274°C, $[\alpha]_D = +5.56^\circ$. The metabolite analysed as $C_{32}H_{46}O_{10}N_4$, and the molecular weight was 602 ± 50 by thermistor drop technique. The infra-red spectrum was characteristic of an amide with $\nu_{max} = 1645$ cm^{-1} and 1570 cm^{-1}, and there was no appreciable ultra-violet absorption beyond end-absorption. The n.m.r. spectrum had a singlet at 2.8τ (6H) which would indicate unsaturation, and so the lack of ultra-violet absorption is surprising. Further peaks occurred at 2.75τ (1H;NH), 4.25τ (1H, quartet$_{ABX}$$J_{AB} = 6$, $J_{AX} = 12$ c.p.s.), 4.76τ (1H singlet CH-O), 6.7τ (3H, singlet, NMe), 7.08τ (3H, singlet, NMe). A series of well defined singlets occurred in the 8.4 to 9τ region, and eight protons occurred at the exceptionally high value of 9.25τ. The amount of material available to us has not permitted any further work to be done on this metabolite.

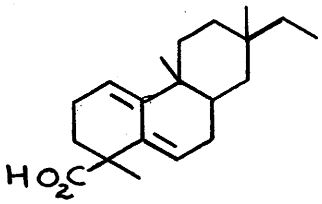
The liquid metabolite rosein V, found in both mycelium and broth, distilled at 124°C and 0.045 mm pressure. It had a refractive index of 1.4565 and $[\alpha]_D = 0^\circ$. Peaks at 1745 cm^{-1} and 1730 cm^{-1} in the infra-red were thought to be typical of ester and $\alpha\beta$-unsaturated ester functions, and the latter assignment was supported by an ultra-violet spectrum with $\lambda_{max} = 216$ mμ. The compound showed only one peak on a 0.75% silicone v.p.c. column and analysed as $C_{15}H_{24}O_5$. One mole of hydrogen was consumed on catalytic reduction, and the product no longer had the 1730 cm^{-1} infra-red absorption, characteristic of the



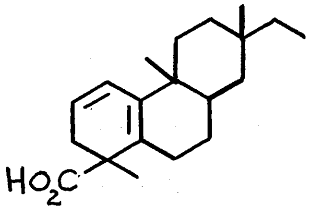
CXI



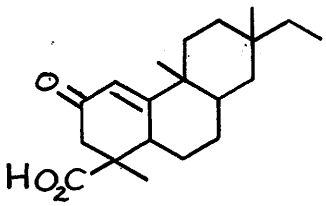
CXII



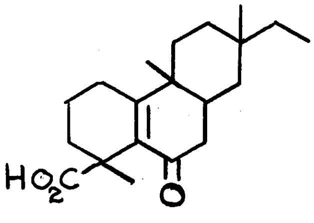
CXXII



CXXIII



CXXIV



CXXV

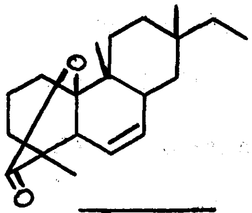
$\alpha\beta$-unsaturated ester, the ultra-violet spectrum becoming transparent. Hydrolysis of rosein V required two moles of base and gave only water soluble products.

From the n.m.r. spectrum of this compound, and some further expxriments, it was concluded that it was not homogeneous, and separation was effected on a polyethylene glycol adipate v.p.c. column to give two metabolites with mass spectroscopic molecular weights of 258 and 342. No further work has been done on these metabolites.

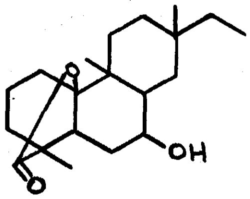
Rosenonolactone and rosololactone. Whalley's work on the structure of rosololactone indicated that structures CXI and CXII were possible (see p. 23), and re-examination of the evidence would indicate that structure CXII is the more likely.

Dehydration of dihydrorosololactone gave a diene $C_{20}H_{30}O_2$, $\lambda_{max} = 238$ mμ. We would suggest that this is the heterodiene CXXII with $\lambda_{calc} = 245$ mμ which can be obtained from alcohol CXII directly. Alcohol CXI would give the homodiene CXXIII, $\lambda_{calc} = 274$ mμ but it is not inconceivable that this homodiene might rearrange to the heterodiene and so this evidence for structure CXII is not rigorous. More compelling evidence is the fact the enone derived by oxidation and lactone opening of dihydrorosololactone has $\lambda_{max} = 248$ mμ. This enone can have structures CXXIV or CXXV with $\lambda_{calc} = 243$ and 248 mμ respectively and the correspondence of λ_{calc} for CXXV to the observed absorption strongly supports this structure for the enone and hence structure CXII for rosololactone.

If rosololactone is in fact the C_6 alcohol CXII, it should



CXXVI

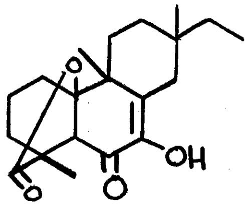


CXXVII

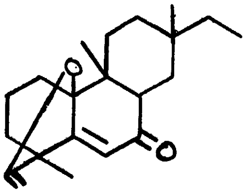
be relatively simple to prove this by conversion of rosololactone and rosenonolactone to a common intermediate which would include some feature indicative of the vicinyl nature of the oxidised carbon atoms in each compound.

The Δ^6 ene CXXVI would be a useful compound through which the two compounds might be interrelated. It should be preparable by α-bromination of the respective ketones, reduction of the bromoketones to bromhydrins, and conversion of these to the common 6-ene by treatment with zinc and acetic acid. Treatment of dihydrorosenonolactone with bromine in acetic acid gave the monobromide $C_{20}H_{29}O_3Br$, m.pt. = 221-223°C, as plates from methanol. Neither the infra-red nor the ultra-violet ketone absorption had shifted to any extent in this compound, due probably to the fact that ring B was in the boat form. The n.m.r. spectrum of the monobromide showed a proton at 5.37τ (C$\underline{H}$BrCO), indicative of C_6 rather than C_8 bromination. Sodium borohydride reduction of the bromoketone gave a bromhydrin, m.pt. = 185-187°C, as needles from methanol. The infra-red absorption at 3400 cm^{-1} (OH) and the n.m.r. spectrum with protons at 5.5τ (C$\underline{H}$Br) and 6.1τ (C$\underline{H}$OH) were in agreement with this assignment. Reduction of the bromhydrin with zinc and acetic acid gave a compound $C_{20}H_{32}O_3$, m.pt. = 186-189°C as plates from methanol. This still contained the alcoholic grouping as evidenced by the strong infra-red absorption at 3500 cm^{-1} and was shown to be the 7-alcohol (CXXVII) by identity with an authentic specimen which we prepared by sodium borohydride reduction of dihydrorosenonolactone.

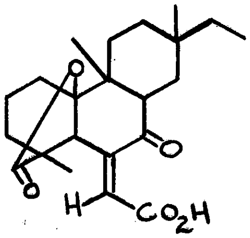
The lack of success with the above route led us to attempt



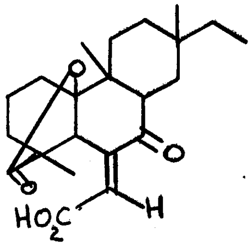
CXXVIII



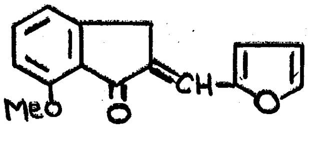
CXXIX



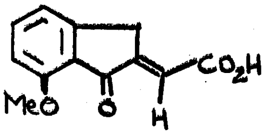
CXXX



CXXXa



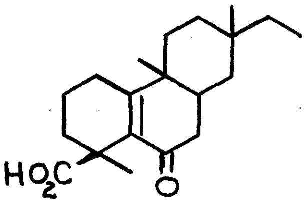
CXXXI



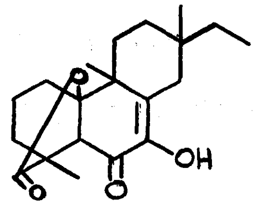
CXXXII

the preparation of the diosphenol CXXVIII as the link compound. Selenium dioxide oxidation of dihydrorosenonolactone gave a solid, $C_{20}H_{28}O_3$, m.pt. = 159-159°C as needles from methanol. The ultra-violet spectrum of λ_{max} = 237 mμ (6,850) suggested that this was the enone CXXIX which has λ_{calc} = 238 mμ, and this was borne out by a 1670 cm^{-1} peak in the infra-red spectrum, and an unsplit proton at 4.23τ in the n.m.r. spectrum.

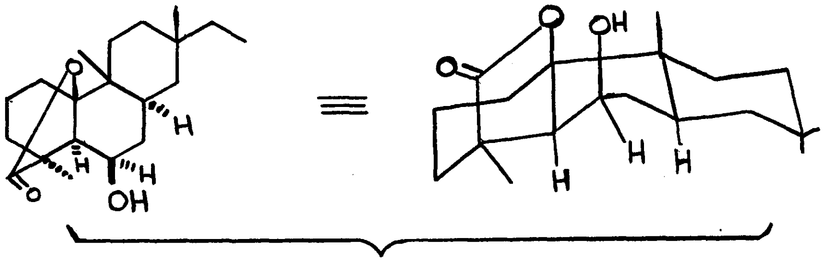
In the hope that ozonolysis of a furfurylidene derivative would provide us with the required diosphenol, we prepared α-furfuryl-dihydrorosenonolactone, $C_{25}H_{32}O_4$, m.pt. = 192 to 193°C, $[\alpha]_D = +195°$, as needles from methanol. The infra-red spectrum had the expected peaks at 1670, 1600 and 1530 cm^{-1}, and the ultra-violet spectrum had absorption at λ_{max} = 319 mμ (23,130). Ozonolysis of this compound in chloroform at -50°C for 30 minutes, followed by an oxidative work up, gave a compound $C_{22}H_{30}O_5$, m.pt. = 228-229°C as needles from ethyl acetate-petroleum-ether (60-80°). The infra-red spectrum of this compound was typical of a carboxylic acid in the 3500-2500 cm^{-1} region, and had peaks at 1770 cm^{-1} (γ-lactone), 1710 cm^{-1} (carboxylic acid) and 1670 cm^{-1} (enone). The ultra-violet spectrum of λ_{max} = 247 mμ (8,000) indicated enone CXXX, λ_{calc} = 242 mμ and an unsplit proton at 3.5τ supported this assignment. A similar unsplit proton occurred at 5.57τ and this might indicate the mixture of <u>cis</u> and <u>trans</u> isomers, CXXX and CXXXa, the proton in CXXXa being deshielded by the ketonic carbonyl. The steric compression in CXXXa would be severe, and so the assignments cannot be made with certainty.



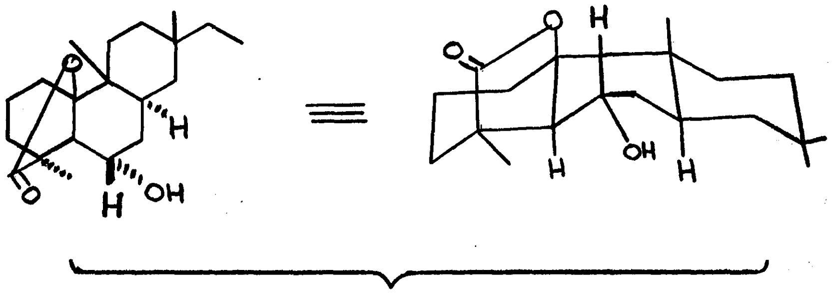
CXXV



CXXIX



CXXXIIIa



CXXXIIIb

We had observed a similar partial ozonolysis of furfurylidene derivatives in some synthetic work in gibberellin chemistry where we prepared the furfuryl-indanone CXXXI, $C_{15}H_{12}O_3$, m.pt. = 166.5 to 167°C as needles from methanol, λ_{max} = 240 mμ (6,200) 288 mμ (6,300) and 360 mμ (29,400). Ozonolysis of CXXXI in chloroform at -50°C for 10 minutes, followed by oxidative work up gave a compound $C_{12}H_{10}O_4$, m.pt.= 207-8°C as needles from ethanol-water. The infra-red in the 3500 to 2500 cm^{-1} region was characteristic of a carboxylic acid, the carbonyl of which appeared at 1700 cm^{-1}. The compound CXXXII had λ_{calc} = 242 mμ by Woodward's rules for the enone chromophore, and λ_{calc} = 256 mμ by Scott's rules$^{(85)}$ for the phenone chromophore. The average λ_{calc} = 249 mμ is in agreement with the observed λ_{max} = 250 mμ (15,120), 288 mμ (9,170), and 345 mμ (5,270), and the singlet at 3.59 τ in the n.m.r. spectrum is in full agreement with the compound being CXXXII.

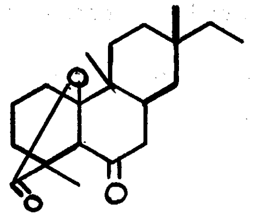
Since ozonolysis had been incomplete at -50°C, furfuryl-dihydrorosenonolactone was ozonised in acetic acid at room temperature and reductively worked up to give a compound $C_{20}H_{28}O_4$, m.pt.= 173-176°C as needles from methanol. The infra-red spectrum had peaks at 1760 cm^{-1} (γ-lactone), 1670 cm^{-1}, (enone), and 1640 cm^{-1} (double bond) and hydroxyl absorption at 3400 cm^{-1}. The compound gave a green-black colour with alcoholic ferric chloride solution, and had λ_{max} = 287 mμ (11,500) shifting to 355 mμ in base. The bathochromic shift is expected for a diosphenol$^{(86)}$, and the only diosphenol possible has λ_{calc} = 274 mμ, the divergence from the observed absorption being due to the boat ring B. Acetylation of the diosphenol gave a gummy acetate with λ_{max} = 254 mμ

and such a hypsochromic shift is expected from the acetylation of a diosphenol. A biproduct of the acetylation reaction was a compound $C_{22}H_{36}O_6$, m.pt. = 189-192°C as needles from methanol. The compound had no ultra-violet absorption, and the infra-red spectrum indicated that it was an anhydride with ν_{max} = 1835 and 1780 cm^{-1}. No further work was done on this compound in view of the small quantities available.

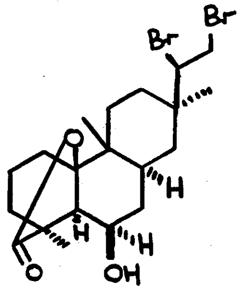
In our hands, oxidation of dihydrorosololactone gave a ketone with m.pt. = 157-9°C (lit.$^{(77)}$ m.pt. = 138°C). In an attempt to prepare the furfurylidine derivative of this compound, a gum was obtained of λ_{max} = 249 $m\mu$. It appeared that the basic conditions used to prepare the furfurylidene derivative had opened the lactone ring to give the enone CXXV. The gum was chromatographed on grade III alumina to give a small yield of a compound $C_{25}H_{32}O_4$, m.pt. = 189-191°C, from methanol as yellow needles, ν_{max} = 1670 cm^{-1} (enone), and λ_{max} = 325 $m\mu$ (8,300). This furfurylidene derivative was ozonised in acetic acid at room temperature and reductively worked up to give a gum, λ_{max} = 286 $m\mu$, shifting to 355 $m\mu$ in base. The gum was spotted on a silica gel chromatoplate which was developed in benzene:ether (1:1) and sprayed with ferric chloride solution. Two green-black spots appeared, the more polar of which had the same Rf as the authentic diosphenol CXXIX from dihydrorosenonolactone.

This would indicate that the hydroxyl group is at C_6 in rosololactone, although further evidence would be required to settle this point completely.

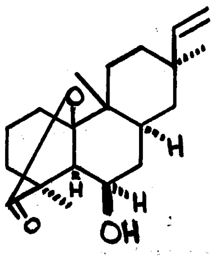
Two possible epimeric alcohols, CXXXIIIa and CXXXIIIb may



CX



CXXXIV

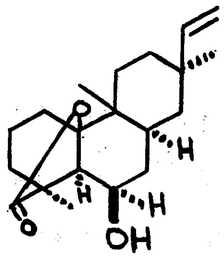


CXXXV

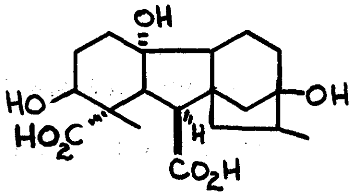
be written for rosololactone. Reduction of the ketone(CX), derived from dihydrorosololactone, with sodium borohydride, regenerated dihydrorosololactone, indicating that CXXXIIIa is the stereochemistry in rosololactone, as hydride attack is expected from the less hindered α-face of the molecule.

The stereochemistry at C_{13} in the rosenonolactone series remained to be determined. Whalley$^{(76)}$ had isolated αethyl-αmethylsuccinic acid as a degradation product of dihydrorosenonolactone (see p. 22) but had not reported a rotation for this compound. The absolute stereochemistry of (+) α-methyl-α-ethyl-succinic acid has been adduced from mixed melting point studies$^{(87)}$, and so the rotation of the compound from dihydrorosenonolactone should permit assignment of the C_{13} stereochemistry. In order to obtain this rotation, we repeated the degradation of dihydrorosenonolactone, but were unable to repeat the last step owing to the poor yields involved.

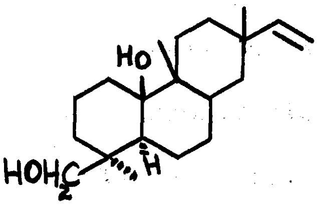
To obtain unambiguous evidence for the stereochemistry at C_{13} in rosololactone, and to verify our other structural and stereochemical assignments, we undertook an X-ray structural analysis of the compound. Treatment of rosololactone with pyridine perbromide in dioxan gave a dibromide, $C_{20}H_{30}O_3Br_2$, with m.pt. = 187-189°C as prisms from ethyl acetate. The compound effervesced at its melting point, and the vinyl peak at 910 cm^{-1} in the infra-red spectrum of rosololactone was now missing. Equi-inclination Weissenberg photographs were taken, and from the data which we estimated visually, our colleagues have deduced the structure CXXXIV for the dibromide. The structure and absolute stereo-



CXXXV



LXXXII



CXXXVI

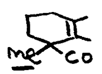
chemistry of rosololactone is thus as represented in CXXXV opposite.

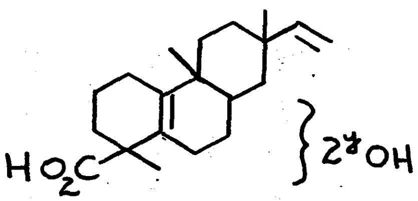
Rosein VI, the acid metabolite isolated from the broth of *T. roseum* crystallised from benzene-petroleum ether (60-80°) as needles, m.pt. = 191-193°C, $[\alpha]_D = 0^\circ$. From the infra-red spectrum with $\nu_{max} = 3400$ cm^{-1} (OH), 1695 cm^{-1} (CO_2H), 1020 cm^{-1} (OH) and 909 cm^{-1} (C=C) we concluded that the compound was probably a hydroxy-acid with a vinylic double bond. The compound analysed as $C_{20}H_{30}O_3$, and this was supported by a mass spectroscopic molecular weight of 318. The loss of water and CO_2 could be observed in the mass spectral cracking pattern. The ultra-violet spectrum had $\lambda_{max} = 203m\mu$ (5,750).

Hydrogenation of the mould acid with 10% palladised charcoal in ethyl acetate resulted in the uptake of one mole of hydrogen, and the dihydro compound, m.pt. = 180-184°C as needles from ether-petroleum ether (60-80°), no longer had the characteristic vinyl absorption at 1630 and 909 cm^{-1}. Hydrogenation with Adams catalyst in acetic acid resulted in a two mole uptake of hydrogen, the second mole being consumed very slowly. Rosein VI is thus probably a tricyclic hydroxy-acid with one vinyl double bond, and one very hindered double bond.

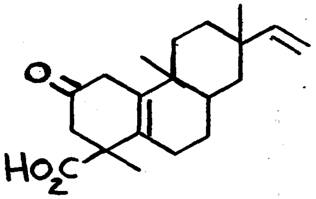
As the hydroxyacid LXXXII from gibberellic acid could be lactonised readily$^{(46)}$, we attempted to lactonise our mould hydroxy-acid under the same conditions, but obtained a quantitative recovery of starting material. This suggested that the hydroxyl group and the carboxylic acid group were not set up for such a lactonisation, and were not at C_4 and C_{10} as in LXXXII. This was supported by the fact that the diol CXXXVI, $C_{20}H_{30}O_2$, m.pt. = 144-145°C, $[\alpha]_D = -29^\circ$ from desoxyrosenonolactone on treatment with lithium aluminium hydride,

TABLE 2.

	$\underline{H}C{=}CH_2$	J_{CIS}	J_{TRANS}	$HC{=}C\begin{smallmatrix}H\\ \underline{H}\end{smallmatrix}$	J_{TRANS}	$HC{=}C\begin{smallmatrix}\underline{H}\\ H\end{smallmatrix}$	J_{CIS}	
ROSEIN VI ESTER-ACETATE	4·16 τ	10 cps.	17.5 cps	5·10 τ	17·5 cps.	5·13 τ	10 cps	8·72 τ
ROSOLOLACTONE	4·18 τ	10 cps.	17·5 cps	5·10 τ	17·5 cps.	5·12 τ	10 cps	8·72 τ



CXXXVII



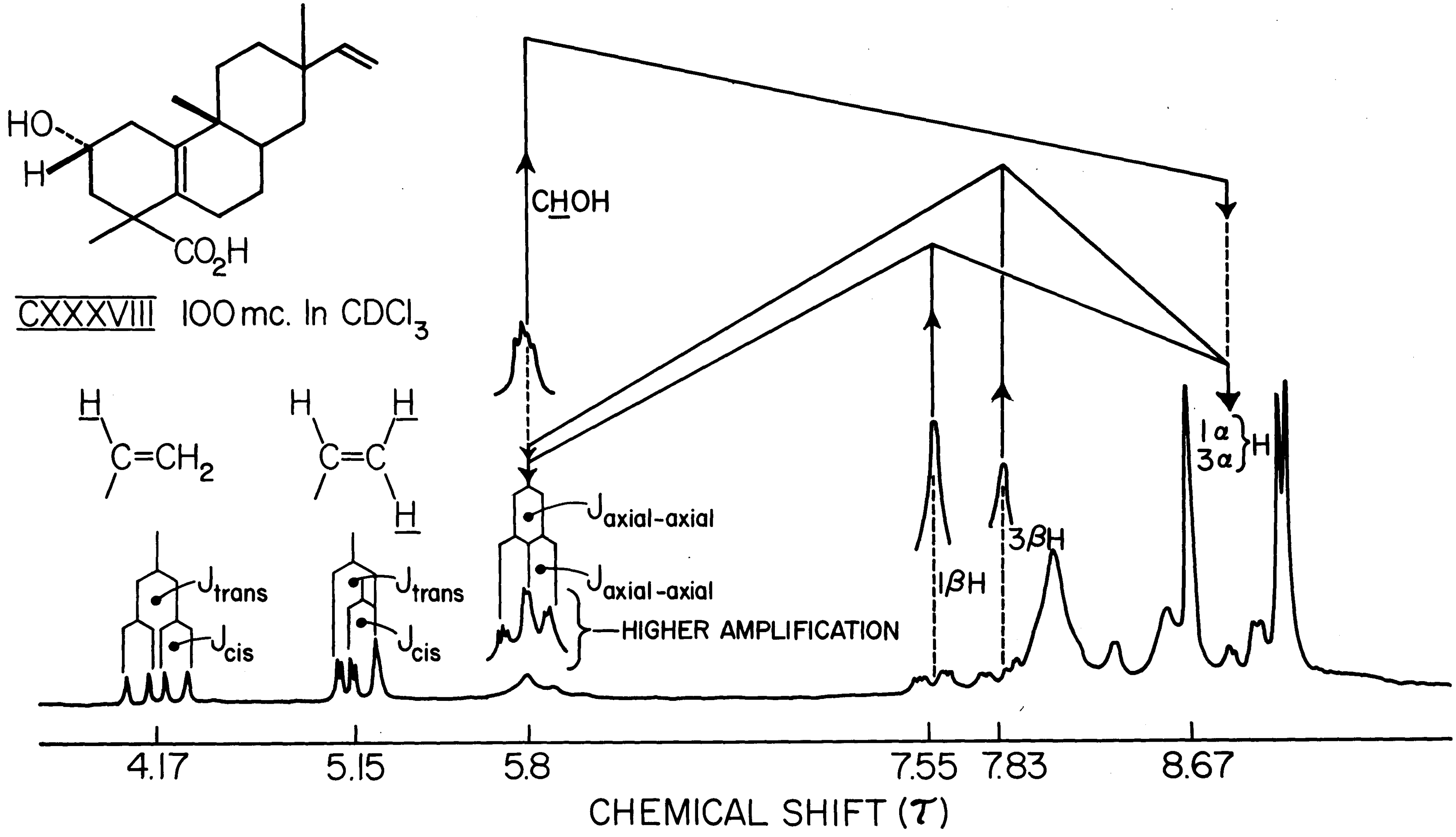
CXXXVIIa

lactonised immediately on oxidation of the primary alcoholic group with chromium trioxide and pyridine.

Acetylation of rosein VI gave an intractable gum. Esterification also gave a gum which chromatographed on silica-gel to give a small yield of a solid m.pt. = 130-134°C. This had no hydroxyl peak in the infra-red spectrum which had peaks at 1720 cm^{-1} (ester), 1125 cm^{-1} (OMe) and 910 cm^{-1} (vinyl) and the ultra-violet spectrum showed only end absorption.

Acetylation and esterification of the mould acid gave a liquid which was chromatographed on grade II alumina. The infra-red spectrum of the acetoxy-ester had peaks at 1720 cm^{-1} (ester, acetate), and 1210 cm^{-1} (acetate) and the n.m.r. spectrum had three-proton singlets at 6.34 and 7.97τ indicative of methyl ester and acetate groupings. A quartet at 4.16τ (1H, J_{cis} = 10 c.p.s. J_{TRANS} = 17.5 c.p.s., vinyl), a doublet at 5.10τ (1H, J_{TRANS} = 17.5 c.p.s., vinyl) a doublet at 5.13τ (1H, J_{cis} = 10 c.p.s., vinyl) and methyl singlets at 8.72τ, 9.08τ and 9.13τ were also apparent in the n.m.r. spectrum. The methyl singlet at 8.72τ is typical of a methyl group on a carbon atom which also bears a carbonyl group, and the chemical shifts for this proton and for the protons associated with the vinyl group are extremely similar to chemical shifts for similar groups in rosololactone as is shown in table 2. opposite. The $\mathrm{C\underline{H}OAC}$ proton occurred in the 5.0τ region.

The n.m.r. spectrum of the ester-acetate would indicate that the compound may be in the rosenonolactone series of diterpenes,

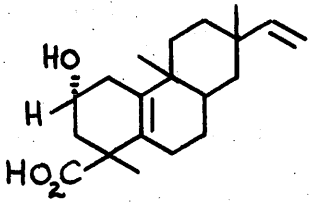

HO
H
CO2H
CXXXVIII 100 mc. In CDCl3
CHOH
H
C=CH2
H
C=C
H
H
Jtrans
Jcis
Jtrans
Jcis
Jaxial-axial
Jaxial-axial
HIGHER AMPLIFICATION
1βH
3βH
1α
3α
H
4.17
5.15
5.8
7.55
7.83
8.67
CHEMICAL SHIFT (τ)

with a secondary hydroxyl group, and structure CXXXVII was suggested.

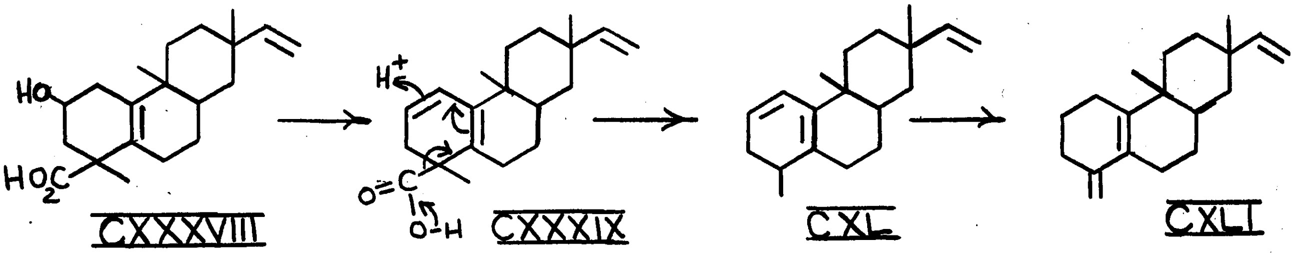
Jones oxidation(92) of rosein VI gave a compound, m.pt. = 217–222°C as needles from methanol, ν_{max} = 1705 cm^{-1} (acid and ketone) and 905 cm^{-1} (vinyl). This was esterified with ethereal diazomethane to give a solid, $C_{21}H_{30}O_3$, m.pt. = 135–136°C, as needles from methanol. The keto-ester had ν_{max} = 1730 cm^{-1} (ester), 1710 cm^{-1} (ketone) and had no ultra-violet absorption above 220 mμ.

100 megacycle n.m.r. spectra of this compound, and of the original hydroxy-acid were now investigated. Both showed the methyl and vinyl protons which had been observed in the ester-acetate. The n.m.r. spectrum of the keto-ester had doublets at 7.03τ and 7.60τ, with coupling constants of 12.5 c.p.s. These can be ascribed to <u>equatorial</u> protons, α to a ketone group. being split by the <u>axial</u> protons on the same carbon atom(88). The only position on the rosenonolactone skeleton which would allow the ketone to have four adjacent hydrogen atoms is the C_2 position, and so the ketone would be CXXXVIIa. The doublet at 7.03τ may be ascribed to the 1β proton and that at 7.60τ to the 3β proton since both are shifted by the ketone group, but the 1β proton is also shifted by the C_5–C_{10} double bond.

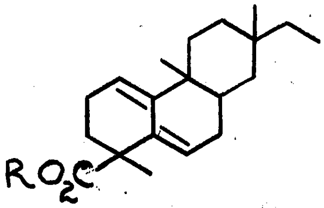
The n.m.r. spectrum of the hydroxyacid rosein VI is shown opposite. An ill-resolved carboxylic acid proton obscured the C$\underline{H}$OH proton at 6.8τ, but this was removed by equilibration with deuterium oxide. The proton, which appeared as a triplet of intensity ratio 1:2:1 centred at 5.8τ, was assigned to an <u>axial</u> proton on the carbon atom bearing the alcohol group since it showed <u>axial</u>-<u>axial</u> spin-spin



CXXXVIII



CXXXVIII CXXXIX CXL CXLI



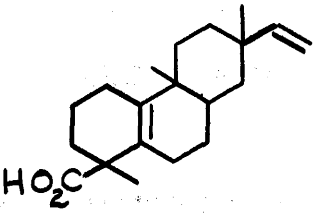
CXXII

coupling ($J = 10$ c.p.s.) with the adjacent axial protons. On the basis of our arguments from the n.m.r. spectrum of the keto-ester, the two adjacent protons would be the 1α and 3α protons, and double irradiation(88) at the frequency of these protons caused spin decoupling of the triplet to a singlet. The triplet and decoupled singlet bands both showed a fine splitting of $J = 2.5$ c.p.s. which could be ascribed to axial-equatorial coupling with the 1β and 3β protons.

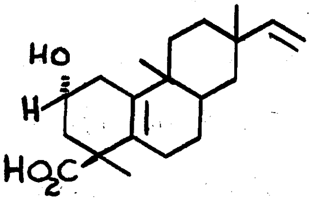
The 1β and 3β protons, which had been observed in the case of the keto-ester, now appeared at $7.55\,\tau$ and $7.83\,\tau$ respectively, but were highly split. Triple irradiation(88) at the frequencies of the 1α and 3α protons and the C$\underline{H}$OH proton caused spin decoupling and two well defined singlets were obtained at $7.55\,\tau$ and $7.83\,\tau$. The structure CXXXVIII follows for the mould acid.

When the mould acid was dehydrated with phosphorus oxychloride and pyridine, a gum was obtained with $\lambda_{max} = 235$ mμ which had no carbonyl absorption in its infra-red spectrum. A further dehydration of rosein VI under the same conditions gave a gum with $\lambda_{max} = 290$ mμ and a very much reduced carbonyl absorption in its infra-red spectrum. These results can be explained on the basis of the decarboxylation-dehydration mechanism CXXXVIII $\rightarrow$ CXXXIX $\rightarrow$ CXL $\rightarrow$ CXLI. The homoannular diene CXL would have $\lambda_{calc} = 289$ mμ and could presumably rearrange to the heteroannular diene CXLI with $\lambda_{calc} = 240$ mμ.

Pyrolysis of the ester-acetate of rosein VI gave a fraction with $\lambda_{max} = 243$ mμ which had still the ester carbonyl absorption in the infra-red. This might well be the diene ester CXXII which we have



CXLII



CXXXVIII

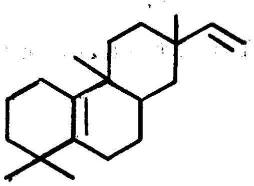
prepared by Whalley's procedure(77). The pyrolysis product and the authentic diene were spotted on a silica-gel chromatoplate, and developed with benzene:ether (1:1). The pyrolysis product of the ester-acetate showed seven spots, the least polar of which had the same mobility as the authentic diene-ester.

The acid CXLII, $C_{20}H_{30}O_2$, m.pt. = 138-148°C as prisms from petroleum ether, was prepared from desoxyrosenonolactone, and esterification with ethereal diazomethane gave the methyl ester $C_{21}H_{32}O_2$ as an oil. The methyl ester was hydrogenated to its dihydro-derivative. The keto-ester of rosein VI was converted to the mercaptal which was reduced with Raney nickel to give a gummy product which showed six spots on elution of a silica-gel chromatoplate with benzene. The least polar spot had an identical Rf to both the ester of acid CXLII, and its dihydro derivative.

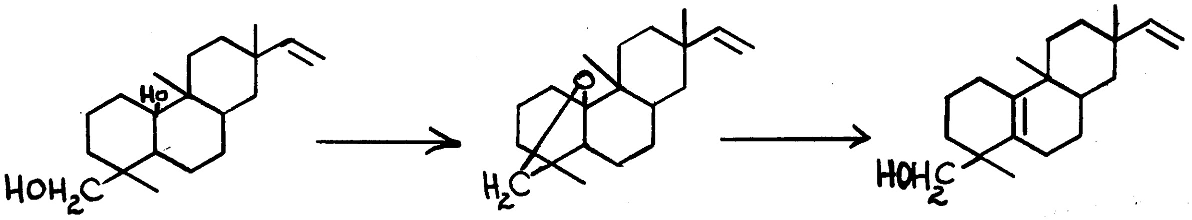
Treatment of the mould acid with concentrated sulphuric acid at -10°C gave a gum which had $\nu_{max} = 1780\ cm^{-1}$ indicative of a γ-lactone. This should have proved the C_5-C_{10} double bond, but the ultra-violet absorption of $\lambda_{max} = 240\ m\mu$ indicated that dehydration had occurred, and so the experiment was ambiguous.

In summary, we have a working structure CXXXVIII for the mould acid. The chemical relation to the rosein skeleton has, however, been made on the basis of thin-layer chromatographic evidence, which is far from being satisfactory. The observation of the various chromophores on dehydration is encouraging.

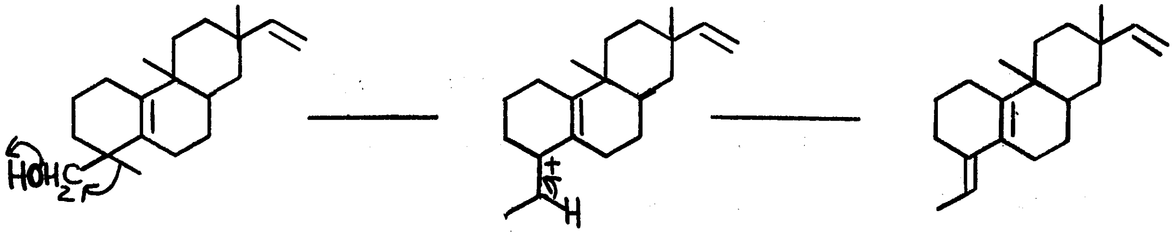
In some earlier work on the rosein VI problem, it was



CXLIII



CXXXVI CXLV CXLIV



CXLIV CXLVI

thought that the hydrocarbon CXLIII might be a useful model compound for comparison with the mould acid. Some attempts to prepare this hydrocarbon resulted in rearrangements which we shall mention briefly.

The diol CXXXVI on treatment with <u>para</u>-toluene-sulphonyl chloride at reflux, followed by lithium aluminium hydride reduction gave a solid, $C_{20}H_{32-34}O$, m.pt. = 45-51°C which had no hydroxyl group in its infra-re spectrum. This compound was treated with mineral acid to give the alcohol CXLIV, $C_{20}H_{32}O$, m.pt. = 106-108°C which we had prepared unambiguously from the diol CXXXVI by dehydration. The diol CXXXVI and the alcohol CXLIV had similar n.m.r. spectra, with two protons exhibiting a quartet at ca. 6.58τ. This was ascribed to two identical protons of an AB system with $J_{AB}/\delta_B - \delta_A = 0.3$ to 1.0(89), as would be expected of the $C\underline{H}_2OH$ group. The solid of m.pt. = 45 to 51°C exhibited a two-proton singlet at 6.28τ characteristic of a cyclic ether of the type CXLV(90). Support for this structure was obtained when oxidation of the dihydro compound gave a gum with an infra-red spectrum characteristic of a γ-lactone.

An attempt to prepare the hydrocarbon CXLIII from the alcohol CXLIV by reduction of the tosylate resulted in a liquid hydrocarbon $C_{20}H_{30}$ with $\lambda_{max} = 246\ m\mu$. This took up two moles of hydrogen and we have tentatively assigned structure CXLVI with $\lambda_{calc} = 245\ m\mu$ to the compound, postulating the rearrangement CXLIV → CXLVI as shown opposite.

Experimental (1)

Experimental

Melting points were taken on the Kofler Block. Infra-red spectra were taken with a Perkin-Elmer Infracord spectrophotometer for Nujol Mulls, ultra-violet spectra were taken with a Perkin-Elmer 202 spectrophotometer for ethanol solutions, and n.m.r. spectra were taken on a Varian A.60 n.m.r. spectrometer for solutions in deuterochloroform. The circular dichroism spectra were taken with a modified(93) Unicam SP500 spectrophotometer. Microanalyses were by Mr J. M. L. Cameron, B.Sc. and his staff at Glasgow University and by Mrs. C. Aldrich at the University of British Columbia.

Methyl bromogibberellate (LXXXVIII)

Methyl gibberellate (190 mg) and pyridine perbromide (135 mg.) were dissolved in dioxan (3 ml.), and ether was added after 30 minutes to give white prisms (170 mg.), m.pt. = 180-200°C, which crystallised from ethanol with m.pt. = 215-217°C (found, C, 54.7%; H, 5.66%. $C_{20}H_{23}O_6Br$ requires C, 54.6%; H:5.24%) and ν_{max} = 3490 (OH), 1758 (γ-lactone), 1745 (5.M ring ketone) and 1730 cm^{-1} (ester).

Bromoepoxynorcafestanone (XCIV)

This was prepared by the method of Djerassi(32). Repeated recrystallisation from ethanol gave prisms, m.pt. = 197-198.5°C (lit.(32) m.pt. = 183-5°C). This gave a deep green Beilstein test.

Gibberic Acid (LXXVI)

This was prepared by the method of Cross(91), and had m.pt. = 154-155°C, $[\alpha]_D = -4°$ (lit.(91) m.pt. = 152.5-154°C, $[\alpha]_D = -7°$).

Epigibberic Acid (LXXVIa)

The following modification of the previously reported route(91) was used. Gibberic acid (1 gm.) and 10% palladised charcoal (1 gm.) were heated at 210°C for 90 minutes in a stream of nitrogen. The product was cooled and digested with ether. The ethereal solution was washed with sodium bicarbonate solution, and the sodium bicarbonate washings were acidified with dilute sulphuric acid and extracted with ether. The extracts were washed with water and dried (anhyd. $MgSO_4$), and the solvent was removed to give a solid (170 mg.) which crystallised from methanol with m.pt. = 253-255°C, $[\alpha]_D = +131°$ (lit.(91) m.pt. = 252-255°C, $[\alpha]_D = +131°$).

Metabolites of T. Roseum Link

20 litres of Czapec-Dox and corn steep liquor(72) was seeded with the spores of T. roseum. The mould was grown for 3 weeks in still culture, and the broth was decanted from the mycelium. A little chloroform was added to both broth and mycelium to effect sterilisation.

The mycelium (190 gm.) was dried, powdered and extracted in a soxhlet extraction apparatus with chloroform. The solvent was removed to give a brown semi-solid which was triturated with petrol. The petrol insoluble portion was washed with hot methanol and centrifuged to give a white crystalline solid which recrystallised from methanol as white prisms of rosenonolactone (14 gm.) m.pt. = 212°C (lit.(75) m.pt. = 214°C). The methanol soluble portion was a brown semi-solid which was filtered through a plug of alumina and recrystallised from ethyl acetate-petroleum ether (60-80°) as fine needles of rosololactone, m.pt. = 186°C (lit.(73) m.pt. = 186°C) (4.3 gm.).

The petrol soluble material (17.06 gm.) was a brown oil and 1 gm. was chromatographed on grade III alumina. Elution with benzene gave a solid (177 mg.) which recrystallised from petroleum ether (60-80°) as prisms m.pt. = 116-7°C, $[\alpha]_D = +60.7^{\circ}$ (found, C. 79.21%; H, 9.99%. $C_{20}H_{30}O_2$ requires C, 79.42%; H, 10.00%). This was desoxyrosenonolactone (lit.(79) m.pt. = 115-116°C, $[\alpha]_D = +57^{\circ}$). Elution with benzene:ether (19:1) gave a liquid product (305 mg.) which we have designated rosein V, and elution with benzene:ether (9:1 and 5:1) gave trichothecin (120 mg.) m.pt. = 115-117°C (lit.(72) m.pt. = 118°C), with $\lambda_{max} = 230$ mμ.

The broth was extracted with chloroform, and the extracts were washed with sodium bicarbonate solution and water and dried (anhyd. Mg SO_4) and the solvent was removed to give a brown gum (15.8 gm). 1 gm. of this gum was chromatographed on grade III alumina. Elution with benzene gave the liquid product rosein V (364 mg.), and elution with benzene:ether (19:1) gave trichothecin (122 mg.).

The sodium bicarbonate washings were neutralised with dilute sulphuric acid and extracted with ethyl acetate. The extracts were washed with water and dried over anhydrous magnesium sulphate, and the solvent was removed to give a brown gum (1.2 gm.) which was chromatographed on silica gel. Elution with benzene:ether (1:1) gave a semi-solid which crystallised from benzene-petroleum ether (60-80°) as needles m.pt. = 191-193°C (113 mg.). This compound was designated rosein VI.

In a further batch of <u>T. roseum</u>, grown on 70 litres of culture medium, a neutral product could be isolated by chromatography of both the mycelium and the broth. This was designated rosein VII and crystallised from ethyl acetate as fibrous needles, m.pt. = 271-274°C, $[\alpha]_D = +5.56$ (found, C, 59.46%; H, 7.21%; N, 8.80%. $C_{32}H_{46}O_{10}N_4$ requires C, 59.5%; H, 7.12%; N, 8.67%), $\nu_{max} = 3320$, 3230 (NH), 1745 (ester), 1645 and 1570 cm^{-1} (amide), $\lambda_{max} = 202\ m\mu$ (14,700). The molecular weight was 602 ± 50 by the thermistor drop technique and a total yield of 90 mg. of this metabolite was obtained. The metabolite was not present in later batches of the mould.

The liquid metabolite rosein V

This distilled at 124°C and 0.045 mm., and $n_D = 1.4565$, $[\alpha]_D = 0^{\circ}$ (found, C, 63.36%; H, 8.60%. $C_{15}H_{24}O_5$ requires C, 63.36%; H, 8.51%), $\nu_{max} = 1745$ (ester), and 1730 cm^{-1} ($\alpha\beta$- unsaturated ester), $\lambda_{max} = 216\ m\mu$ (6,745). V.p.c. on a 0.75% silicone column at 185°C showed one peak with a retention time of 30 minutes.

Hydrogenation of rosein V

Rosein V (47 mg.) was added to presaturated 10% palladised charcoal (25 mg.) in ethyl acetate (6 ml.) and 3.28 ml. of hydrogen was taken up after shaking for 2½ hours in an atmosphere of hydrogen. The solution was filtered through celite and the solvent was removed to give a gum (43 mg.) with $\nu_{max} = 1740\ cm^{-1}$ (ester).

Hydrolysis of Rosein V

Rosein V (157 mg.) was dissolved in methanol and refluxed with 15 ml. of 0.1N sodium hydroxide solution for 2 hours. The solution was neutralised to methyl red with 5.9 ml. of 0.1N hydrochloric acid. Excess acid was added, and the solution was extracted with ethyl acetate. No organic soluble product was obtained.

Separation of Rosein V

Rosein V separated on a polyethylene glycol adipate v.p.c. column at 189°C to give two fractions with retention times of 59 minutes and 95 minutes respectively. The fractions had mass spectroscopic molecular weights of 248 and 342.

Bromodihydrorosenonolactone

Dihydrorosenonolactone (2 gm.) was dissolved in glacial acetic acid (10 ml.) and 3.5 ml. of a solution of bromine (3 gm.) in acetic acid (10 ml.) was added. The solution was left at room temperature for 30 minutes, water was added, and the aqueous solution was extracted with ether. The extracts were washed with sodium bicarbonate solution and water and dried (anhyd. Mg SO_4), and the solvent was removed to give a gum which triturated with methanol to give a solid (260 mg.). The product crystallised from methanol as plates, m. pt. = 221-223°C (found, C, 60.17%; H, 7.16%; Br, 20.33%. $C_{20}H_{29}O_3Br$ requires C, 60.4%; H, 7.3%; Br, 20.15%), ν_{max} = 1770 (γ lactone), and 1705 cm^{-1} (ketone). The n.m.r. spectrum had a singlet at 5.37τ.

Dihydrorosenonolactone bromhydrin

The bromoketone (160 mg.) was dissolved in methanol (10 ml.) and added to a solution of sodium borohydride (160 mg.) in water (1.5 ml.) and methanol (5 ml.). After one hour at room temperature, the solution was added to excess water and extracted with ether. The extracts were washed with water and dried (anhyd. Mg SO_4) and the solvent was removed to give a white solid (142 mg.) which crystallised from methanol with m.pt. = 185–187.5°C, ν_{max} = 3400 (OH), 1770 and 1740 cm^{-1} (split lactone). The n.m.r. spectrum had protons at 5.5τ (C$\underline{H}$-Br) and 6.1τ (C$\underline{H}$OH).

Reduction of the bromhydrin

The bromhydrin (93 mg.) was dissolved in glacial acetic acid (10 ml.) and powdered A.R. zinc (250 mg.) was added over a period of one hour at reflux. The solution was added to iced water, and extracted with ether. The extracts were washed with sodium bicarbonate solution and water and dried (anhyd. Mg SO_4). The solvent was removed to give a gum which was chromatographed on Spense alumina. Elution with benzene:ether (4:1) gave a solid (17 mg.) which crystallised from ethyl acetate as prisms, m.pt. = 186–189°C (found C, 75.16%; H, 9.93%. $C_{20}H_{32}O_3$ requires C, 74.96%; H, 10.06%), ν_{max} = 3500 (OH) and 1730 cm^{-1} (lactone), with no ultra-violet absorption.

Reduction of dihydrorosenonolactone

Dihydrorosenonolactone (100 mg.) was dissolved in methanol (5 ml.), and added to a solution of sodium borohydride (100 mg.) in water (1 ml.) and methanol (3.5 ml.). After one hour at room temperature, the solution was added to dilute hydrochloric acid and extracted with ether. The extracts were washed with water and dried (anhydr. Mg SO_4), and the solvent was removed to give a solid (87 mg.) which crystallised from methanol as prisms, m.pt. = 193–4°C, undepressed on admixture with the product of the previous reaction. The infra-red and n.m.r. spectra of these two compounds were identical.

The enone CXXIX

Dihydrorosenonolactone (250 mg.) was dissolved in ethanol (10 ml.), and refluxed with selenium dioxide (300 mg.) for 22 hours. Sulphur dioxide gas was passed through the solution which was filtered through celite, added to excess water, and extracted with ether. The extracts were washed with water, and dried (anhydr. $MgSO_4$), and the solvent was removed to give a semi-solid with $\nu_{max} = 1760$ (lactone), 1710 (ketone), and 1670 cm^{-1} (enone), and $\lambda_{max} = 235$ $m\mu$ (3,260).

Since the oxidation was obviously incomplete, the product was dissolved in ethanol (10 ml.) and refluxed with selenium dioxide (250 mg.) for a further four days. The reaction was worked up as above to yield a solid which crystallised from methanol as needles, m.pt. = 159-159.5°C (found, C, 75.82%; H, 9.14%. $C_{20}H_{28}O_3$ requires C, 75.91%; H, 8.92%), $\nu_{max} = 1760$ (lactone), and 1670 cm^{-1} (enone), and $\lambda_{max} = 237$ $m\mu$ (6,850). The n.m.r. spectrum had an unsplit proton at 4.23τ.

Furfuryldihydrorosenonolactone

Dihydrorosenonolactone (2.2 gm.) was dissolved in ethanol (14 ml.) and 15% aqueous sodium hydroxide (3.4 ml.), and freshly distilled furfuraldehyde (450 mg.) was added to the solution which was left overnight at room temperature. The solution was filtered to give a solid (680 mg.) which crystallised from methanol as yellow needles, m.pt. = 192-193°C, $[\alpha]_D = +195°$ (found C, 75.5%; H, 8.3%. $C_{25}H_{32}O_4$ requires C, 75.72%; H, 8.13%), $\nu_{max} = 1770$ (lactone), 1670 (enone) and 1600 cm^{-1} (aromatic), and $\lambda_{max} = 319$ $m\mu$ (23,130).

Ozonolysis of furfuryldihydrorosenonolactone at low temperature

Furfuryldihydrorosenonolactone (100 mg.) was dissolved in AR chloroform (30 ml.) and ozonised at -50°C. An aliquot showed that the 322 mμ band in the ultraviolet spectrum had disappeared after 5 minutes, and the solution was ozonised for a total of 30 minutes and the solvent was removed at low temperature. Glacial acetic acid (10 ml.), 30% hydrogen peroxide (5 ml.) and conc. sulphuric acid (2 drops) were added to the gum and the solution was left overnight at room temperature and heated for 15 minutes on the steam bath. Excess sodium bicarbonate solution was added and the solution was washed with ether. The aqueous phase was neutralised with dilute sulphuric acid and extracted with ethyl acetate. The extracts were washed with water and dried (anhyd. $MgSO_4$), and the solvent was removed to give a gum which was chromatographed on silica gel. Elution with benzene:ether (9:1) gave a solid (20 mg.) which was crystallised from ethyl acetate-petroleum ether (60-80°) with m.pt. = 228-229°C (found C, 70.71%; H, 8.40%. $C_{22}H_{30}O_5$ requires C, 70.56%; H, 8.08%), ν_{max} = 1770 (lactone), 1710 (carboxylic acid) and 1670 cm^{-1} (enone), and λ_{max} = 245 mμ (8,160). The n.m.r. spectrum had singlets at 3.5τ and 5.7τ.

2-furfuryl-7-methoxy indan-1-one (CXXXI)

7 methoxy-indan-1-one (1 gm.) was dissolved in ethanol (10 ml.) and 15% sodium hydroxide solution (3 ml.) and freshly distilled furfuraldehyde (0.75 gm.) was added. After 5 minutes at room temperature, a yellow solid (1.85 gm.) was filtered off. This crystallised from ether as needles, m.pt. = 166.5-167°C (found C, 74.52%; H, 5.06%.

$C_{15}H_{12}O_3$ requires C, 74.9%; H, 5.03%), ν_{max} = 1695 (indanone), and 1630 cm^{-1} (double bond), and λ_{max} = 240 mμ (6,200), 288 mμ (6,300) and 360 mμ (29,400).

The indanone acid (CXXXII)

2-furfuryl-7methoxyindan-1-one (1 gm.) was dissolved in AR chloroform (50 ml.) and ozonised at -50°C for ten minutes. The solvent was removed at reduced pressure, and glacial acetic acid (40 ml.), 30% hydrogen peroxide (5 ml.) and conc. sulphuric acid (2 drops) were added. The reaction was left overnight, and added to excess potassium bicarbonate solution. The basic solution was washed with ether, neutralised with conc. sulphuric acid, and extracted with ether. The extracts were washed with water, and dried (anhyd. Mg SO_4), and the solvent was removed to give a solid (340 mg.) which crystallised from ethanol as yellow needles, m.pt. = 207-208°C (found C, 65.5%; H, 5.34%. $C_{12}H_{10}O_4$ requires C, 65.45%; H, 5.5%), ν_{max} = 1700 cm^{-1} (carboxylic acid) and λ_{max} = 250 mμ (15,120), 288 mμ (9,170) and 345 mμ (5,270). The n.m.r. spectrum had a singlet at 3.59τ.

Ozonolysis of furfuryldihydrorosenonolactone at a higher temperature

Furfuryldihydrorosenonolactone (1 gm.) was dissolved in acetic acid and ozonised at room temperature for 2 hours. Powdered zinc was added, and the solution was left overnight at room temperature, and filtered through celite. Most of the acetic acid was removed under reduced pressure, and excess sodium bicarbonate solution was added. The basic solution was extracted with ethyl acetate, and the extracts were washed with water and dried (anhydr. $MgSO_4$). The solvent was removed to give a solid (529 mg.) which crystallised from methanol as needles, m.pt. = 173-176°C (found C, 72.59%; H, 8.77%. $C_{20}H_{28}O_4$ requires C, 72.26%; H, 8.49%), ν_{max} = 3400 (enol), 1760 (lactone), 1670 (enone) and 1640 cm^{-1} (olefin), and λ_{max} = 287 mμ, shifting to 355 mμ in base. The diosphenol showed a strong black-green colour with alcoholic ferric chloride solution.

Acetylation of the diosphenol

The mother liquors of the diosphenol (λ_{max} = 287 mμ) were left overnight at room temperature with acetic acid (2 ml.) and AR pyridine (2 ml.). The solution was added to sodium bicarbonate solution and extracted with ether, and the extracts were washed with water and dried (anhyd. $MgSO_4$). The solvent was removed to yield a gum with λ_{max} = 254 mμ (5,510).

Trituration of the gum with methanol gave a solid which was crystallised from methanol as needles, m.pt. = 189-192°C (found C, 67.75%; H, 7.45%. $C_{22}H_{30}O_6$ requires C, 67.67%; H, 7.74%), ν_{max} = 1835 and 1780 cm^{-1} (anhydride), 1770 cm^{-1} (γ-lactone). There was no ultra-violet absorption beyond end absorption.

Dihydrorosonolactone

This was prepared by Walley's method$^{(77)}$, and had m.pt. = 157-159°C (lit.$^{(77)}$ m.pt. = 138°C).

Furfuryldihydrorosonolactone

Dihydrorosonolactone (500 mg.) was dissolved in ethanol (3 ml.) with 15% sodium hydroxide solution (0.75 ml.) and freshly distilled furfuraldehyde (200 mg.). The solution was left overnight at room temperature and added to aqueous sodium metabisulphite solution. This solution was extracted with ether, and the extracts were washed with water and dried (anhyd. $MgSO_4$). The solvent was removed to give a gum, $\lambda_{max} = 249$ mμ, which was chromatographed on grade III alumina. Elution with ether gave a solid (40 mg.) which crystallised from methanol as yellow needles, m.pt. = 189-191°C (found C, 75.6%; H, 8.0%. $C_{25}H_{32}O_4$ requires C, 75.7%; H, 8.13%), $\nu_{max} = 1770$ (γ-lactone) and 1670 cm^{-1} (enone), and $\lambda_{max} = 325$ mμ (8,300).

Ozonolysis of furfuryldihydrorosonolactone

Furfuryldihydrorosonolactone (5 mg.) was dissolved in glacial acetic acid and ozonised at room temperature for 3 hours. Powdered zinc was added, and the solution was left overnight at room temperature, filtered through celite, and added to sodium bicarbonate solution. The basic solution was extracted with ethyl acetate, and the extracts were washed with water and dried (anhyd. $MgSO_4$). The solvent was removed to give a gum with $\lambda_{max} = 286$ mμ, shifting to 355 mμ in base.

A thin-layer silica-gel chromatoplate was spotted with samples of the gummy product and the diosphenol from dihydrorosenonolactone, and developed with benzene:ether (1:1). The plate was dried and sprayed with alcoholic ferric chloride solution. The gummy product showed two spots, the least polar of which had the same Rf as the diosphenol from dihydrorosenonolactone.

Reduction of dihydrorosonolactone

Dihydrorosonolactone (150 mg.) was dissolved in methanol (10 ml.) and added to a solution of sodium borohydride (150 mg.) in water (1.5 ml.) and methanol (5 ml.). After one hour at room temperature, excess water was added, and the solution was extracted with ether. The extracts were washed with water and dried (anhydr. $MgSO_4$) and the solvent was removed to give a solid (133 mg.) which crystallised from ethyl acetate with m.pt. = 182-183°C and had an identical infra-red spectrum to that of dihydrorosololactone.

The ketone CIV

This was prepared as reported previously(75) but in much lower yield.

Oxidation of the ketone CIV

The sweet-smelling liquid ketone CIV (350 mg.) was treated with conc. nitric acid (3 ml.) and water (3 ml.), and heated for five minutes on the steam bath. The reaction was allowed to proceed spontaneously, and was then heated for a further 45 minutes on the steam bath, cooled, and added to excess sodium bicarbonate solution. The solution was washed with ether, neutralised with dilute sulphuric acid, and extracted with ethyl acetate. The extracts were washed with water and dried (anhyd. $MgSO_4$), and the solvent was removed to give a gum. Chromatography of the gum and attempted sublimation of the main fractions from the column failed to yield any α-ethyl-α-methyl succinic acid as had been reported(76).

Dibromorosololactone

Rosololactone (200 mg.) was dissolved in dioxan (8 ml.) with pyridine perbromide (300 mg.) and left for 3 hours at room temperature. The solution was added to aqueous sodium thiosulphate solution and extracted with ether. The extracts were washed with water and dried (anhyd. $MgSO_4$), and the solvent was removed to give a semi-solid which yielded a solid (127 mg.) on trituration with methanol. This solid crystallised from ethyl acetate as prisms, m.pt. = 187-189°C, $[\alpha]_D = -9.7^\circ$ (found C, 50.59%; H, 6.05%; Br, 33.52%. $C_{20}H_{30}O_3Br_2$ requires C, 50.2%; H, 6.26%; Br, 33.45%), $\nu_{max} = 3400$ (OH), and 1750 cm^{-1} (γ-lactone).

Rosein VI, the mould acid

The mould acid crystallised from benzene-petroleum ether (60-80°) as fibrous needles, m.pt. = 191-193°C, $[\alpha]_D = \pm 0^\circ$ (found C, 75.39%; H, 9.30%. $C_{20}H_{30}O_3$ requires C, 75.43%; H, 9.50), $\nu_{max} =$ 3400 (OH), 3070 (vinyl CH), 1695 (carboxylic acid), 1020 (OH) and 909 cm^{-1} (vinyl), and $\lambda_{max} = 202.5$ mμ (5,750). The mass spectrum had a parent at 318, and the loss of angular methyl, water and carbon dioxide was observed in the cracking pattern.

Dihydrorosein VI

Rosein VI (17.8 mg.) was added to a presaturated suspension of 10% palladised charcoal (8 mg.) in ethyl acetate (11 ml.). One mole of hydrogen was taken up in 45 minutes and no further uptake was observed. The solution was filtered through celite, and the solvent was removed to give a solid (15 mg.) which crystallised from ether-petroleum ether (60-80°) with m.pt. = 180-184°C, $\nu_{max} = 3380$ (OH), 1695 (CO_2H), and 1020 cm^{-1} (OH), and $\varepsilon_{210} = 1,400$.

Tetrahydrorosein VI

Rosein VI (18.7 mg.) was added to a presaturated suspension of platinum oxide (Adams, 50 mg.) in acetic acid (6 ml.). 2.14 ml. of hydrogen were taken up over 2 hours and a total of 2.57 ml. (1 M = 1.32 ml.) had been taken up after 18 hours. The solution was filtered through celite, added to water and extracted with ether. The extracts were washed with water and dried (anhyd. $MgSO_4$), and the solvent was removed to give a solid which crystallised from ethyl acetate-petroleum ether (60-80°) with m.pt. = 228-230°C, ν_{max} = 3400 (OH) and 1700 cm^{-1} (CO_2H).

Attempted lactonisation of Rosein VI

Rosein VI (14 mg.) was dissolved in AR ethyl acetate (16 ml.) and conc. hydrochloric acid (3 drops), and refluxed for 2 hours. More ethyl acetate was added and the solution was washed with water and dried (anhyd. $MgSO_4$), and the solvent was removed to give a solid (9 mg.), m.pt. = 184-192°C which had an identical infra-red spectrum to starting material. The melting point did not depress on admixture with starting material.

The diol CXXXVI

Desoxyrosenonolactone (500 mg.) was dissolved in anhydrous ether (20 ml.), and added dropwise to a stirred suspension of lithium aluminium hydride (300 mg.) in anhydrous ether. Stirring was continued, at reflux, overnight and excess $LiAlH_4$ was destroyed by dropwise addition of an ethereal ethyl acetate solution. The solution was filtered through celite, washed with water and dried (anhyd. $MgSO_4$), and the solvent was removed to give a solid (424 mg.) which crystallised from petroleum-ether (60-80°) with m.pt. = 144-145°C, $[\alpha]_D = -29°$ (found

C, 78.63%; H, 10.90%. $C_{20}H_{34}O_2$ requires C, 78.4%; H, 11.18%), ν_{max} = 3100 (OH), 1640 (olefin) and 910 cm^{-1} (vinyl).

Oxidation of diol CXXXVI

The diol CXXXVI (134 mg.) in AR pyridine (1 ml.) was added to AR chromium trioxide (130 mg.) in AR pyridine (1 ml.) and left overnight at room temperature. The solution was added to excess water and extracted with ethyl acetate. The extracts were washed with water and dried (anhyd. $MgSO_4$) and the solvent was removed to give a solid (72 mg.) which crystallised from petroleum ether (60-80°), m.pt. = 116°C, undepressed on admixture with desoxyrosenonolactone.

Acetylation of Rosein VI

Rosein VI (13 mg.) was dissolved in AR pyridine (1 ml.) and AR acetic anhydride (1 ml.) and left overnight at room temperature. The solution was added to dilute aqueous hydrochloric acid, and extracted with ether. The extracts were washed with water and dried (anhyd. $MgSO_4$), and the solvent was removed to give a gum with ν_{max} = 1730-1710 (CO_2H and acetate) and 1210 cm^{-1} (acetate).

Diazomethane treatment of rosein VI

Rosein VI was dissolved in excess ethereal diazomethane and left for 4 hours at room temperature. A few drops of acetic acid were added to destroy any remaining diazomethane, and the solvent was removed to give a gum which was chromatographed on grade III alumina to yield a solid (5 mg.), m.pt. = 130-134°C, ν_{max} = 1720 (ester), 1125 (OMe) and 910 cm^{-1} (vinyl). The solid had no ultra-violet absorption above 220 $m\mu$.

Rosein VI ester-acetate

Rosein VI (50 mg.) was dissolved in AR pyridine (1 ml.) and acetic anhydride (1 ml.), and left overnight at room temperature. The solution was added to water and extracted with ethyl acetate. The extracts were washed with dilute hydrochloric acid and water and dried (anhyd. $MgSO_4$), and the solvent was removed to give a gum which was dissolved in excess ethereal diazomethane and left overnight at room temperature.

The solution was washed with sodium bicarbonate solution and water and dried (anhyd. $MgSO_4$) and the solvent was removed to give a liquid (40 mg.) which was chromatographed twice on grade II alumina. This had $\nu_{max} = 1710$ (ester-acetate), and 1210 cm^{-1} (acetate), and the n.m.r. spectrum had protons at 4.16 τ (1H, quartet, $J_{CIS} = 10$ c.p.s., $J_{TRANS} = 17.5$ c.p.s.), 5.10 τ (1H, doublet, $J_{TRANS} = 17.5$ c.p.s.), 5.13 τ (1H; doublet $J_{cis} = 10$ c.p.s.), 6.34 τ (3H, singlet, OMe), 7.97 τ (3H, singlet, OAc), 8.72 τ (3H, singlet, Me), 9.08 τ (3H, singlet, Me), and 9.13 τ (3H, singlet, Me).

The keto ester CXXXVIII

Rosein VI (20 mg.) in acetone (2 ml.) was treated dropwise with 0.2 ml. of a solution of AR chromium trioxide (260 mg.) in conc. sulphuric acid (0.2 ml.) and water (1 ml.) over one minute at room temperature with stirring. Stirring was continued for a further 4 minutes and the solution was added to water and extracted with ether. The extracts were washed with water and dried (anhyd. $MgSO_4$), and the solvent was removed to give a solid (18 mg.) which crystallised from methanol as needles m.pt. = 217-222°C, ν_{max} = 1705 cm^{-1} (ester and ketone), and 905 cm^{-1} (vinyl).

The acid, m.pt. = 217-222°C was dissolved in excess ethereal diazomethane and left overnight at room temperature. Removal of the solvent gave a solid which crystallised from methanol as needles (6 mg.), m.pt. = 135-136°C (found C, 76.61%; H, 9.08%. $C_{21}H_{30}O_3$ requires C, 76.32%; H, 9.15%), ν_{max} = 1730 (ester), 1710 (ketone) and 910 cm^{-1} (vinyl). The compound had no ultra-violet absorption above 220 mμ and the n.m.r. spectrum is discussed on p.36.

Phosphorus oxychloride treatment of Rosein VI

Rosein VI (20 mg.) was dissolved in AR pyridine (0.3 ml.) with freshly distilled phosphorus oxychloride (0.075 ml), and left overnight at room temperature. The solution was added to excess water and extracted with ether. The extracts were washed with water and dried (anhyd. $MgSO_4$), and the solvent was removed to give a gum, ν_{max} = 1710 (low intensity, CO_2H), 1630, 1620, 1580, 1540 and 820 cm^{-1}, and λ_{max} = 290 mμ.

Rosein VI (3 mg.) was treated with freshly distilled phosphorus oxychloride (2 drops) in AR pyridine (0.3 ml.) at room temperature, and the mixture was worked up as above to give a semi-solid with ν_{max} = 1620, 1605, 1530 cm^{-1} and λ_{max} = 235 mμ.

Pyrolysis of Rosein VI ester acetate

Rosein VI ester acetate (9.4 mg.) was pyrolysed at 0.1 mm. pressure. A fraction distilled at 150-160°C with no appreciable ultra-violet absorption, while a further fraction distilled at 180-200°C with

$\lambda_{max} = 243\ m\mu$ and $\nu_{max} = 1720\ cm^{-1}$ (ester).

The diene CXXII

This was prepared by the method of Whalley(77), and diazomethane treatment gave the methyl ester.

The pyrolysis product of $\lambda_{max} = 243\ m\mu$ from the above reaction, and the diene methyl ester were spotted on a silica-gel thin layer chromatoplate, which was developed with benzene:ether (1:1). The plate was dried and left in an atmosphere of phosphorus pentachloride overnight. Ultra-violet irradiation showed seven spots in the pyrolysis product, the fastest moving spot having the same Rf as the diene ester.

The Acid CXLII

Desoxyrosenonolactone (200 mg.) was dissolved in ethanol (60 ml.) and 12N hydrochloric acid (5 ml.) and refluxed for 3 hours. Most of the ethanol was removed at reduced pressure, and the solution was added to water and extracted with ether. The extracts were washed with water and dried (anhyd. $MgSO_4$), and the solvent was removed to give a solid which crystallised from petroleum ether (60-80°) as prisms m.pt. = 138-140°C (108 mg.) (found C, 79.6%; H, 10.06%. $C_{20}H_{30}O_2$ requires C, 79.42%; H, 10.00%), $\nu_{max} = 1695$ (CO_2H), 1640 (olefin) and 907 cm^{-1} (vinyl).

Treatment of the acid with excess ethereal diazomethane overnight gave the methyl ester as a liquid which distilled at 90-100°C and 0.1 mm. pressure (found C, 78.80%; H, 9.95%. $C_{21}H_{32}O_2$ requires C, 79.7%; H, 10.19%), $\nu_{max} = 1740$ (ester), 1630 (olefin) and 910 cm^{-1} (vinyl).

The dihydroester of CXLII

The methyl ester of acid CXLII (68 mg.) was added to a presaturated suspension of 10% palladised charcoal (30 mg.) in ethyl acetate (7 ml.). 5.2 ml. of hydrogen was taken up, and the solution was filtered through celite. The solvent was removed to give the dihydroester as an oil.

Reduction of the keto-ester of Rosein VI

The keto-ester (1.8 mg.) was dissolved in glacial acetic acid (0.3 ml.) and 40% boron trifluoride etherate (5 drops) and ethane dithiol (4 drops) were added to the solution which was left overnight at room temperature and added to water. The solution was extracted with ether, and the extracts were washed with water and dried (anhyd. $MgSO_4$). The solvent was removed to give a gum.

The gummy mercaptal was dissolved in dioxan (1.5 ml.) and water (1.5 ml.), and W2 Raney nickel (20 mg.) was added. The suspension was refluxed overnight, added to excess water, and extracted with ether. The extracts were washed with water and dried (anhyd. $MgSO_4$), and the solvent was removed to give a gum with $\nu_{max} = 1740$ cm^{-1} (ester).

The gummy product and the methyl ester and dihydro-methyl ester of the acid CXLII were spotted on a silica-gel thin layer chromatoplate which was developed with benzene. The plate was dried and left in an atmosphere of phosphorus pentachloride overnight. Ultra-violet irradiation showed six spots for the gummy reduction product, the fastest moving of which had the same Rf as both model compounds.

Lactonisation of Rosein VI

Rosein VI (6 mg.) was dissolved in conc. sulphuric acid (1 ml.) and left at -10^{0}C for 2 days. The red solution was added to sodium bicarbonate solution and extracted with ether. The extracts were washed with water and dried (anhydr. $MgSO_4$), and the solvent was removed to give a gum, $\nu_{max} = 3500$ (OH) and 1780 cm^{-1} (lactone) and $\lambda_{max} = 240$ $m\mu$.

The Ether CXLV

The diol CXXXVI (100 mg.) and freshly recrystallised para-toluene-sulphonyl chloride (200 mg.) were refluxed in dry pyridine (5 ml.) for 5 hours. Dilute sulphuric acid solution was added, and the solution was extracted with ether. The extracts were washed with water and dried (anhydr. $MgSO_4$), and added dropwise to a stirred suspension of lithium aluminium hydride (300 mg.) in anhydrous ether (20 ml.). The solution was stirred at reflux for six hours, excess $LiAlH_4$ was destroyed by dropwise addition of an ethereal ethyl acetate solution, and dilute sulphuric acid was added. The ether layer was washed with water and dried (anhydr. $MgSO_4$), and the solvent was removed to give a gum which was distilled at 100-120^{0} and 0.1 mm pressure to yield a white solid (38 mg.), m.pt. = 45-51^{0}C (found C, 82.69%; H, 11.55%. $C_{20}H_{34}O$ requires C, 83.27%; H, 11.18%), $\nu_{max} = 1630$ and 910 cm^{-1} (vinyl) with a singlet (2H) at 6.28τ in the n.m.r. spectrum.

Acid treatment of the ether CXLV

The ether (40 mg.) was refluxed with ethanol (10 ml.) and conc. hydrochloric acid (1 ml.) for 4 hours and the solution was added to water and extracted with ether. The extracts were washed with water and dried (anhyd. $MgSO_4$) and the solvent was removed to give a solid (32 mg.) which crystallised from petroleum ether (60-80°) with m.pt.= 98 -102°C and $\nu_{max} = 3350$ (OH), 1630 and 910 cm^{-1} (vinyl).

The alcohol CXLIV

The diol CXXXVI (100 mg.) was refluxed with ethanol (30 ml.) and 12N hydrochloric acid (3 ml.) for 4 hours. The solution was added to water and extracted with ether, and the extracts were washed with water and dried (anhyd. $MgSO_4$). The solvent was removed to give a solid (71 mg.) which crystallised from petroleum-ether as needles m.pt.= 106-108°C (found C, 83.42%; H, 11.09. to $C_{20}H_{32}O$ requires C, 83.27%; H, 11.18%). The infra-red spectrum of this compound was identical to that of the product of the previous reaction, and a mixed melting point was undepressed.

Reduction and oxidation of the ether CXLV

The ether (90 mg.) was added to a presaturated suspension of 10% palladised charcoal (30 mg.) in ethyl acetate (6 ml.), and 1 mole of hydrogen was taken up. The solution was filtered through celite and the solvent was removed to give a gum which was dissolved in AR pyridine (1 ml.) and added to AR chromium trioxide (90 mg.) in pyridine (1 ml.). The solution was left overnight at room temperature, added to dilute sulphuric acid, and extracted with ether. The extracts were washed with water and dried (anhyd. $MgSO_4$) and the solvent was removed to give a gum, $\nu_{max} = 1780$ cm^{-1} (γ-lactone).

The diene CXLVI

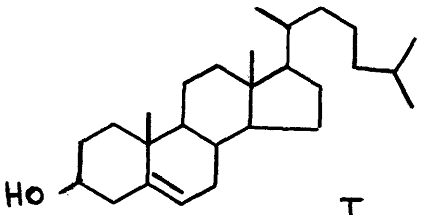
The alcohol CXLIV (160 mg.) was dissolved in dry pyridine (5 ml.)

with freshly recrystallised *para*- toluene sulphonyl chloride (160 mg.) and refluxed for four hours. The solution was added to dilute sulphuric acid and extracted with ether. The extracts were washed with water and dried (anhyd. $MgSO_4$), and added dropwise to a stirred suspension of lithium aluminium hydride (200 mg.) in anhydrous ether (30 ml.) Stirring was continued at reflux for five hours and the excess $LiAlH_4$ was destroyed by dropwise addition of ethereal ethyl acetate. The solution was washed with dilute sulphuric acid and water and dried (anhyd. $MgSO_4$), and the solvent was removed to give a gum which distilled at 105-115°C and 0.1 mm. pressure (found C, 89.33%; H, 11.06%. $C_{20}H_{30}$ requires C, 88.82%; H, 11.18%), $\nu_{max} = 1630$, 910 and 885 cm^{-1}, and $\lambda_{max} = 246$ mμ (3,600).

Reduction of the diene, using 10% palladised charcoal in ethyl acetate, resulted in absorption of two moles of hydrogen.

Part 2

An attempted *in vitro* removal of the 14α methyl group of lanostenol



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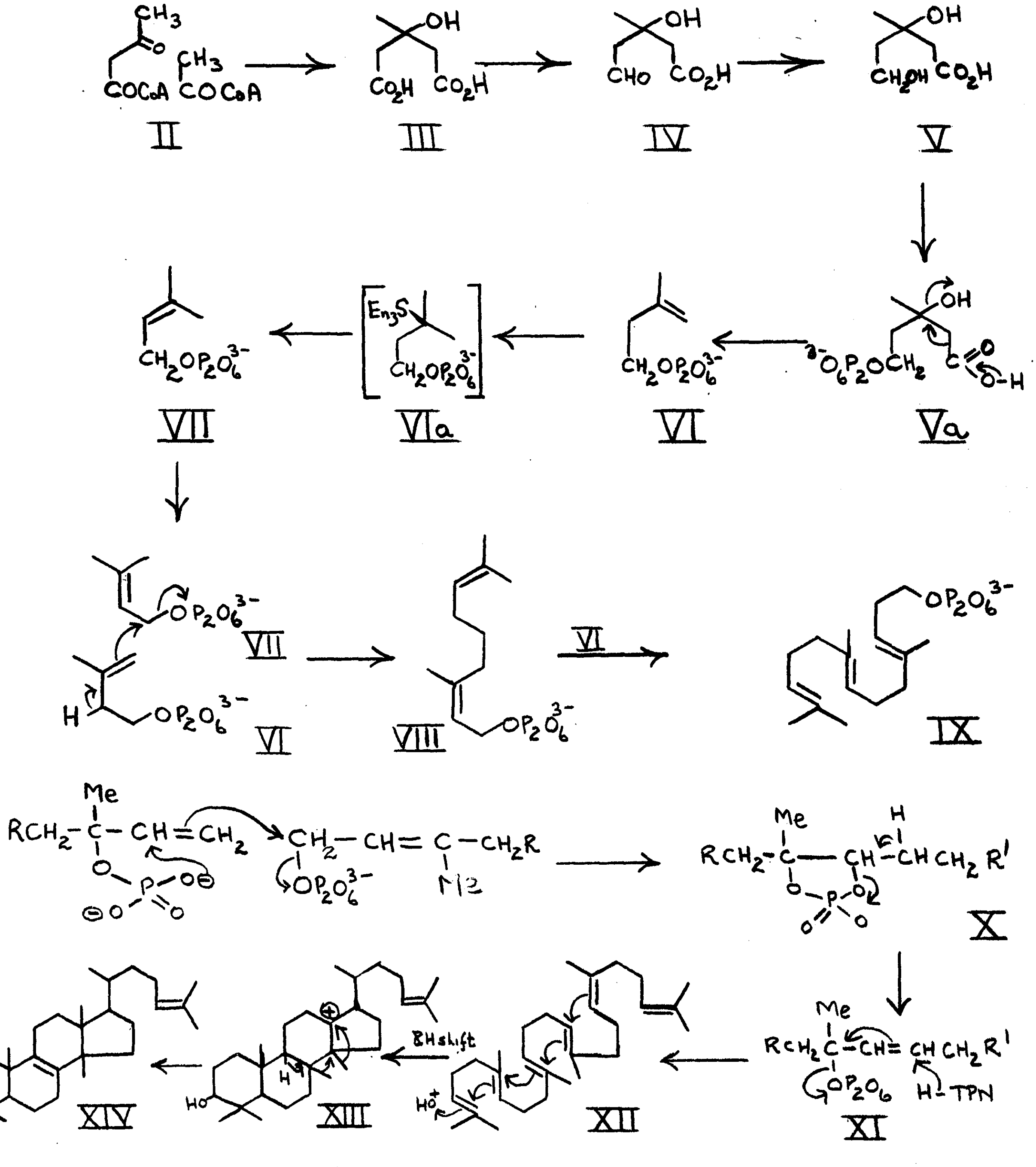


CH3
COCoA
II
OH
CO2H
III
CHO
IV
CH2OH
V
OP2O6 3-
Va
VI
EnzS
VIa
VII
VIII
IX
Me
RCH2
TPN
X
XI
XII
8H shift
XIII
HO
XIV

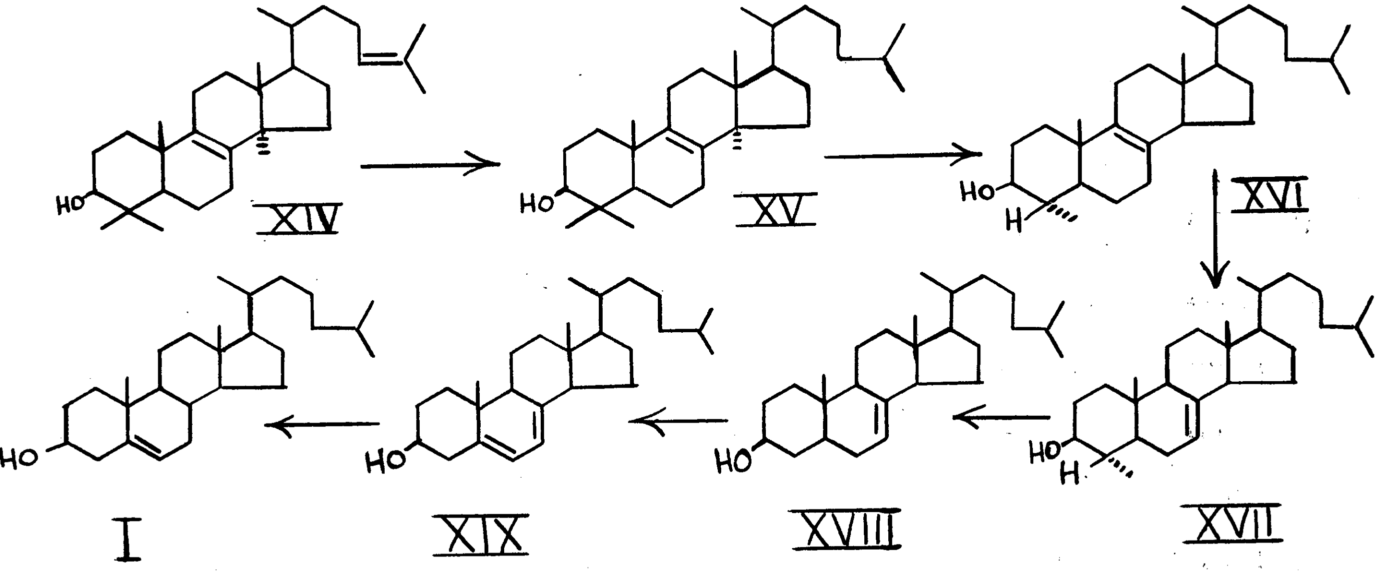
Historical

The biogenesis of cholesterol (I) is one of the most carefully worked out biogenetic pathways, and the topic has been well reviewed(94)(95)(96)(97). In the first steps, acetate condenses with acetoacetyl-coenzyme A (II) to give β hydroxy-β-methylgluaric acid (III)(98) which is reduced via mevaldic acid (IV) to yield (+)-mevalonic acid (V)(98)(99). Mevalonic acid is phosphorylated and concertedly decarboxylated and dehydrated to isopentenylpyrophosphate (VI)(100)(101) which, under the influence of a thiol-containing enzyme, will isomerise to dimethylallylpyrophosphate (VII)(102).

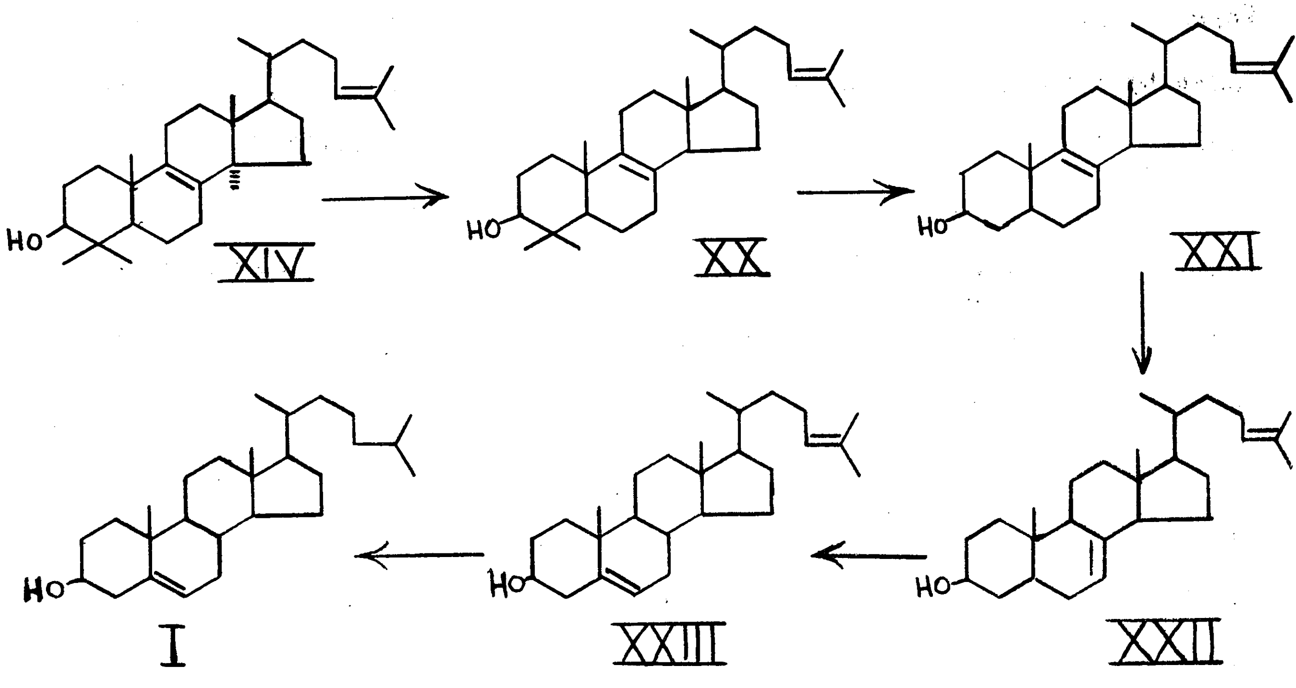
Condensation of isopentenylpyrophosphate (VI) with dimethylallylpyrophosphate (VII) gives rise to geranylpyrophosphate (VIII) which can condense with another molecule of isopentenylpyrophosphate (VI) to give farnesylpyrophosphate (IX). Two molecules of farnesylpyrophosphate condense by a mechanism involving addition of one hydrogen atom from TPNH, such as (IX) → (X) → (XI) → (XII) to yield squalene (XII)(103).

Squalene (XII) cyclises concertedly by a process initiated by atmospheric oxygen probably as the $HO^{\oplus}$ cation on C_3(104)(105), to give the hypothetical intermediate (XIII) which can undergo two 1.2 methyl shifts(106)(107)(108) to yield lanosterol (XIV).

The steps in the conversion of lanosterol to cholesterol have recently been established. Woodward(109) first suggested lanosterol as an intermediate in cholesterol biogenesis, and this was later shown to be the case by labelling experiments(110). The lanosterol to cholesterol conversion requires rearrangement of the annular double



Scheme 1 for cholesterol biosynthesis



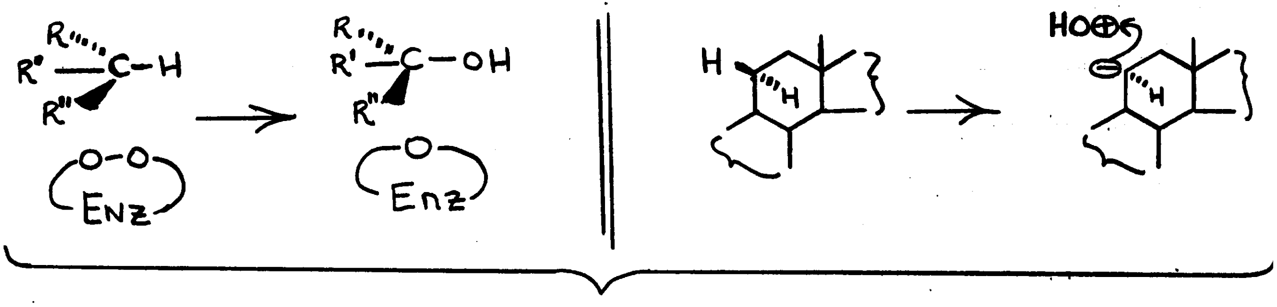
Scheme 2 for cholesterol biosynthesis

bond from the $C_{8(9)}$ position to the C_5 position, reduction of the side chain double bond, and loss of three methyl groups. The methyl groups are lost as carbon dioxide(111) in a sequence which requires oxygen and is believed to involve oxidation of the methyl groups to carboxyl groups(112).

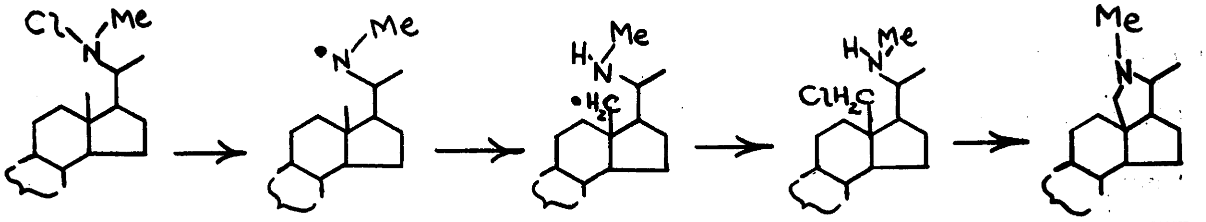
Δ^7-Cholestenol (XVIII) is known to be converted to cholesterol(113)(114) and 4α-methyl-Δ^7-cholestenol (XVII) has been observed in rat tissues and converted to cholesterol in vivo(115)(116)(117). Kandutsch and Russel(118) have identified lanosterol (XIV), 24.25 dihydrolanosterol (XV), 4α-methyl-Δ^8 cholestenol (XVI), Δ^7cholestenol (XVIII), and 7-dehydrocholesterol (XIX) in mouse tumours with cholesterol, and found that these compounds could be converted to cholesterol in vivo. 24.25-Dihydrolanosterol (XV) has recently been obtained from lanosterol in rat liver homogenates(119). From these results, the scheme (XIV)→(XV)→(XVI)→(XVII)→(XVIII)→(XIX)→(I) can be put forward as a mode of cholesterol bioxynthesis. Bloch has shown that oxidation-reduction of the C_3 alcoholic function occurs during this process(120).

An alternative scheme for cholesterol biosynthesis exists, since 14 desmethyllanosterol (XX)(111)(121), zymosterol (XXI)(122)(123), and desmosterol (XXIII)(124)(125) have been isolated and converted to cholesterol in vivo. The scheme involves reduction of the side-chain double bond at a later stage, and may be represented as XIV→XX→XXI→XXII→XXIII→I.

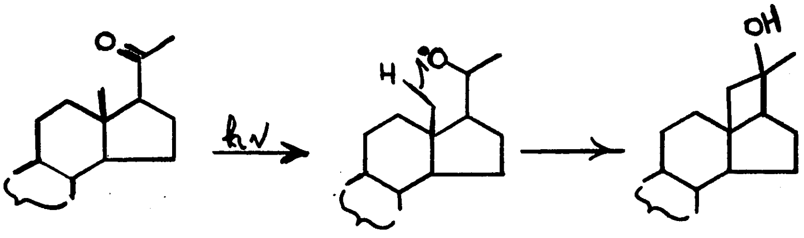
The loss of the methyl group in the lanosterol-squalene interconversion has been of considerable interest. Since 18 and 19



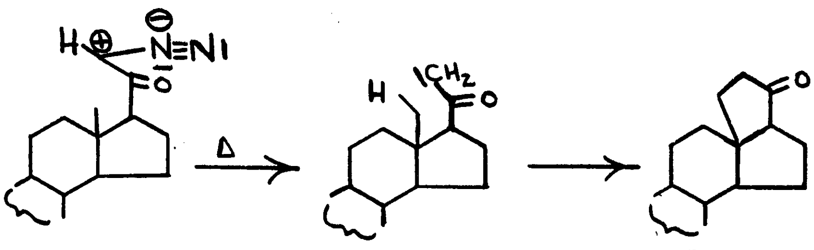
XXIV



XXV XXVI XXVII XXVIII XXIX



XXX XXXI XXXII

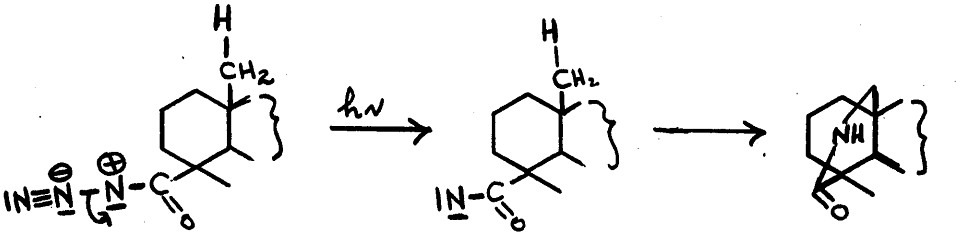


XXXIII XXXIV XXXV

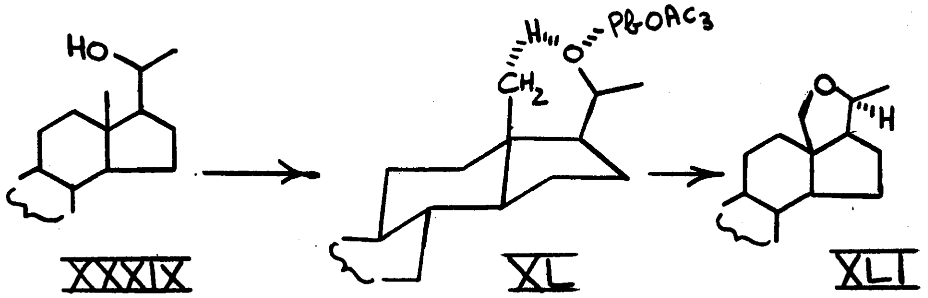
hydroxylated desoxycortisones have been found in Nature(126) and 19 hydroxysteroids are considered to be likely precursors of ring A *nor*-steroids such as oestrone(127)(128), it is reasonable to assume that the first step in the demethylation may be hydroxylation of the methyl groups. Investigation of biological hydroxylation to the 7α(129), 11β(130)(131), and 12β(132) hydroxy steroids has indicated that the hydroxylation occurs by replacement of hydrogen without inversion of stereochemistry, and Corey(133) has suggested electrophilic oxygen as the active agent, comparing the enzyme-oxygen complex to a peroxide or peracid, as in XXIV opposite.

In vitro functionalisation of non-active methyl groups, by intramolecular attack by groups near these groups in space, has been achieved by a variety of methods. The first reported functionalisation of inactive methyl groups was the use of the Loeffler-Freitag reaction(134)(135) to prepare conessine derivatives as shown in XXV→ XXIX opposite. The mechanism is of the free radical chain type(136), and radical abstraction by nitrogen is favoured by the juxtaposition of the two groups involved. A similar radical abstraction reaction involving oxygen rather than nitrogen as the abstracting radical, was the photolysis of C_{20} ketones which gave rise to cyclobutanol products as in XXX→ XXXII opposite(137).

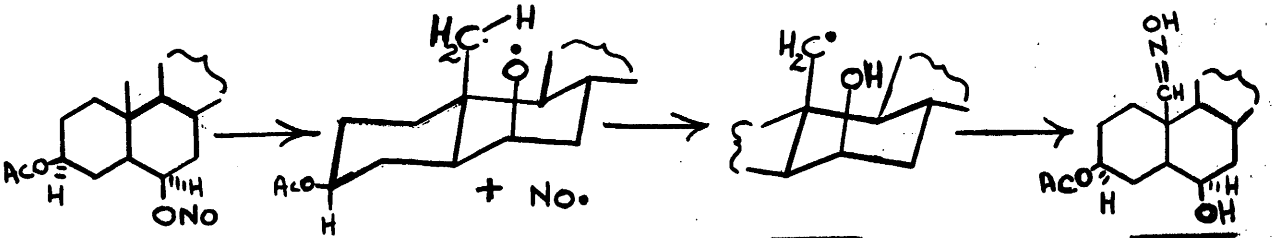
Pyrolysis of 21.diazo-5α-pregnan-20-one (XXXIII) resulted in the formation of a carbon-carbon bond between the 18 methyl group and C_{21}(138) by carbene insertion as shown in XXXIII→XXXV opposite. Photolysis of azides will cause nitrene insertion in a similar manner



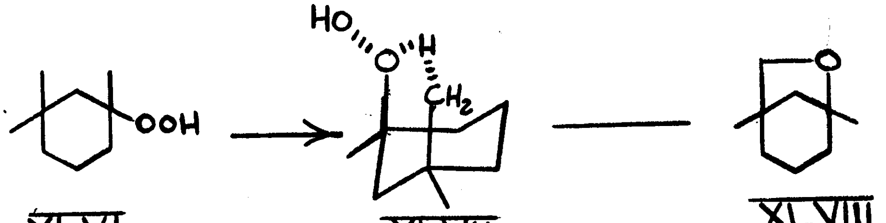
XXXVI XXXVII XXXVIII



XXXIX XL XLI



XLII XLIII XLIV XLV



XLVI XLVII XLVIII

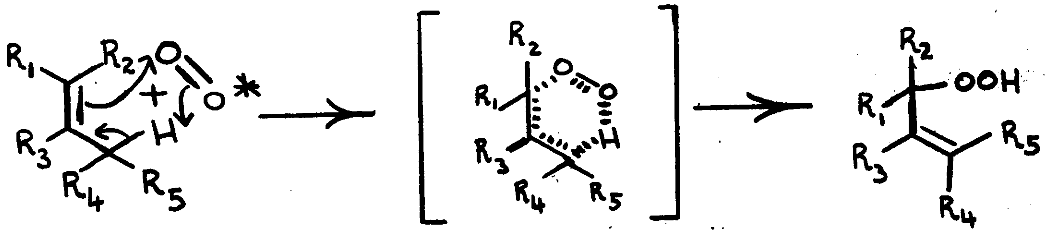
as in XXXVI$\rightarrow$XXXVIII opposite, and use has been made of this in Barton's conessine synthesis(139) and Edwards' diterpene alkaloid synthesis(23).

Treatment of steroidal alcohols with lead tetra-acetate will give rise to cyclic ethers such as XLI, and the reaction is thought to involve the transition state XL(90)(140)(141). The reaction has been used to functionalise the C_{18} methyl group in an aldosterone synthesis(142). Hypochlorites can be photolysed to give functionalisation of suitably placed inert methyl groups(143)(144), and lead tetraacetate treatment of hypohalites gives similar results(145).

Photolysis of nitrites has been used to functionalise both 18 and 19 methyl groups(146)(147) by a mechanism such as XLII$\rightarrow$XLV opposite. The radical nitrite is thought to be held in a solvent cage. The reaction has been used in a partial synthesis of aldosterone(148).

The methods of functionalising methyl groups, summarised above, have all involved some sort of free radical reaction, and are thus not good analogies for Corey's model for biological hydroxylation which involves attack of cationic oxygen. A closer analogy to this model was the reaction of 1.3.4 trimethylcyclohexane hydroperoxide (XLVI) with <u>para</u>-nitrobenzenesulphonyl chloride in pyridine and methylene chloride, which resulted in the ether (XLVIII) via the transition state XLVII(149).

$$[\mathrm{DYE}] \xrightarrow{h\nu} [\mathrm{DYE}]^{*} \xrightarrow{O_2} [\mathrm{DYE.O_2}]^{*} \longrightarrow O_2^{*}$$



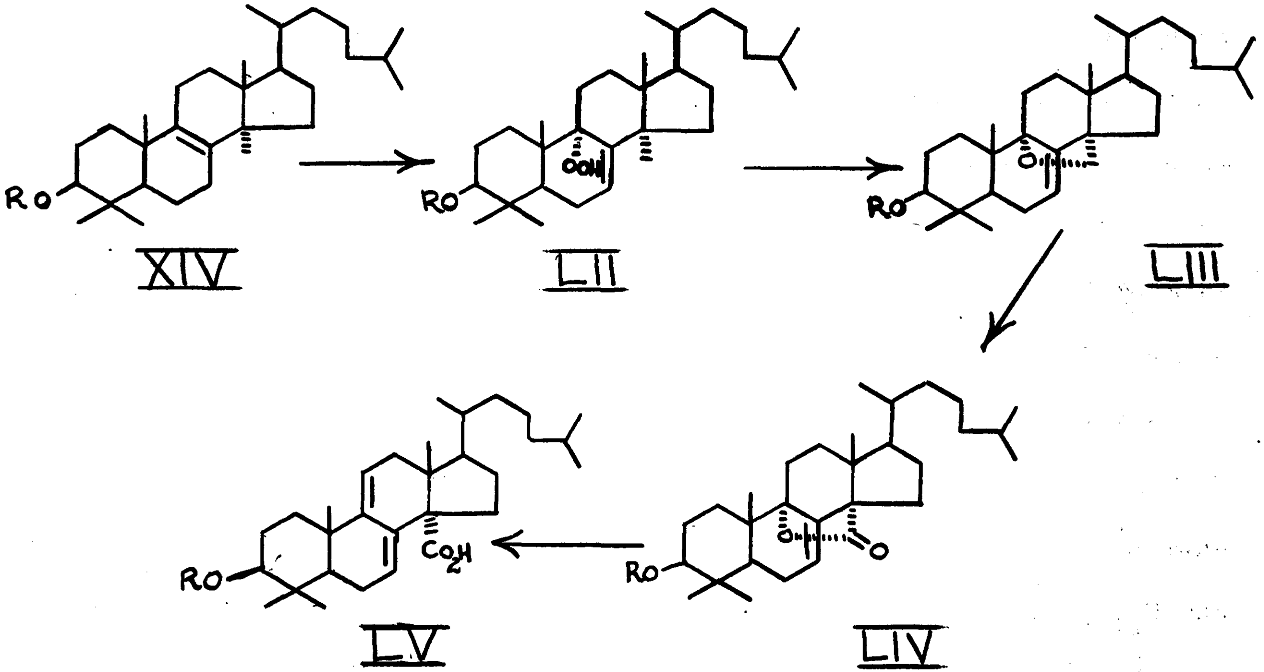
XLIX L LI

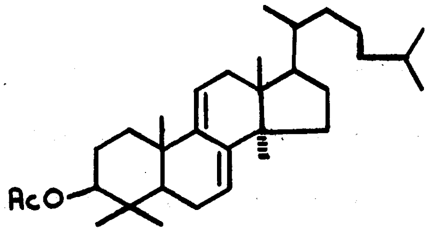
Theoretical

As we have seen, the first stage in the conversion of lanosterol to cholesterol is the removal of the 14α methyl group. To achieve in vitro oxidation of this methyl, a suitable group would have to be introduced in a 1.3 diaxial relationship to it. The $\Delta^{8(9)}$ double bond makes the 9α and 7α positions particularly labile, and these are, therefore, the best positions for substitution with a view to removal of the 14α methyl group as they are both in a 1.3 diaxial relationship to it. Since molecular oxygen is involved in biological hydroxylation(150)(151)(152), a reaction involving molecular oxygen would be a closer in vitro analogy.

Autoxidation of olefins is known to give allylic hydroperoxidation(153)(154), and lanosterol would be expected to oxidise in the C_7 and C_{11} positions. Autoxidation of lanosterol is known, however, to give the 7β and 11β hydroperoxides(155), and this would be useless as a means of obtaining the 7α hydroperoxide. Oxygenation of olefinic double bonds in the presence of light and a photosensitiser gives hydroperoxides in which the double bond has rearranged(156). The proposed mechanism(156) of this reaction involves excitation of the photosensitiser by light and the formation of an excited photosensitiser-oxygen complex. The complex breaks down to yield excited oxygen which will react with the double bond to give the rearranged allylic hydroperoxide L1 via the six membered transition state L(157).

Various steroidal olefins have been hydroperoxidised in this manner and attack has usually been from the less hindered α-face of the molecule(156). In dihydrolanosterol the C_8 position is more hindered





LVI

than the C_9 position due to the proximity of the 14α methyl group and so 9α hydroperoxylanost-7ene-3β ol (L11) would be the expected product of photosensitised oxidation. This compound could be used to functionalise the C_{14} methyl group under the influence of a powerful leaving group such as the <u>para</u>-nitrobenzene sulphonyl group. The C_9-C_{14} methyl ether (L111) would be the expected product by analogy to the Corey reaction(149) (see p. 65), and this ether could be oxidised to the unsaturated lactone (L1V) which would open to the acid (LV) from which the C_{14} group would be readily lost by decarboxylation. In this sequence, the C_{14} methyl group would be removed, and the double bond would be shifted to the 7 position. Both of these are steps in the <u>in vivo</u> conversion of lanosterol to cholesterol.

Dihydrolanosterol was oxygenated in pyridine in the presence of light and haematoporphyrin, and the resultant product, which liberated iodine from sodium iodide solution, was reduced and acetylated. Chromatography of the resultant gum gave 3β-acetoxylanosta-7.9(11)-diene (LVI) as the only solid product. This result might indicate that 9 hydroperoxidation had in fact occurred since the 9α-alcohol would be readily dehydrated under acetylation conditions. The photo-oxygenation was, therefore, repeated on dihydrolanosterol acetate, and reduction and chromatography gave 3β-acetoxylanosta-7.9(11)-diene, m.pt. = 147-149°C, $[\alpha]_D = +91.1^\circ$, $\lambda_{max} = 236\ m\mu$, $245\ m\mu$ and $254\ m\mu$ (lit.(158) m.pt. = 160-169°C, $[\alpha]_D = +89^\circ$). The mass spectrum of this sample showed a peak at mass number 455 which was not present in the mass spectrum of an authentic sample (M = 468) but no meaning could be attached to this information.

An attempt to isolate the 9α hydroperoxide by reverse phase partition chromatography between n-heptane and aqueous ethanol on a column of non-wetting supercell was unsuccessful. The lability or intractability of any 9α-hydroperoxide formed in the photo-oxygenation reaction might be overcome by carrying out the reaction in the presence of para-nitrobenzene-sulphonyl chloride so that the peroxide might react with the 14α-methyl group in situ.

When the in situ reaction was carried out, a gum was obtained which was chromatographed on grade III alumina. Elution with petroleum ether gave unchanged para-nitrobenzene-sulphonyl chloride and 3β-acetoxylanosta-7.9(11) diene. Petrol:benzene (9:1) gave a solid which crystallised from petroleum ether as needles, m.pt. = 142-144°C, $[\alpha]_D = +164^{\circ}$, in 1.5% yield. This compound analysed as $C_{32}H_{52}O_3$ and was designated I.P.I.

Elution with petrol:benzene (4:1) gave a compound I.P.2 which crystallised from petroleum ether as needles, m.pt. = 146-151°C, $[\alpha]_D = +19^{\circ}$. The infra-red spectrum had peaks at 1650 cm^{-1} and 1580 cm^{-1} and the ultra-violet absorption was at 255 mμ, which showed that the compound was an enone. It was, in fact, 3β-acetoxylanost-8-ene-7-one (lit.$^{(158)}$ m.pt. = 149-155°C, $[\alpha]_D = +22^{\circ}$).

Elution with benzene:ether (19:1) gave a solid which crystallised from ethyl acetate as needles m.pt. = 175-176°C, $[\alpha]_D = +35.2^{\circ}$. The compound analysed as $C_{32}H_{52}O_4$ and was designated I.P.3. It has been obtained in as much as 14% yield.

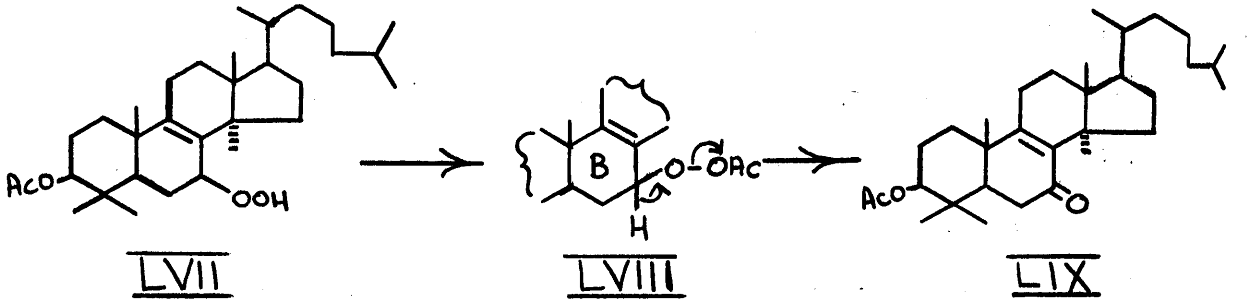
When repeat runs of this reaction were chromatographed, a compound was eluted after the elution of compound I.P.1. and before the elution of 3β-acetoxylanost-8ene-7one. This crystallised from petroleum ether with m.pt. = 182-183°C, $[\alpha]_D = -4.5^\circ$, $C_{32}H_{52}0_3$, and was designated I.P.4.

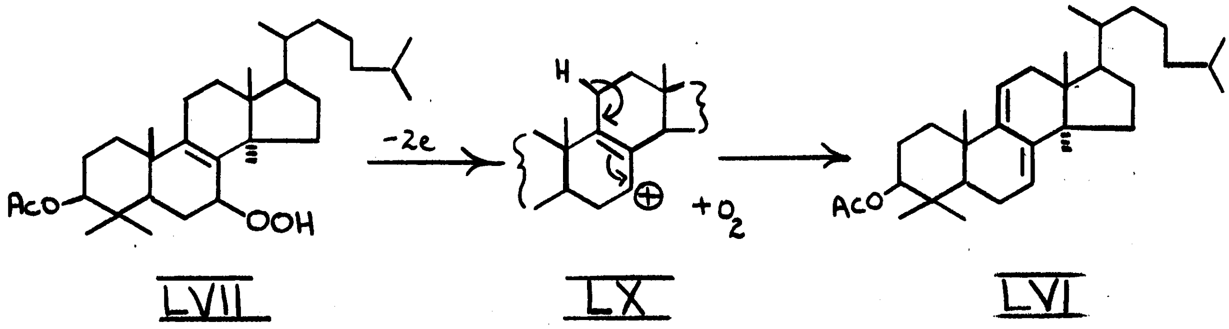
Rechromatography of the fractions which had eluted between the elution of 3β-acetoxylanosta-7.9(11)-diene and compound I.P.1 yielded a new compound, I.P.5., which crystallised from petroleum ether as prisms, m.pt. = 171.5-174°C, $[\alpha]_D = +94.3^\circ$, $C_{32}H_{52}0_3$, in 0.25% yield.

The products I.P.1., I.P.4., and I.P.5., analysed correctly for the required cyclic ether (L111). Oxidation of these compounds under conditions which had yielded lactones from similar ethers$^{(90)}$ failed to give products with lactone absorption in the infra-red.

The compound I.P.3., $C_{32}H_{52}0_4$. m.pt. = 175-176°C, $[\alpha]_D = +35.2^\circ$ had an infra-red spectrum with peaks at 3500 cm^{-1} (OH), 1737 cm^{-1} (acetate) and 1634 cm^{-1} (olefin) and no ultra-violet absorption above 220 mμ. The 3500 cm^{-1} peak in the infra-red spectrum was taken as being indicative of the presence of an alcoholic function in the molecule, and the compound was treated with acetic anhydride in pyridine in the hope of obtaining the acetate. The resultant product was, however, 3β-acetoxylanost-8ene-7one, $C_{32}H_{50}0_3$, $\lambda_{max} = 257$ mμ (13,750), $\nu_{max} = 1720$ cm^{-1} (acetate), 1650 and 1570 cm^{-1} (enone) with m.pt. = 145-149°C, undepressed on admixture with an authentic sample of the enone.

Treatment of the compound I.P.3, with manganese dioxide


AcO
OOH
LVII
B
O-OAc
H
AcO
O
LVIII
LIX


AcO
OOH
LVII
-2e
H
⊕
+O2
AcO
LX
LVI

gave 3β-acetoxylanosta-7.9(11)-diene, λ_{max} = 236, 244 and 251 mμ, with m.pt. = 164-167°C undepressed on admixture with an authentic sample. If the compound had been an allylic alcohol, an enone would have resulted from this oxidation, and it was, therefore, concluded that the compound was not an alcohol. The 3500 cm^{-1} peak in the infra-red spectrum could be explained if the compound were a hydroperoxide, and this assignment was supported by the fact that the compound gave a red colouration with ferrous thiocyanate solution(159).

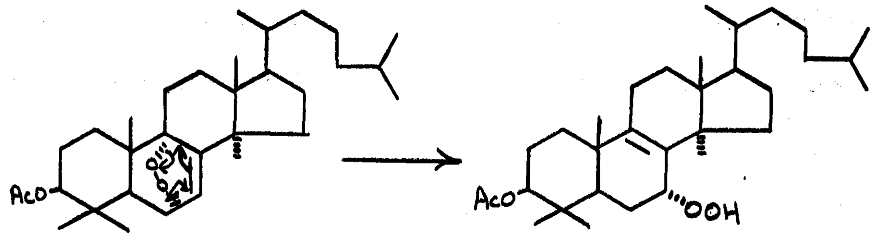
The results of the acetylation and oxidation experiments could only be explained if the compound were 3β-acetoxylanost-8-ene-7-hydroperoxide (LVII). Acetylation of LVII would be followed by loss of the acetate ion from LVIII to give the enone LIX as shown opposite. Oxidation of hydroperoxides with multivalent metal ions has been observed to give rise to an alkyl cation and molecular oxygen(160), and so the formation of the 7.9(11)-diene (LVI) can be rationalised as in LVII → LX → LVI opposite.

The hydroperoxide I.P.3 was not identical to 3β-acetoxylanost-8ene-7β hydroperoxide which had been isolated by Horn and Ilse(155) from the autoxidation of 3β-acetoxylanost-8ene, and it was highly likely that this compound was the 7α epimer. The n.m.r. spectrum of I.P.3 had the $\mathrm{C\underline{H}OOH}$ and $\mathrm{C\underline{H}OAc}$ protons at ca.5.6τ and it was impossible to impute the stereochemistry from this. The differences in molecular rotation between 3β-acetoxylanost-8ene and the compounds I.P.3 and 7β-hydroperoxy-3β acetoxylanost-8 ene were measured, and the difference between these was of the same order as that for the 7α and 7β steroidal

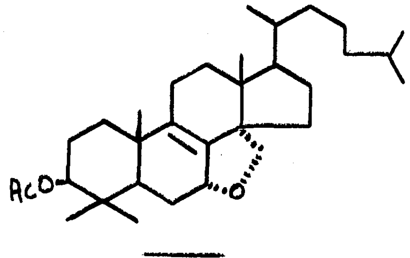
TABLE 1.

3β-acetoxylanost-8 ene	$[M]_D = +271^\circ$
7β-hydroperoxylanost-8 ene-3β- acetate	$[M]_D = +335^\circ$
Product I.P.3	$[M]_D = +176^\circ$

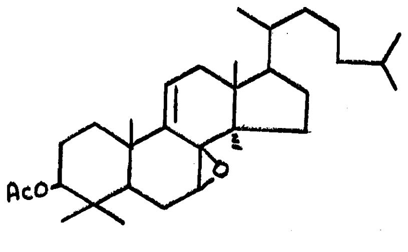
$$\left.\begin{array}{l}\Delta_{I.P.3} = -95^\circ\\ \Delta_{7\beta OOH} = +64^\circ\end{array}\right\} \Delta\Delta = +159^\circ \qquad \left.\begin{array}{l}\Delta_{7\alpha OH} = -59^\circ\\ \Delta_{7\beta OH} = +110^\circ\end{array}\right\} \Delta\Delta = +169^\circ$$



LII LVIIa



LXI



LXIa

alcohols$^{(161)}$, as shown in table 1 opposite. The compound I.P.3 is therefore 3β-acetoxylanost-8-ene-7α-hydroperoxide (LVlla).

Since allylic hydroperoxidation$^{(155)}$ had yielded the 7α-hydroperoxide, it was possible that compound I.P.3 had been formed by rearrangement of the first-formed 3β-acetoxylanost-7ene-9α-hydroperoxide (LII) as shown opposite. The rearrangement of tertiary to secondary hydroperoxides had been reported by Schenk$^{(162)}$ and Lythgoe$^{(163)}$, and the mechanism requires retention of configuration.

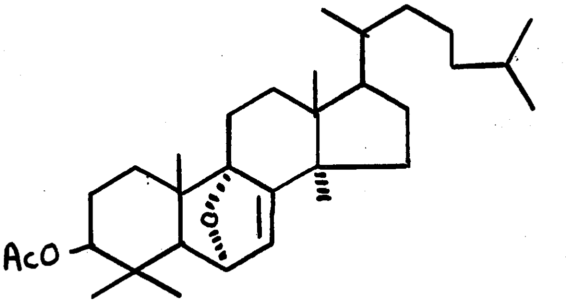
In the hope of functionalising the C_{14} methyl group via the C_7- C_{14}-methyl ether (LXI), 3βacetoxylanost-8ene-7α hydroperoxide was treated with <u>para</u>-nitrobenzene-sulphonyl chloride in pyridine but no reaction occurred, the only compound to be isolated being starting material.

The compound I.P.4., $C_{32}H_{52}O_3$, m.pt.= 182-183°C, $[\alpha]_D$= -4.5°, had only end absorption in the ultra-violet, and the infra-red spectrum had peaks at 1720 and 1240 cm^{-1} typical of the acetate group. The n.m.r. spectrum exhibited one olefinic proton at 4.36 τ which was split symmetrically by two other protons with a coupling constant of 3 c.p.s. The 3α proton occurred as a quartet at 5.72τ and a doublet at 6.9τ (J = 4 c.p.s.) was ascribed to the proton of a trisubstituted 1.2 epoxide which was allylic to a double bond. Treatment of I.P.4 with boron trifluoride etherate gave 3β-acetoxylanost-8ene-7one, and so the compound was concluded to be 3β-acetoxy-lanost-9(11)-ene-7.8-epoxide (LXla). This was borne out by comparison of its physical properties with those of the authentic compound (lit.$^{(158)}$ m.pt.=

185-186°C, $[\alpha]_D = -8°$).

The compound I.P.5., $C_{32}H_{52}O_3$, m.pt. = 171.5-174°C, $[\alpha]_D =$ $+94.3°$ had a mass spectroscopic molecular weight of 484 in agreement with the analysis. The infra-red spectrum had peaks at 1730 and 1240 cm^{-1} (acetate), and the ultra-violet spectrum had only end absorption. The n.m.r. spectrum of I.P.5 had an olefinic proton at 4.36τ, split symmetrically by two other protons with a coupling constant of 4 c.p.s. The 3α proton quartet was at 5.6τ, and a proton at 7.08τ was ascribed to the proton of a trisubstituted 1.2 epoxide allylic to a double bond. Treatment of I.P.5 with boron trifluoride etherate gave 3β-acetoxylanost-8ene-7one and so the compound is 3β-acetoxylanost-9(11)-ene-7.8-epoxide (LXla), stereoisomeric with the compound I.P.4.

No stereochemistry had been assigned to the compound I.P.4. by the previous workers(164) but, since it had been formed by epoxidation of the 7.9(11) diene, it was more likely to be the α-epoxide. Reduction of the epoxides I.P.4 and I.P.5 to the corresponding C_7 alcohols would allow the stereochemistries to be assigned by the method of molecular rotation differences. Treatment of the epoxide I.P.4 with lithium aluminium hydride gave 3β-acetoxylanost-8-ene-7one as the only product, due to opening of the epoxide ring in base without reduction. On catalytic hydrogenolysis, platinum and acetic acid had to be used before uptake of hydrogen was observed, and compound I.P.4 gave 3β-acetoxylanosta-7.9(11)-diene under these conditions. Reduction of the compound I.P.5 under the same conditions gave a small yield of a compound with no ultra-violet absorption above 220 mμ.



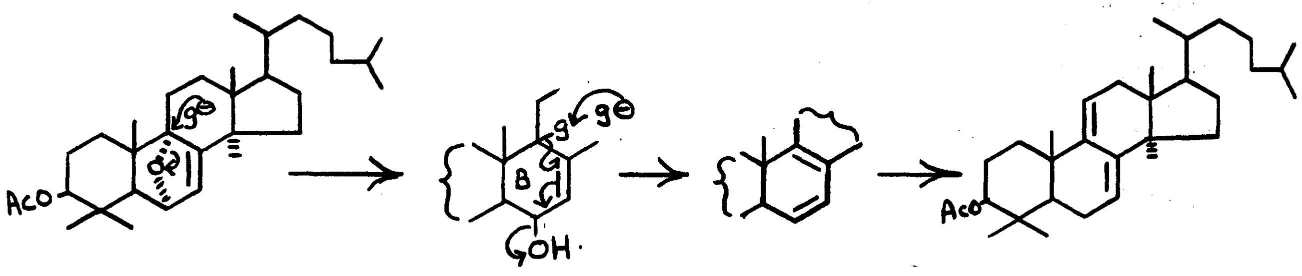
LXII

The formation of the 7.9(11)-diene from the compound I.P.4 and not from I.P.5 supports the assignment of an α-configuration to the former epoxide since the 7α-8α-epoxide would open to the 7α alcohol which would dehydrate readily by a trans-diaxial mechanism to give the 7.9(11)-diene. The compound I.P.4 is, therefore, 3β-acetoxylanost-9(11)-ene-$7\alpha.8\alpha$-epoxide, and I.P.5 is 3β-acetoxylanost-9(11)-ene-$7\beta.8\beta$-epoxide.

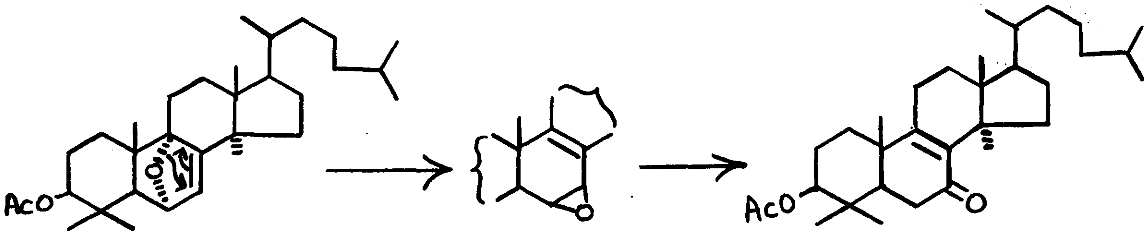
The compound I.P.1., $C_{32}H_{52}O_3$, m.pt. = 142-144°C, $[\alpha]_D = +164°$, had an infra-red spectrum with peaks at 1730 and 1240 cm^{-1} (acetate), and 825 cm^{-1}, and an ultra-violet spectrum with $\lambda_{max} = 210\ m\mu$ (9,050). The mass spectroscopic molecular weight of 484 was in agreement with the analysis, and since it had no carbonyl or hydroxyl functions, the compound was concluded to be an ether.

Treatment of the compound I.P.1 with chromium trioxide in acetic acid gave 3β-acetoxylanost-8ene-7one, and reduction with potassium iodide in acetic acid gave 3β-acetoxylanosta-7.9(11)-diene. A 60-megacycle n.m.r. spectrum of the compound I.P.1 now became available to us and the working structure LXII was obtained from it. The 3α-axial proton on the same carbon atom as the acetate function occurred as a quartet at 5.41τ with $J_{axial-axial} = 8$ c.p.s. and $J_{axial-equatorial} = 3$ c.p.s. as expected. The olefinic proton at C_7 was assigned to a doublet at 5.28τ ($J_{67} = 13$ c.p.s.) and the C_6 proton was assigned to a quartet at 5.17τ with $J_{67} = 13$ c.p.s. and $J_{56} = 10$ c.p.s.

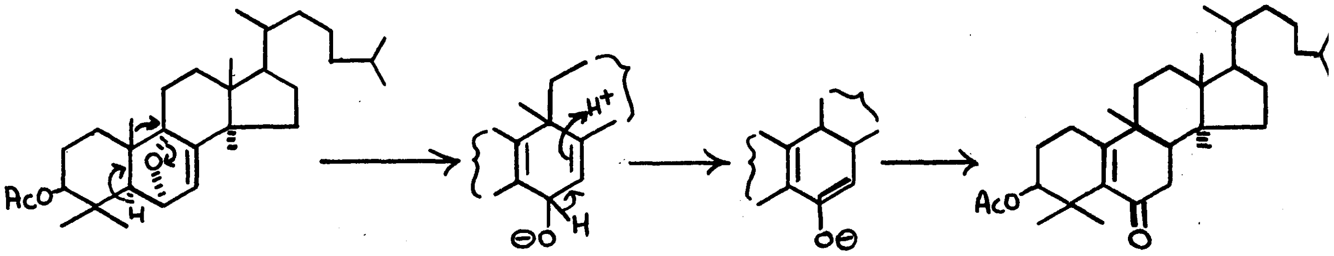
The structure LXII is extremely strained and although cyclohex-1-ene-3.6 ethers are known as the products of Diels-Alder reactions with furan rings, there is a high Pitzer strain between the 18 and 19



LXII LXIII LXIV LVI



LXII LXV LIX



LXII LXVI LXVII LXVIII

methyl groups. The reduction of I.P.1 with potassium iodide in acetic acid can be explained on the basis of this structure by the sequence LXII → LXIII → LXIV → LVI, as the homoannular diene LXIV will rearrange to the more stable heteroannular diene LVI(165).

The reaction with chromium trioxide in acetic acid to yield the 8-ene-7one can only be explained by the involved acid catalysed rearrangement LXII → LXV → LIX.

Although the structure LXII was regarded with suspicion, it was appealing in that, if the expected 7ene-9α hydroperoxide (LII) were in fact formed, then the C_6 position would be nucleophilic due to its allylic nature and so it might conceivably compete with the C_{14} α-methyl group for the oxygen cation at C_9.

The ether I.P.1 was treated with boron trifluoride etherate to give two homogeneous products. The first of these was an enone, $C_{32}H_{52}O_3$, m.pt. = 162-163°C, $[\alpha]_D = -19.2^{\circ}$ with peaks in the infra-red spectrum at 1720 cm^{-1} (acetate) and 1645 and 1600 cm^{-1} (enone). The ultra-violet absorption at 253 $m\mu$ (11,200) substantiated that this compound was an enone, and the n.m.r. spectrum showed no enone olefinic proton so that the enone double bond was tetrasubstituted. The only enones, $C_{32}H_{52}O_3$, with the lanosterol skeleton which have a tetrasubstitued enone double bond are the 8-ene-7one and the 8-ene-11 one, and this corresponded to neither of these. Rearrangement of the lanosterol skeleton was indicated, and an enone LXVIII might be derived from the working structure LXII as shown opposite. LXVIII would fit all of the physical data to hand.

The second product from boron trifluoride treatment of the ether I.P.1 was a ketol, $C_{32}H_{54}O_4$, m.pt. = 176-177°C, $[\alpha]_D$=-61.7°, as plates from petroleum ether. The infra-red spectrum had peaks at 3500 cm^{-1} (OH), 1710 cm^{-1} (acetate) and 1680 cm^{-1} (ketone) and the ultra-violet spectrum was transparent. The alcoholic grouping was considered to be secondary in view of a proton at 6.8τ in the n.m.r. spectrum. This proton was coupled with two protons on an adjacent carbon atom and was considered to be <u>axial</u> since the quartet had similar spin-spin coupling to the 3α <u>axial</u> proton at 5.51τ. Since this proton is <u>axial</u>, the alcoholic group must be <u>equatorial</u>. It is impossible to derive a ketone-secondary alcohol easily from the working structure LXII which must therefore be incorrect.

In summary, the oxidation product I.P.1 is an ether and, since it is formed from the oxygenation of 3β-acetoxylanost-8ene, we feel that one terminus of the ether linkage is at C_7 or C_9. A 1.2-epoxide is ruled out in view of the lack of a proton in the 7τ region of the n.m.r. spectrum, and the formation of a ketone-secondary alcohol with boron trifluoride etherate would indicate that the ether termini are very probably secondary. None of the various hypothetical structures which are possible has fitted all of the physical and chemical data available and so we decided to tackle the problem by X-ray methods. The compound I.P.1 was readily brominated to give the dibromide $C_{32}H_{52}O_3Br_2$ as lathes from petroleum ether, m.pt. = 198-199.5°C, $[\alpha]_D$= +79.6°, and we await receipt of an unambiguous structure for the dibromide by X-ray analysis.

Experimental (2)

Experimental

Melting points were taken on the Koffler Block. Infra-red spectra were taken on the Perkin-Elmer Infracord spectrophotometer for Nujol Mulls and ultra-violet spectra were taken on the Perkin-Elmer 202 spectrophotometer for solutions in ethanol. N.m.r. spectra were taken on the Varian A60 n.m.r. spectrometer for solutions in deuterochloroform. Microanalyses were by Mr. J. M. L. Cameron, B.Sc., and his staff. The phrase "worked up in the usual way" refers to addition of the reaction mixture to water, extraction with ether, washing the extracts with water, drying (anhyd. $MgSO_4$), and removing the solvent on the steam bath in vacuo.

Oxygenation of dihydrolanosterol

Dihydrolanosterol (1 gm.) and haematoporphyrin (8 mg.) were dissolved in AR pyridine (20 ml.). The solution was irradiated with two 20 watt, 250 volt fluorescent tubes, and oxygen was bubbled through it for three days. The solvent was removed in vacuo and the product (992 mg.) was dissolved in a solution of sodium iodide (5.6 gm.) in anhydrous ether (10 ml.), ethanol (50 ml.) and acetic acid (15 drops). The solution became red on standing overnight at room temperature and the solvent was removed. Ether and water were added to the residue, and the reaction was worked up in the usual way, a sodium thiosulphate wash being used to remove traces of iodine.

The gummy product (766 mg.) was dissolved in acetic anhydride (5 ml.) and pyridine (5 ml.) and left overnight at room temperature. The reaction was worked up in the usual way to give a gum (793 mg.) which was chromatographed on grade III alumina. Elution with petroleum ether

(60-80°): benzene (9:1) gave 3β-acetoxylanosta-7.9(11)-diene (20 mg.) as white needles from petroleum ether (60-80°), m.pt. = 161-165°C, ν_{max} = 1730 (OAC), 1230 (OAC) and 810 cm^{-1} (olefin), λ_{max} = 236 mμ, 244 mμ and 253 mμ.

Elution with petroleum ether (60-80°):benzene (1:1) gave a solid (2 mg.) which crystallised from petroleum-ether (60-80°) with m.pt. = 191-199°C. The compound had no ultra-violet absorption above 220 mμ and had ν_{max} = 1730 (OAC), 1715 (C=O), 1670, 1630, 1245 (OAC) and 855 cm^{-1}.

Oxygenation of 3β-acetoxylanost-8ene.

Dihydrolanosterol acetate (1 gm.) and haematoporphyrin (8 mg.) were dissolved in AR pyridine (20 ml.) and the solution was irradiated with two 20 watt, 250 volt fluorescent tubes for three days while a steady stream of oxygen was passed through it. The solvent was removed in vacuo and the resultant gum was dissolved in a solution of sodium iodide (3 gm.) in ether (12 ml.), ethanol (60 ml.) and glacial acetic acid (18 drops) and left overnight at room temperature. The deep red solution was taken to small volume and ether was added. The ethereal solution was washed with sodium thiosulphate solution and water and dried (anhyd. $MgSO_4$) and the solvent was removed to give a gum (1.1 gm.) which showed eight spots on an alumina chromatoplate.

The gum was chromatographed on grade III alumina. Elution with petroleum ether (60-80°):benzene (4:1) gave a solid (243 mg.) which crystallised from petroleum ether (60-80°) as needles, m.pt. = 147-149°C, $[\alpha]_D$ = +91.1°, λ_{max} = 236, 245 (10,300) and 254 mμ. This was very similar in properties to 3β-acetoxylanosta-7.9(11)-diene (lit.$^{(158)}$ m.pt. = 164-166°C, $[\alpha]_D=+89°$), but a peak was evident at 455 in the mass spectrum.

Elution with benzene:ether (1:1) gave a solid (176 mg.) which crystallised from petroleum:ether (60-80°) as needles, m.pt. = 159-168°C. This was shown to be a mixture of two compounds by thin layer chromatography, and a preparative thin layer alumina chromatoplate was developed with benzene:ether (1:1) to give 3β-acetoxylanost-8ene-7one, m.pt. = 140°C, ν_{max}= 1725 (OAC) and 1660 cm^{-1} (enone), λ_{max} 255 mμ, together with a small amount of a compound, m.pt. = 146-176°C, ν_{max}= 3450 (OH), 1740 (OAC), 1230 (OAC) and 855 cm^{-1}. This was later to be identified with 3β-acetoxylanost-8ene-7α-hydroperoxide (I.P.3.)

Oxygenation of dihydrolanosterol acetate in the presence of para-nitrobenzene sulphonyl chloride

3β-acetoxylanost-8ene (10 gm.), para- nitrobenzenesulphonyl chloride (4.66 gm.) and haematoporphyrin (130 mg.) were dissolved in AR pyridine (200 ml.) and oxygen was passed through the solution, which was irradiated with two 20 watt, 250 volt fluorescent tubes, for six days. The solvent was removed under reduced pressure, and the reaction was worked up in the usual way to give a semi-solid which was chromatographed on grade III alumina.

Elution with petroleum ether (60-80°) gave unchanged para-nitrobenzene-sulphonyl chloride, followed by 3β-acetoxylanosta-7.9(11)-diene which crystallised from petroleum ether as needles, m.pt. = 160-165°C, λ_{max}= 236, 245 and 254 mμ.

Elution with petroleum ether (60-80^{o});benzene (9:1) gave a solid, I.P.1., which crystallised from petroleum ether (60-80^{o}) as needles (153 mg.), m.pt. = 142-144^{o}C, $[\alpha]_D$= +164^{o} (found C, 79.00%; H, 10.53%. $C_{32}H_{52}O_3$ requires C, 79.28%; H, 10.81%), ν_{max}= 1730 and 1240 cm^{-1} (acetate) and 820 cm^{-1}, λ_{max}= 210 mμ (9,050).

Elution with petroleum ether (60-80^{o}):benzene (4:1) gave a compound (1.6 gm.) which crystallised from petroleum ether (60-80^{o}) as needles, m.pt. = 146-151^{o}C, $[\alpha]_D$= +19^{o}, ν_{max} = 1730 (OAC), 1650 and 1570 (enone) and 1230 cm^{-1} (OAC), λ_{max}= 255 mμ (13,050). This was 3β-acetoxylanost-8ene-7one (lit.(158) m.pt. = 149-155^{o}C, $[\alpha]_D$= +22^{o}).

Elution with benzene:ether (19:1) gave a solid, I.P.3., which crystallised from ethyl acetate as needles (540 mg.), m.pt. = 175-176^{o}C, $[\alpha]_D$= +35.2^{o} (found C, 76.96%; H, 10.41%. $C_{32}H_{52}O_4$ requires C, 76.75%; H, 10.47%), ν_{max}= 3500 (OOH), 1737 and 1270 (OAC) and 855 cm^{-1}. The compound had only end absorption in the ultra-violet.

When the reaction was repeated with 3β-acetoxylanost-8-ene (10 gm.), a compound, I.P.4., was eluted immediately after the elution of the product I.P.1. This compound (200 mg.) crystallised from petroleum ether (60-80^{o}) with m.pt. = 182-183^{o}C, $[\alpha]_D$ = -4.5^{o}, (found C, 79.69%; H, 10.74%. $C_{32}H_{52}O_3$ requires C, 79.28%; H, 10.81%), ν_{max}= 1720 and 1240 cm^{-1} (OAC). The ultra-violet spectrum had no absorption above 220 mμ.

The fractions which eluted after the 7.9(11)-diene and before the product I.P.1., using the residues from the reaction of a total of 40 gm. 3β-acetoxylanost-8ene, were rechromatographed on grade III alumina. Elution with petroleum ether (60-80°) gave 3β-acetoxylanosta-7.9(11)-diene, m.pt. = 156-159°C, λ_{max} = 236, 245 and 254 mμ. Elution with petroleum ether (60-80°): benzene (19:1 and 9:1) gave a solid which crystallised from petroleum ether (60-80°) as prisms (101 mg.), m.pt.= 171.5-174°C, $[\alpha]_D = +94.3^{\circ}$ (found C, 79.34%; H, 10.17%. $C_{32}H_{52}O_3$ requires C, 79.28%; H, 10.17%), ν_{max} = 1730 and 1240 cm^{-1} (acetate) and 980 cm^{-1}. The compound was designated I.P.5., and had no ultra-violet absorption above 220 mμ, and a mass spectroscopic molecular weight of 484.

Chromic acid oxidation of I.P.1., I.P.4., and I.P.5.

The following general procedure was used to oxidise these compounds(90).

The ether (12 mg.) was dissolved in acetic acid (1 ml.) and a solution of AR chromium trioxide (20 mg.) in 95% acetic acid (1.5 ml.) was added at reflux. The solution was refluxed for a further 15 minutes, added to excess methanol and worked up in the usual way. The products were all gums, and in no case was a peak observed above 1740 cm^{-1} in the infra-red spectrum.

Attempted acetylation of the compound I.P.3.

The compound I.P.3 (20 mg.) was dissolved in acetic anhydride (1 ml.) and pyridine (1 ml.) and left overnight at room temperature. The solution was worked up in the usual way to give a semi-solid (16 mg.) which crystallised from petroleum ether (60-80°) as needles, m.pt.= 145-149°C (found C, 79.2%; H, 10.1%. $C_{32}H_{50}O_3$ requires C, 79.6%; H, 10.4%), ν_{max} = 1720 (OAC), 1650 and 1570 (enone), 1235 (OAC) and

895 cm^{-1}, $\lambda_{max} = 257$ $m\mu$ (13,750). The melting point was undepressed on admixture with an authentic sample of 3β-acetoxylanost-8ene-7one.

Manganese dioxide treatment of the compound I.P.3

The compound I.P.3 (37 mg.) was dissolved in chloroform (4 ml.) and shaken with manganese dioxide (350 mg.) for three days at room temperature. The solution was filtered through celite and the solvent was removed to give a gum which was chromatographed on grade III alumina. Elution with petroleum ether (60-80°) gave a solid (3 mg.), m.pt.= 160 -163°C, ν_{max} = 1730 and 1230 (OAC) and 820 cm^{-1} (olefin), λ_{max} = 236, 244 (19,400) and 252 $m\mu$. The infra-red and ultra-violet spectra were identical to those of 3β-acetoxylanosta-7.9(11)-diene, and a mixed melting point was undepressed.

Treatment of I.P.3 with para-nitrobenzene-sulphonyl chloride

The compound I.P.3 (500 mg.) and *para*-nitrobenzene-sulphonyl chloride (250 mg.) were dissolved in dry pyridine (15 ml.) and the solution was left at room temperature for two days. The reaction was worked up in the usual way to give a semi-solid which was chromatographed on grade III alumina. Elution with petroleum ether (60-80°) gave unchanged *para*-nitrobenzene-sulphonyl chloride, while elution with benzene:ether (19:1 and 9:1) gave unchanged I.P.3. No intermediate fractions were obtained.

Treatment of I.P.4 with boron trifluoride

The compound I.P.4 (80 mg.) was dissolved in 45% boron trifluoride etherate (4 ml.) and left for three days at room temperature. The reaction was worked up in the usual way to give a gum which was chromatographed on grade III alumina. Elution with benzene gave a solid (35 mg.) which crystallised from petroleum ether (60-80^o) as needles, m.pt. = 156-160^{o}C, λ_{max} = 257 mμ, ν_{max} = 1720 (OAC), 1650 and 1570 (enone), and 1230 cm^{-1} (acetate). The compound was 3β-acetoxylanost-8ene-7-one since the infra-red and ultra-violet spectra and the melting point were identical to those of an authentic sample.

Treatment of I.P.5 with boron trifluoride

The compound I.P.5 (50 mg.) was dissolved in acetic acid (2.5 ml.) with 45% boron trifluoride etherate (10 drops) and left at room temperature for three hours. The reaction was worked up in the usual way to give a solid (47 mg.) which crystallised from petroleum ether (60-80^o) as needles, m.pt. = 152-154^{o}C, $[\alpha]_D$ = + 18.6^o, ν_{max} = 1735 (OAC), 1650 and 1580 (enone), and 1245 cm^{-1} (OAC), λ_{max} = 257 mμ (9,250). The compound was 3β-acetoxylanost-8ene-7one as its physical properties were identical to those of an authentic sample.

Lithium aluminium hydride treatment of the compound I.P.4.

Treatment at room temperature resulted in recovery of starting material, and so the reaction was undertaken at reflux.

The compound I.P.4 (100 mg.) was dissolved in dry tetrahydrofuran and refluxed with lithium aluminium hydride (130 mg.) with stirring for two days. Excess lithium aluminium hydride was destroyed by dropwise addition of ethyl acetate and the reaction was worked up in the usual way to give a gum which crystallised from petroleum ether (60-80^o) as needles (40 mg.), m.pt. = 141-149^{o}C, $[\alpha]_D$ = + 30^o, ν_{max} = 1730 (OAC), 1650 and 1580 (enone) and 1240 cm^{-1} (OAC), λ_{max} = 255 mμ.

This compound was identical to 3β-acetoxylanost-8ene-7one in every respect.

Catalytic reduction of I.P.4.

The compound I.P.4 (39 mg.) was added to a suspension of presaturated platinum oxide (62 mg.) in acetic acid (6 ml.) and shaken in an atmosphere of hydrogen until one mole had been absorbed. The solution was filtered through celite and worked up in the usual way to give a solid (32 mg.) which crystallised from petroleum ether $(60-80^{\circ})$ as needles, m.pt. = $160-162^{\circ}$C, ν_{max} = 1730 and 1240 (acetate) and 810 cm^{-1} (olefin), λ_{max} = 237, 245 and 253 mμ. The compound was identical to 3β-acetoxylanosta-7.9(11)-diene in every respect.

Catalytic reduction of I.P.5.

The compound I.P.5. (17 mg.) was added to a suspension of presaturated platinum oxide (25 mg.) in acetic acid (6 ml.) and shaken in an atmosphere of hydrogen until 0.8 ml. of hydrogen had been consumed. The solution was filtered through celite and worked up in the usual way to yield a semi-solid which crystallised from petroleum ether $(60-80^{\circ})$ with m.pt. = $134-145^{\circ}$C (2 mg.), ν_{max} = 1730 and 1240 cm^{-1} (acetate) and no ultra-violet absorption above 220 mμ.

Chromium trioxide oxidation of the compound I.P.1.

The compound I.P.1 (11 mg.) was dissolved in glacial acetic acid (0.6 ml.) and a solution of chromium trioxide (40 mg.) in 85% acetic acid (1 ml.) was added. The solution was left overnight at room temperature, added to methanol, and worked up in the usual way to give a solid which crystallised from petroleum ether $(60-80^{\circ})$ as needles (6 mg.), m.pt. = 138-143°C, ν_{max} = 1720 (OAC) 1650 and 1570 (enone), 1240 (OAC) and 895 cm^{-1}, λ_{max} = 255 $m\mu$ (15,550). The melting point was undepressed on admixture with an authentic sample of 3β-acetoxylanost-8ene-7one.

Potassium iodide reduction of I.P.1.

The compound I.P.1 (21 mg.) was heated with a saturated solution of potassium iodide in acetic acid (5 ml.) on the steam bath for thirty minutes. The red solution was added to a solution of sodium thiosulphate and sodium bicarbonate and worked up in the usual way to give a semi-solid with λ_{max}= 235, 244 (3,200), and 253 $m\mu$. The reaction was obviously incomplete, and so the product was heated for a further four hours on the steam bath with a saturated solution of potassium iodide in acetic acid (10 ml.). The reaction was worked up in the usual way to give a gum (12 mg.) which was chromatographed on grade III alumina.

Elution with petroleum ether (60-80°) gave a solid (6 mg.) which crystallised from petroleum ether (60-80°) as needles, m.pt.= 166-169°C, λ_{max} = 236, 244 (13,500) and 253 $m\mu$. The infra-red spectrum of this compound was identical to that of 3β-acetoxylanosta-7,9(11)-diene, and a mixed melting point was undepressed.

Boron trifluoride treatment of I.P.1.

The compound I.P.1. (150 mg.) was dissolved in glacial acetic acid (7 ml.) with 45% boron trifluoride etherate (17 drops) and the solution was left for four hours at room temperature and worked up in the usual way to give a gum which was chromatographed on grade III alumina.

Elution with petroleum ether (60–80°) gave a semi-solid (56 mg.) which crystallised from petroleum ether (60–80°) as needles (14 mg.), m.pt. = 162–163°C, $[\alpha]_D = -19.2^\circ$ (found C, 79.34%; H, 10.47%. $C_{32}H_{52}O_3$ requires C, 79.28%; H, 10.81%), $\nu_{max} = 1720$ (OAC) 1645 and 1600 (enone) and 1260 cm^{-1} (OAC), $\lambda_{max} = 253$ $m\mu$ (11,200).

Elution with petroleum ether (60–80°):benzene (19:1 to 1:1) gave a solid (35 mg.). Sublimation and repeated recrystallisation from petroleum ether gave a white solid, m.pt. = 187–192°C, $\lambda_{max} = 260$ $m\mu$ (885), but this was not pure.

Elution with benzene gave a solid which recrystallised from petroleum ether (60–80°) as plates (32 mg.), m.pt. = 176–177°C, $[\alpha]_D = -61.7^\circ$ (found C, 76.15%; H, 10.73%. $C_{32}H_{54}O_4$ requires C, 76.44%; H, 10.83%), $\nu_{max} = 3500$ (OH), 1710 (OAC), 1680 (ketone) and 1280 cm^{-1} (OAC). The compound had no ultra-violet absorption and had a negative Beilstein halogen test. The n.m.r. spectrum had the usual quartet at 5.51 τ due to the $C_{3\alpha}$ proton, and a quartet at 6.80 τ with $J_{axial-axial} = 10$ c.p.s. and $J_{axial-equatorial} = 5$ c.p.s. was assigned to the <u>axial</u> proton on a carbon atom bearing an alcohol group.

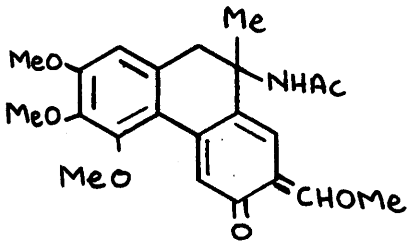
Bromination of I.P.1.

The compound I.P.1 (29 mg.) was dissolved in dioxan (5 ml.) with pyridine perbromide (29 mg.) and the solution was left for three hours at room temperature, added to sodium thiosulphate solution and worked up in the usual way to yield a solid. The product crystallised from petroleum ether as lathes (27 mg.), m.pt. = 198–199°C, $[\alpha]_D = +79.6^\circ$ (found C, 59.95%; H, 7.93%. $C_{32}H_{52}O_3Br_2$ requires C, 59.7%;

H, 8.03%), ν_{max} = 1720 and 1242 (OAC) and 890 cm^{-1}. The ultra-violet spectrum was transparent.

Part 3

In vitro synthesis of colchicine



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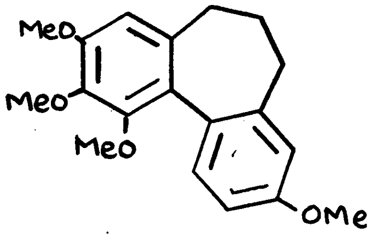

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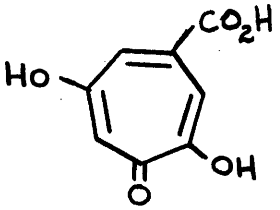

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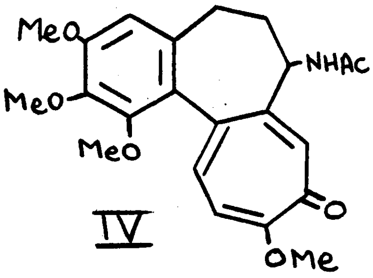

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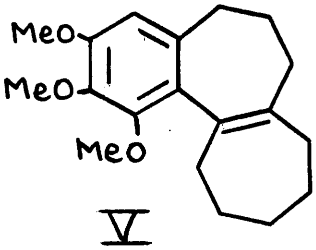

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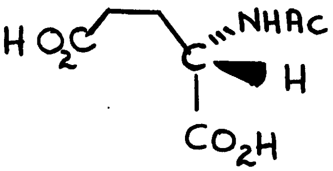

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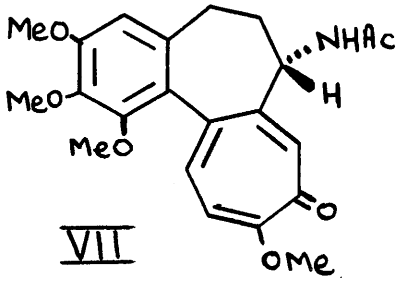

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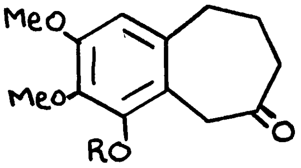

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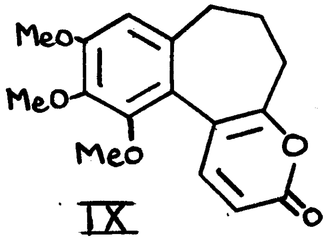
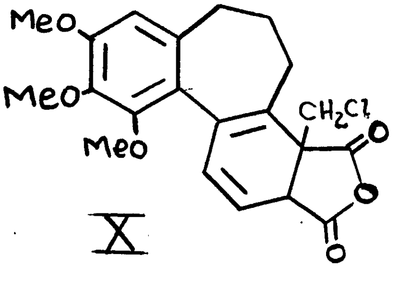


IX



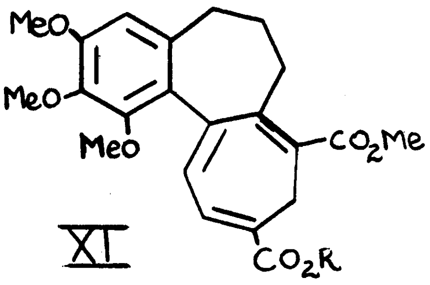
X

Historical

The alkaloid colchicine and its relatives are found in plants of the Lilliaceae family. Pure crystalline colchicine, free of solvent was first described in 1915(166), and structure I was assigned to it by Windaus in 1924(167). A seven membered ring B was proposed by Cook(168) and this was substantiated by two independent syntheses(169)(170) of the degradation product N-acetylcolchinol methyl ether (II). The tropolone nature of ring C was suggested by Dewar(171) to explain its reactivity being similar to that of the mould metabolite stipitatic acid (III) for which he had postulated tropolone as a new aromatic system(172), and he proposed that colchicine would have the structure IV. This suggestion was upheld by the synthesis(173) of the colchicine degradation product V. The observation(174) that N acetyl-L-glutamic acid (VI) was a degradation product of colchicine led to the stereochemistry VII for the alkaloid. Several reviews are available on the structural studies on colchicine(175)(176)(177).

Many syntheses of colchicine have been attempted, and the work in this field up to 1959 has been reviewed by Nozoe(178). Three total syntheses of the alkaloid have recently been achieved.

Eschenmoser(179) condensed the β suberone (VIII, R = H), obtained from purpurogallin, with propiolic ester. The resultant enol-ester (VIII, $R = O_2CC \equiv CH$) underwent internal Michael condensation, followed by lactonisation and methylation to yield the lactone IX. Diels-Alder condensation of the lactone IX with chloromethylmaleic anhydride gave the adduct X, the diester of which could be ring-expanded in base



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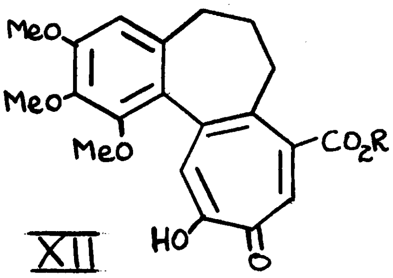

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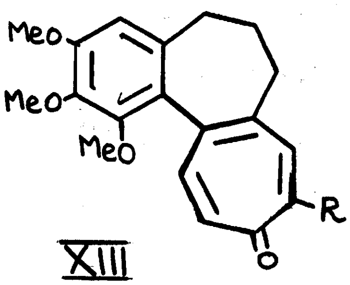
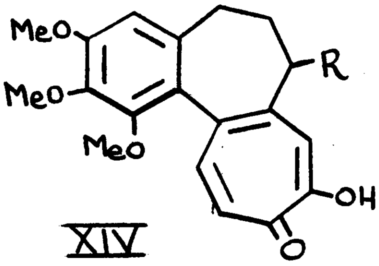
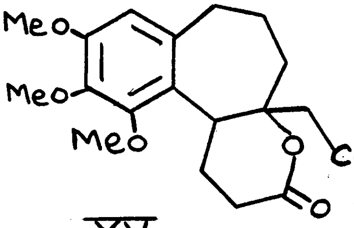


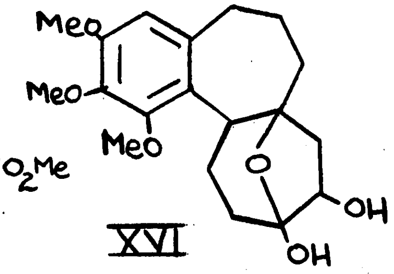
XIII



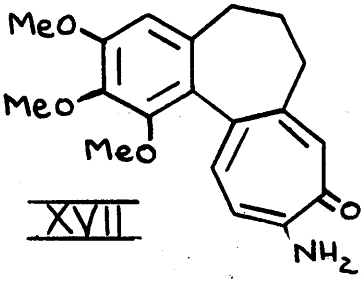
XIV



XV



XVI



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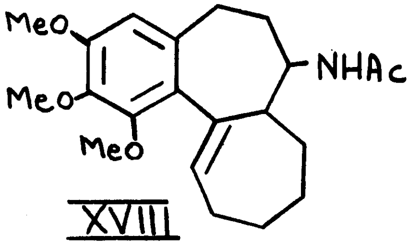
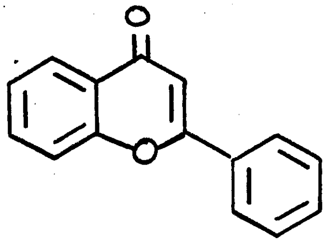
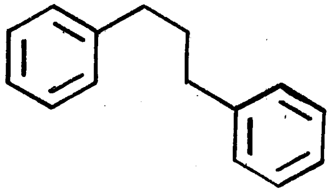


XVIII



XIX



XX

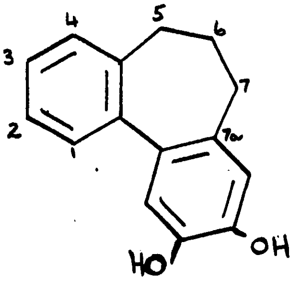
to the cycloheptatriene (XI, R=Me). The acid (XI, R=H) could be obtained by basic hydrolysis, and it was treated with osmium tetroxide to give the tropolone XII which was decarboxylated and tosylated. Treatment of the tosyl derivative with ammonia gave the amino derivative (XIII, $R=NH_2$) which was converted to desacetylamidocolchiceine (XIII, R=OH). This was converted to desacetylcolchiceine (XIV, $R=NH_2$) via the 7-bromo compound (XIV, R=Br) and this compound was resolved to give (-) desacetylcolchiceine from which (-) colchicine had already been synthesised(174).

Van Tamelin(180) used the benzsuberone (VIII, R=Me) as his starting material. Michael addition of acrylonitrile, followed by Reformatsky reaction with bromoacetic ester and lactonisation gave the lactone (XV) which underwent a novel acyloin reaction to give the hemiketal XVI. The hemiketal was oxidised, dehydrated and aromatised to give (±) desacetylamidocolchiceine (XIII, R=OH) which was readily converted to colchicine.

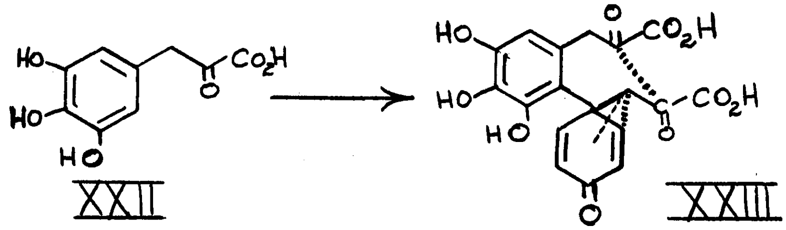
Nakamura(181) prepared colchiceinamide (XVII), which had previously been converted to colchicine(182), from the colchicine degradation product XVIII. He later synthesised XVIII to complete the total synthesis(183).

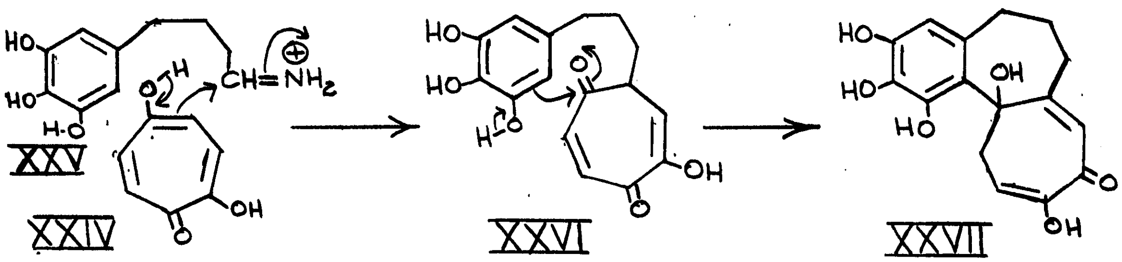
The biogenesis of colchicine has been the subject of much speculation over the past few years, although this has been accompanied by a disproportionately small amount of experimental work.

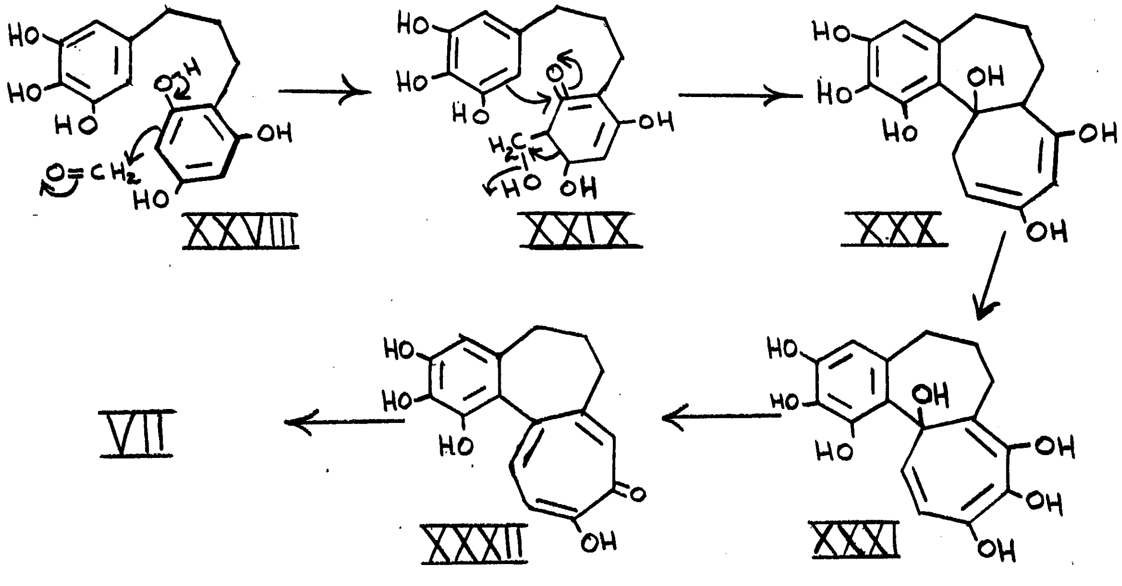
Anet(184)(185)(186) suggested that colchicine might be related to the flavones (XIX) through the common precursor (XX). This

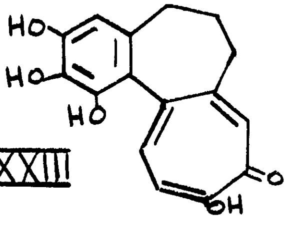

1
2
3
4
5
6
7
7a
HO
OH

XXI


HO
HO
HO
CO_2H
XXII
HO
HO
HO
CO_2H
CO_2H
XXIII


HO
HO
H-O
XXV
H
$CH=NH_2$
OH
XXIV
HO
HO
H-O
OH
XXVI
HO
HO
HO
OH
OH
XXVII


HO
HO
HO
H
OH
$O=CH_2$
HO
XXVIII
HO
HO
HO
H_2C
HO
OH
OH
XXIX
HO
HO
HO
OH
OH
OH
XXX
HO
HO
HO
OH
OH
OH
OH
XXXI
HO
HO
HO
OH
XXXII
VII


HO
HO
HO
OH

XXXIII

precursor could couple to XXI which would undergo ring enlargement by formaldehyde or its equivalent. Feeding experiments have shown that phenylalanine is the precursor of ring C and its appended three-carbon side chain in the flavanoids(187)(188)(189), and so, if the Anet-Robinson scheme for colchicine biogenesis is valid, feeding of $[3C^{14}]$ phenylalanine to the crocus should result in colchicine labelled at position C_5.

Belleau(190) postulated that colchicine is derived from phenylalanine via the oxidative coupling of two molecules of 3.4.5-trihydroxyphenylpyruvic acid (XXII) to yield the quinone (XXIII) which would undergo ring cleavage followed by cyclisation, as indicated opposite, to give the colchicine skeleton. This proposal would require colchicine to be labelled at positions C_5 and C_{7a} on feeding $[3C^{14}]$- phenylalanine to the crocus.

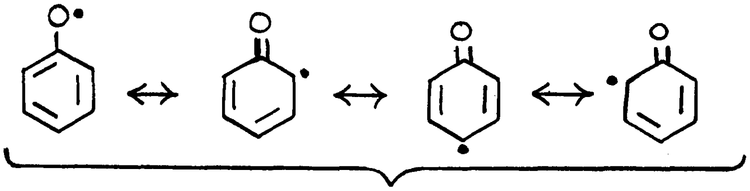
Wenkert(191) proposed that colchicine is formed by condensation of shikimate-derived 5-hydroxytropolone (XIV) with the prephenate-derived protonated Schiff's base XXV, via intermediates XXVI and XXVII as shown. Feeding $[3C^{14}]$phenylalanine would be expected to yield colchicine labelled at C_5 if this hypothesis were valid.

Leete(192) has fed $[3C^{14}]$phenylalanine to *Colchicum byzantium* and obtained colchicine labelled exclusively in the C_5 position. The Anet-Robinson and Belleau schemes were ruled out on this basis, but Leete has proposed a modified Anet-Robinson scheme which would account for the observed labelling. The precursor XXVIII, in which ring A and its appended three-carbon side chain would be phenylalanine-derived and ring C would be acetate-derived, could react with formaldehyde as shown to yield colchicine by the sequence XXVIII → XXIX → XXX → XXXI → XXXII → VII. This scheme involves two unnecessary changes in oxidation level, although the genesis of the tropolone ring from three acetate units and one formaldehyde unit would be in agreement with the biogenesis of tropolonoid mould metabolites(193)(194). The fact that Battersby(195) was unable to incorporate $[1C^{14}]$acetic acid in the tropolone ring of colchicine may have been due to experimental difficulties.

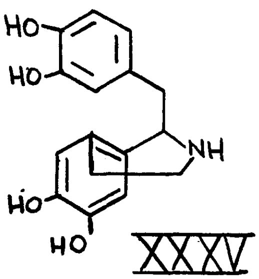
Battersby(195) was unable to incorporate $[2C^{14}]$tyrosine in the ring system of colchicine, and all of the label appeared in the N-acetyl group. Since Leete(192) had incorporated $[3C^{14}]$phenylalanine, Battersby argued that the C_6-C_3 precursor had broken down to C_6-C_1 and C_2 units before being incorporated. Although it is reasonable to assume splitting of tyrosine into C_6-C_1 and C_2 units, there is no reason to suppose that the C_6-C_1 unit is incorporated into the alkaloid or indeed that tyrosine is a precursor.

Scott(196) has proposed a scheme for colchicine biogenesis involving intramolecular oxidative coupling of the hypothetical precursor XXXIII which has a preformed tropolone ring. The feeding of suitable precursors would be required before it could be ascertained whether the tropolone ring were formed before or after the oxidative coupling reaction.

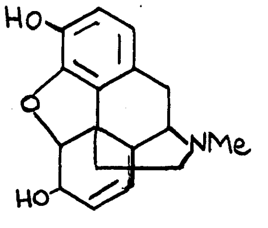
The concept(197) of phenolic oxidative coupling has been well reviewed(197)(198)(199). In such coupling reactions, phenols are treated with one-electron oxidising agents such as potassium ferricyanide,



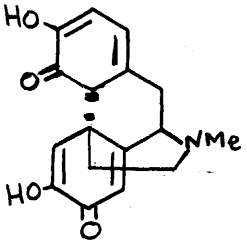
XXXIV



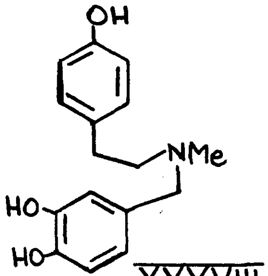
XXXV



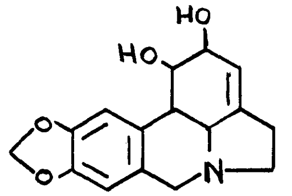
XXXVI



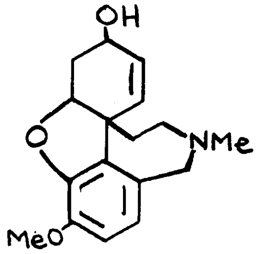
XXXVII



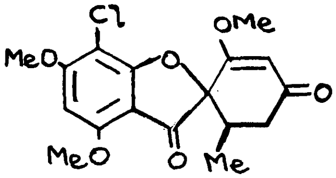
XXXVIII



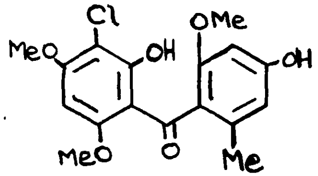
XXXIX



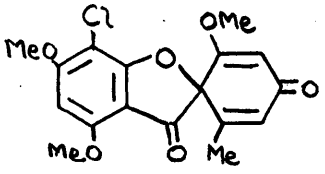
XL



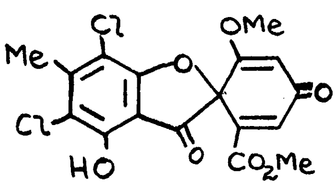
XLI



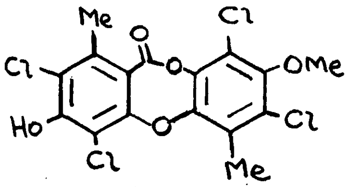
XLII



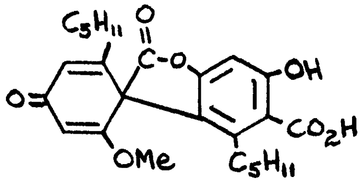
XLIII



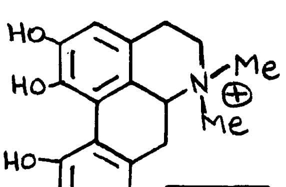
XLIV



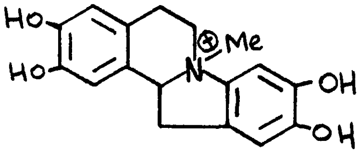
XLV



XLVI



XLVII

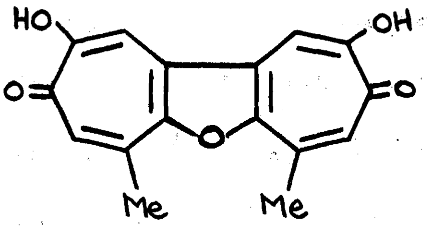


XLVIII

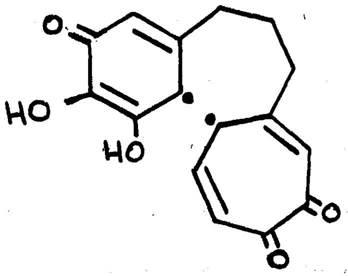
manganese dioxide and lead dioxide to yield resonance-stabilised radicals, such as XXXIV, which will couple to give carbon-carbon, carbon-oxygen or oxygen-oxygen bonds. The coupling of such radicals, formed by oxidative enzymes, has been recognised as being an important biosynthetic process, and the conversion of labelled norlaudonosoline (XXXV) to morphine (XXXVI) in Papaver somniferum has indicated an in vivo para-para radical coupling of the diradical XXXVII(200). Norbelladine (XXXVIII) has been incorporated into lycorine (XXXIX)(201), galanthamine (XL)(202) and other Armarylidaceae alkaloids which is also indicative of a phenolic oxidative coupling mechanism in the biogenesis.

Birch(203) has shown that acetic acid is incorporated in griseofulvin (XLI) in the expected manner, and that the labelled precursor XLII will yield griseofulvin in vivo(204). An in vitro conversion of this precursor to the intermediate spirodienone XLIII has been achieved by Scott(205)(206) in his total synthesis of the metabolite.

Various other in vitro phenolic radical coupling reactions have been performed to yield the expected natural products. Barton(207) has synthesised galanthamine (XL) from norbelladine (XXXVIII) using manganese dioxide as the oxidant, and geodin (XLIV)(208), diploicin (XLV)(209) and picrolichenic acid (XLVI)(210) have been synthesised in like manner. Recently Frank(211) has achieved the in vitro synthesis of an aporphine alkaloid XLVII from the quaternary salt of norlaudanosoline (XXXV) using ferric chloride as the oxidant. Thirty years before, Robinson(212) and Schopf(213) had attempted this coupling and obtained only the dehydrolaudanosoline XLVIII which was later shown to be a naturally occurring type(214).



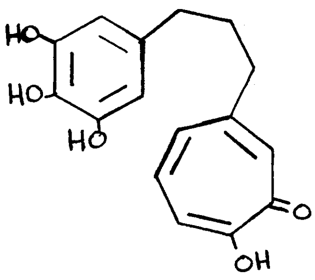
XLIX



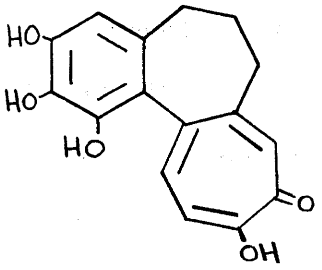
L

Although radical coupling reactions of phenols are well established both in vitro and in vivo, radical coupling of tropolone with phenol, as would be required in the scheme for colchicine biosynthesis proposed by Scott(196), is without precedent. No precursor corresponding to XXXIII has been isolated from the plant to indicate that this is the mode of biogenesis, and the only example of tropolone radical(227) coupling has been the isolation of the bitropolone XLIX on nitrous acid oxidation of 5 hydroxy-4 methyl-tropolone(215).

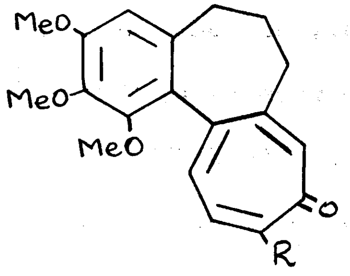
There are no theoretical grounds for supposing that the diradical L would not couple and indeed we shall show in the sequel that such a coupling is extremely facile.



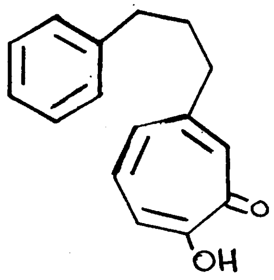
XXXIII



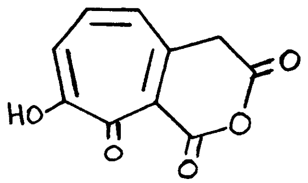
LI



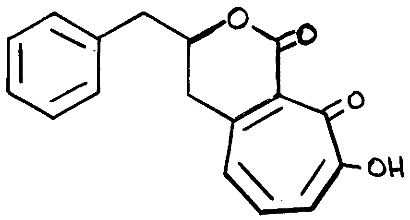
XIII, (R=OH)



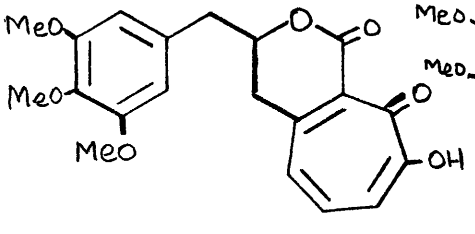
LII



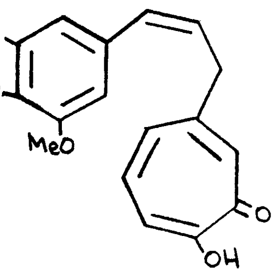
LIII



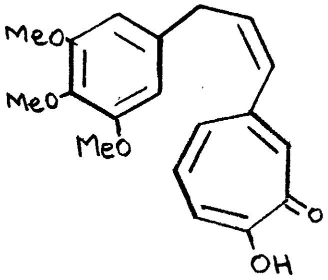
LIV



LV



LVI



LVII

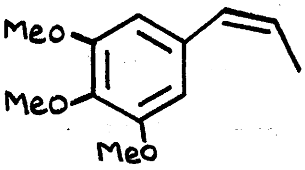
Theoretical

The scheme for colchicine biogenesis proposed by Scott(196) suggests a synthetic route to the alkaloid. Synthesis of the precursor (XXXIII) followed by oxidative coupling with a one-electron oxidising agent would yield the tricyclic product (LI) which should be convertable to desacetylamidocolchiceine (XIII, R = OH) by methylation and hydrolysis. Desacetylamidocolchiceine has been converted to colchicine by other workers(174)(179)(180), and so a total synthesis would have been achieved.

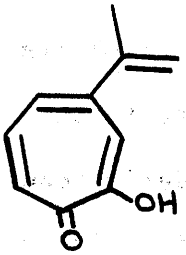
Nozoe(216) has prepared the phenyl analogue (LII) of the precursor (XXXIII) by condensation of phenylacetaldehyde with the anhydride of 3 carboxy-4carboxymethyltropolone (LIII)(217)(218) to give the lactone LIV which was decarboxylated and reduced to yield LII. He attempted to condense 3.4.5-trimethoxyphenylacetaldehyde with the anhydride LIII but failed to obtain the expected lactone.

In our hands(219), 3.4.5 trimethoxyphenylacetaldehyde, prepared by Rosenmund reduction of the acid chloride, condensed with the anhydride LIII to give the desired lactone in 72% yield.

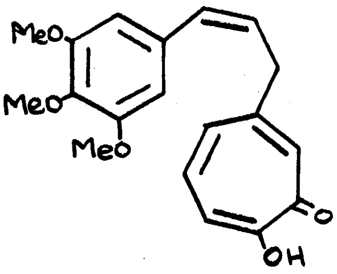
The lactone LV was decarboxylated by heating with copper bronze to 265°C at 0.05 mm. pressure. The resultant gum was triturated with methanol to give a solid which crystallised from methanol as yellow-green prisms, m.pt. = 115-116°C in 48% yield. The product, $C_{19}H_{20}O_5$. no longer exhibited the lactone carbonyl peak which had been typical of the starting lactone in the infra-red spectrum, and had $\lambda_{max} = 270\ m\mu$ and 380 $m\mu$, shifting to 345 $m\mu$ and 420 $m\mu$ in base. Of the two possible structures, LVI and LVII, for the decarboxylation product,



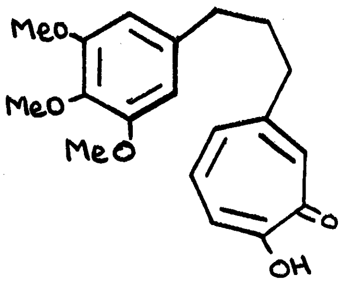
LVIII



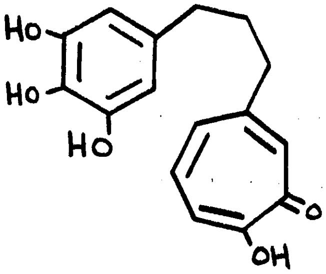
LIX



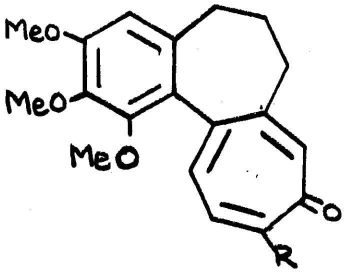
LVI



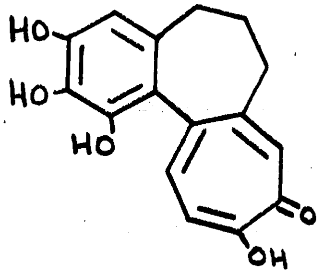
LX



XXXIII



XIII (R=OH)



LXI

LVI was preferred since the absorption at 270 mμ in the ultra-violet spectrum was nearer to the $\lambda_{max} = 264$ mμ absorption of isoelemicin (LVIII) than to the $\lambda_{max} = 254$ mμ absorption of β-dolabrin (LIX)(220). The styrene protons occurred as a multiplet with the benzene ring protons in the 3.5τ region of the n.m.r. spectrum and this was of no help in assigning the position of the double bond.

Hydrogenation of the styrene (LVI), using 10% palladium charcoal in ethyl acetate and one mole of hydrogen, led to the dihydro-compound (LX) which crystallised from ethanol as brown plates, $C_{19}H_{22}O_5$, m.pt. = 113-115°C, in 75% yield. The product had an ultra-violet absorption of $\lambda_{max} = 334$ mμ shifting to 408 mμ in base. The 270 mμ absorption, characteristic of the original styrene was no longer present.

Demethylation of LX was effected by refluxing in 48% hydrobromic acid for thirty minutes, and the desired trihydroxy precursor (XXXIII) was obtained as brown prisms, m.pt. = 128-132°C, from water in 39% yield. The product, $C_{16}H_{16}O_5$, sublimed as a white solid with $\lambda_{max} = 325$ and 350 mμ shifting to 338 and 398 mμ in base, and $\nu_{max} = 3500$ cm^{-1} (OH). The infra-red absorption at 1150 cm^{-1} (one), characteristic of starting material, had disappeared, as had the nine-proton singlet at 6.15τ (OMe) in the n.m.r. spectrum. Treatment of the trihydroxy precursor (XXXIII) with diazomethane followed by hydrolysis gave back the trimethoxy compound (LX).

Desacetylamidocolchiceine (XIII, R=OH) was prepared from colchicine by the method of Eschenmoser(179), and it was demethylated by refluxing in 48% hydrobromic acid for four hours. The resultant trihydroxy compound (LXI) crystallised from methanol as yellow-green prisms, m.pt. = 238-240°C in 57.5% yield. It analysed as $C_{16}H_{14}O_5$ and had $\lambda_{max} = 363\ m\mu$ shifting to $402\ m\mu$ in base. There were no protons in the methoxyl region of the n.m.r. spectrum.

The trihydroxy tricyclic compound (LXI) was treated with methyl iodide and potassium carbonate in acetone, and the product was hydrolysed in dilute acid to give desacetylamidocolchiceine (XIII, R=OH) in good yield. As this compound had been converted to colchicine by previous workers(174)(179)(180), phenolic oxidative coupling of the precursor XXXIII to the tricyclic compound LXI would complete the formal total synthesis of colchicine. With this end in view, and with the object of providing a laboratory analogy for Scott's hypothesis for the biogenesis of colchicine, we have subjected both the precursor XXXIII and the expected product LXI to treatment with a variety of one-electron oxidising agents.

Air oxidation of basic solutions of pyrogallol(221) and of methyl gallate(222) had resulted in the radical dimerisation of these compounds, and it was thought that this would be a good analogy for the desired coupling reaction. An aqueous sodium carbonate solution of the tricyclic compound (LXI) was treated with air for two days to give a poor recovery of a gum which, from its ultra-violet spectrum and paper chromatographic characteristics, was essentially starting material. Aeration of solutions of the bicyclic precursor (XXXIII) in aqueous barium hydroxide and 3N. ammonium hydroxide gave starting material as the only isolable product. An aqueous sodium carbonate solution of

XXXIII was aerated, and aliquots were taken at three, twenty four, and forty eight hours. The ultra-violet spectra of the aliquots were identical to the spectrum of starting material. Paper chromatography revealed that the product was very impure but none of the coupled tricyclic material could be detected.

Treatment of acetone solutions of both the bicyclic (XXXIII) and the tricyclic (LXI) compounds with lead dioxide at room temperature resulted in complete destruction of the tropolone chromophore, even after two minutes.

Ethanolic ferric chloride has proved to be an effective one-electron oxidant in the past, and treatment of the tricyclic compound (LXI) with this oxidant gave good recoveries of starting material. The bicyclic precursor XXXIII was treated in the same way, and aliquots were taken at two, five, fifteen, and thirty minutes and one, three and eighteen hours. The ultra-violet spectra of these aliquots showed a progressive shift to longer wavelength with time, as would be expected if we were entering the tricyclic series. No tricyclic material was detected by paper chromatography. Starting material, Rf 0.81, was present in all of the aliquots, and a blue fluorescent spot of Rf 0.5 was observed in the later aliquots.

The tricyclic compound LXI was treated with a fourfold excess of manganese dioxide in acetone, and aliquots were taken at five, ten, twenty, forty and one hundred and twenty minutes. Paper chromatography of these aliquots showed diminishing amounts of starting material with time, and the forty minute aliquot exhibited a yellow-blue spot of Rf 0.57. Similar treatment of the bicyclic precursor XXXIII showed diminishing amounts of starting material with time, and the ultra-violet absorption typical of tropolone-containing compounds had degenerated completely after thirty minutes. When a 1:1 ratio by weight of manganese dioxide to starting material was used, paper chromatography of aliquots from the reaction of the tricyclic material showed decreasing amounts of starting material with time, and increasing amounts of a material which appeared as a yellow-green spot of Rf 0.50. The bicyclic material was treated in the same way to give starting material, together with a small amount of a compound with m.pt. = 184-191°C whose ultra-violet spectrum was identical to that of starting material.

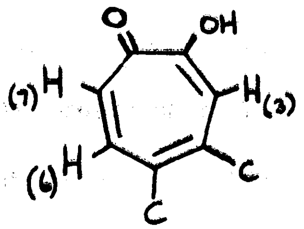
An aqueous sodium carbonate solution of the tricyclic compound LXI was treated with 3.3 moles of potassium ferricyanide in an atmosphere of nitrogen, and samples were taken at five, ten, fifteen, thirty, sixty and one hundred and twenty minutes. The ultra-violet spectra of these aliquots showed no change, but paper chromatography revealed a yellow-green fluorescent spot of Rf 0.57 which had also been observed in the manganese dioxide oxidation products. Treatment of a sodium bicarbonate solution of the precursor XXXIII with 3.3 moles of potassium ferricyanide in a nitrogen atmosphere was also followed by aliquots, taken at five, fifteen, thirty and sixty minutes. It was evident from paper chromatography that starting material remained until the thirty minute aliquot, and the sixty minute aliquot showed the yellow-green spot of Rf 0.56.

The yellow-green spot, Rf 0.56, had now been observed on oxidation of the tricyclic compound LXI with manganese dioxide and potassium ferricyanide, and of the bicyclic compound XXXIII with potassium ferricyanide. It became important, therefore, to isolate and identify the compound responsible for this spot.

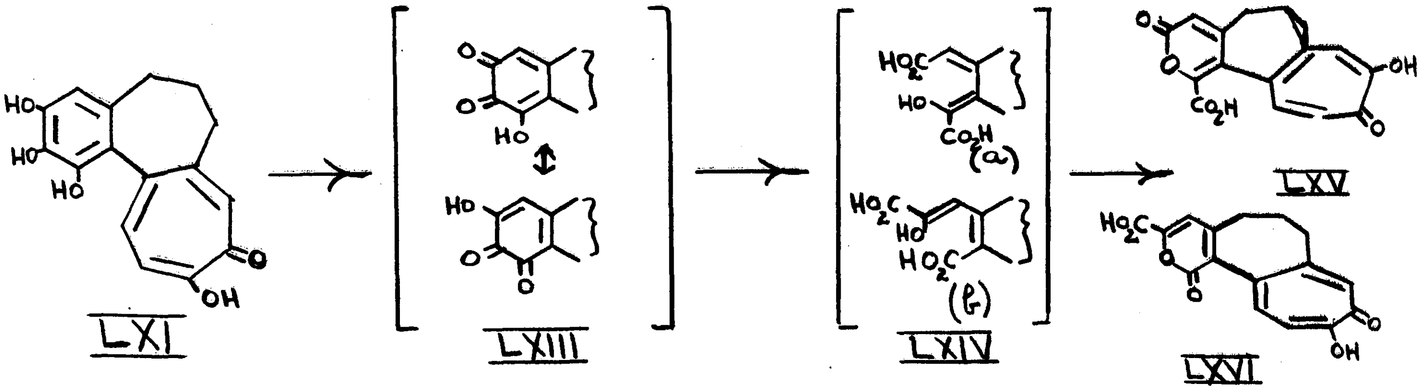
A sodium carbonate solution of the tricyclic compound LXI was treated for forty minutes with 3.3 moles of potassium ferricyanide in an atmosphere of nitrogen to give a solid which crystallised from ethanol as needles, m.pt. = 284-286°C, in 35% yield. The infra-red spectrum had peaks at 3500 cm^{-1} (OH), 3250 cm^{-1} (OH), 1750 cm^{-1} and 1700 cm^{-1} (split CO_2H), and the ultra-violet absorption was at 233 $m\mu$ and 365 $m\mu$, shifting to 239 $m\mu$ and 404 $m\mu$ in base.

A sodium bicarbonate solution of the precursor XXXIII was treated with 3.3 moles of potassium ferricyanide in an atmosphere of nitrogen for one hour, to give a solid which crystallised from ethanol as needles, m.pt. = 286-290°C in 5% yield. The melting point of the product was undepressed on admixture with the oxidation product of the tricyclic compound LXI, and the infra-red and ultra-violet spectra and paper chromatographic behaviour of the two compounds were identical.

The oxidation product common to both the bicyclic and tricyclic compounds was recrystallised from ethanol as yellow needles with m.pt.= 284-286°C. It analysed as the ethanol solvate, $C_{16}H_{12}O_6 \cdot EtOH$, and was obtained free of solvent as $C_{16}H_{12}O_6$ by sublimation. The ultra-violet spectrum with λ_{max} = 233 $m\mu$ and 365 $m\mu$, shifting to 239 $m\mu$ and 404 $m\mu$ in base, was almost identical to the ultra-violet spectrum of the tricyclic compound LXI, and the infra-red spectrum was typical



LXII



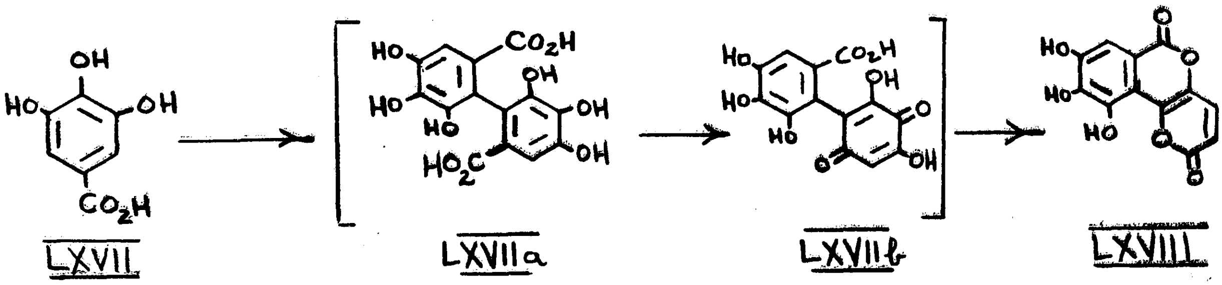
LXI

LXIII

LXIV

LXV

LXVI

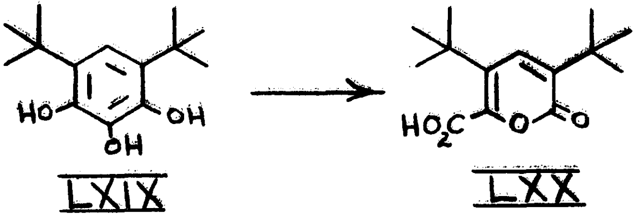


LXVII

LXVIIa

LXVIIb

LXVIII



LXIX

LXX

of a pyrone-acid with ν_{max} = 3550 cm^{-1} (OH), 3250 cm^{-1} (OH), 1750 cm^{-1} and 1700 cm^{-1} (split CO_2H). The analysis was supported by a mass spectroscopic molecular weight of 300 and the mass spectral cracking pattern indicated that carbon monoxide and carbon dioxide were lost at the ion source. Loss of carbon monoxide is a mass spectral characteristic of tropolones and loss of carbon dioxide is indicative of the presence of a carboxylic acid function which was confirmed by the effervescence of the compound with aqueous sodium bicarbonate solution. The n.m.r. spectrum was typically that of a 4.5 disubstituted tropolone with a singlet at 2.68 τ for the C_3 proton and an AB quartet centred at 2.48 τ and 2.82 τ for the C_6 and C_7 protons (see LXII opposite) with coupling constants of 12 c.p.s. and an intensity ratio of 1:2:2:1. A further proton occurred as a singlet at 2.7 τ (226).

The above evidence establishes that the oxidation product is the pyrone LXV (or LXVI), formed from the tricyclic compound LXI by oxidation and ring fission as shown in LXI → LXIII → LXIVa and b → LXV and LXVI opposite. Precedent for pyrone formation from pyrogallol rings under oxidative conditions was found in the formation of galloflavin (LXVIII) from gallic acid (LXVII) on aeration of basic solutions of the latter(223)(224). A closer analogy is the formation of the pyrone carboxylic acid (LXX) from 4.6-ditertiarybutyl pyrogallol (LXIX) on aeration of its alkaline solution(225).

The structure LXV is preferred to LXVI for the pyrone since its ultra-violet spectrum is more in keeping with the cross-conjugated system than the through conjugated system.

The formation of this pyrone from the precursor XXXIII indicates that the expected phenolic oxidative coupling between the pyrogallol and tropolone moieties has occurred and so the laboratory analogy for Scott's biogenetic hypothesis(196) has been achieved. The facile overoxidation of the tricyclic product LXI has prevented completion of the total synthesis and some attempts were now made to prevent overoxidation.

When the theoretical amount of potassium ferricyanide for the completion of the oxidative coupling was used (two moles), the precursor XXXIII yielded only starting material in the first aliquots (Rf 0.75) and some pyrone (Rf 0.47) in the later aliquots. The same result was observed when only 0.5 moles of potassium ferricyanide was used, and it was concluded that the rate of pyrone formation from the tricyclic material was faster than the rate of oxidative coupling of the bicyclic precursor.

In the hope that the reaction would be pH-dependent, the precursor XXXIII was treated with two moles of potassium ferricyanide solution at pH.7 in an atmosphere of nitrogen. Aliquots taken at five, fifteen, thirty, sixty and one hundred and twenty minutes showed decreasing amounts of starting material, Rf 0.78, and increasing amounts of the pyrone, Rf 0.57.

The extremely facile oxidation of the coupled material LXI has prevented its isolation, and an approach in which two of the pyrogallol hydroxyl functions are blocked might be indicated before the total synthesis of colchicine can be completed by this route. In the present work, we have shown that oxidative coupling between a phenol and a tropolone ring is feasible and so no theoretical objection to the synthesis remains.

In summary, the *in vitro* analogy for Scott's biogenetic hypothesis has been achieved and the total synthesis of colchicine is but one step from completion. Indeed, if, by improved paper chromatographic techniques, we can detect some of the tricyclic compound LXI in any of the aliquots from the oxidation of precursor XXXIII, the synthesis will have been achieved. It is not inconceivable that some of the compound LXI may be present in these aliquots in view of the mechanism for the formation of the pyrone and the very similar paper chromatographic behaviour of the bicyclic and tricyclic compounds.

Experimental (3)

Experimental

Melting points were done on the Koffler block. Infra-red spectra were taken with a Perkin-Elmer Infracord spectrophotometer for nujol mulls and ultra-violet spectra were taken with a Carey 14 spectrophotometer for ethanolic solutions. N.m.r. spectra were taken with a Varian A 60 spectrometer for solutions in deuterochloroform or dimethylsulphoxide. Microanalyses were by Mrs. C. Aldrich of the University of British Columbia and Alfred Bernhardt and Associates, Mülheim. Unless otherwise stated, paper chromatography was done on Whatman No. 3 paper, buffered by spraying with 0.5 M aqueous potassium dihydrogen phosphate solution and developed with a butanol:acetic acid:water (60:40:35) system. The phrase "worked up in the usual way" refers to addition of the reaction mixture to 6N sulphuric acid, extraction with chloroform and ether, washing the extracts with water and removing the solvent on the steam bath *in vacuo*.

3 carboxy-4 carboxymethyltropolone

This was prepared as described by Haworth and Hobson(217).

The anhydride (LIII)

This was prepared as described by Crow, Haworth and Jeffries(218).

3.4.5. trimethoxyphenylacetaldehyde

This was prepared as described by Davidson(219).

The lactone LV

This was prepared as described by Davidson(219).

The styrene LVI

The lactone (LV) (200 mg.) was mixed with a little copper bronze and heated to 265°C at 0.05 mm. The gummy distillate was triturated with methanol to give a solid (85 mg., 48.2%) which crystallised from ethanol as yellow-green prisms, m.pt. = 115-116°C (found C, 69.30%; H, 5.99%. $C_{19}H_{20}O_5$ requires C, 69.50%; H, 6.14%), ν_{max} = 3200 (tropolone OH), and 1590 cm^{-1} (aromatics), λ_{max} = 270 and 380 mμ, shifting to 345 and 420 mμ in base. The n.m.r. spectrum had the three tropolone ring protons in the 2.5-3τ region, and the two benzene ring protons as a singlet at 3.59τ. The two olefinic protons were also in the 3.5τ region. The methoxyl nine-proton singlet occurred at 6.15τ, and the "allylic-benzylic" protons were at 6.48τ as a doublet, J = 5 c.p.s.

<u>The Dihydro-compound LX</u>

The styrene LVI (25 mg.) was added to a presaturated suspension of 10% palladium charcoal (26 mg.) in ethyl acetate (5 ml.) and hydrogenated until 1.98 ml. of hydrogen was consumed. The solution was filtered through celite and the solvent was removed to give a solid (18 mg.) which crystallised from ethanol as light brown plates, m.pt. = 113-115°C (found C, 68.98%; H, 6.52%. $C_{19}H_{22}O_5$ requires C, 69.07%; H, 6.71%), ν_{max} = 3200 (tropolone OH), 1590 (aromatics) and 1150 cm^{-1} (OMe), λ_{max} = 334 mμ, shifting to 337 and 408 mμ in base.

<u>The precursor XXXIII</u>

The dihydro compound LX (300 mg.) was refluxed for thirty minutes with 48% hydrobromic acid (30 ml.). The solvent was removed <u>in vacuo</u> and the resultant gum was digested in hot water from which it crystallised as brown prisms. The product sublimed as a white solid,

$\lambda_{max} = 325$ and 350 mμ, shifting to 338 and 398 mμ in base (found C, 66.51%; H, 5.78%. $C_{16}H_{16}O_5$ requires C, 66.66%; H, 5.59%). The n.m.r. spectrum had six aliphatic protons in the 7.5τ region, two benzene ring protons as a singlet at 3.83τ, and the three tropolone protons in the 2.7 to 3τ region.

Methylation and hydrolysis of XXXIII

The precursor XXXIII (10 mg.) was dissolved in the minimum amount of ethanol and excess ethereal-diazomethane was added. The solution was left overnight at room temperature and the solvent was removed to give a gum which was left with 0.1N sulphuric acid (7 ml.) on the steam bath for one hour and worked up in the usual way to give a gum which showed a spot, Rf 0.95, on paper chromatography on the usual system. The gum sublimed at 140°C and 0.5 mm. pressure to give a solid which crystallised from methanol as plates, m.pt. = 105-113°C (2 mg.), undepressed on admixture with authentic LX.

Desacetylamidocolchiceine (XIII, R = OH)

This was prepared by the method of Eschenmoser(179).

Demethylation of desacetylamidocolchiceine

Desacetylamidocolchiceine (200 mg.) was refluxed with 48% hydrobromic acid (20 ml.) for four hours, and the solution was taken to dryness *in vacuo*. The resultant black gum was digested with ether and water. The aqueous phase was extracted with ether and chloroform, and the combined organic layers were washed with brine, and the solvent was removed to give a solid (101 mg.) which crystallised from ethanol as yellow-green prisms, m.pt. = 238-240°C (found C, 67.31%; H, 5.20%.

$C_{16}H_{14}O_5$ requires C, 67.12%; H, 4.93%), ν_{max} = 3500 (OH) and 1590 cm^{-1} (aromatics), λ_{max} = 363 mμ, shifting to 402 mμ in base. The n.m.r. spectrum had a singlet at 3.75 τ for the proton on the benzene ring and a singlet at 2.67 τ for the isolated C_3 proton on the tropolone ring. The C_6 and C_7 tropolone protons exhibited an AB spectrum centred on 2.53 τ and 2.86 τ with J_{AB} = 12 c.p.s.

Methylation and hydrolysis of LXI

The tricyclic compound LXI (10 mg.), methyl iodide (0.5 ml.) and dry AR potassium carbonate (500 mg.) were refluxed in acetone (10 ml.) for eighteen hours. The solution was filtered, taken to small bulk and added to excess water. The solution was extracted with ether and the extracts were washed with dilute sodium hydroxide solution and water and dried (anhyd. $MgSO_4$). The solvent was removed to give a gum to which water (10 ml.) and sulphuric acid (0.5 ml.) were added. The solution was heated on the steam bath for one hour and worked up in the usual way to give a solid (6 mg.) on trituration with methanol. This had m.pt. = 157-160°C, undepressed on admixture with desacetylamidocolchiceine.

Air and sodium carbonate on LXI

The compound LXI (10 mg.) was dissolved in water (10 ml.) containing AR sodium carbonate (80 mg.) and air was bubbled through the solution. Aliquots were taken at 3, 24 and 48 hours and worked up in the usual way. The ultra-violet spectra all had λ_{max} = 390 mμ, shifting to 402 mμ in base. The experiment was repeated on LXI (10 mg.) for 24 hours, and the product (3 mg.) was paper chromatographed on the usual ascending system to show a brown fluorescent spot of Rf 0.81, identical to starting material.

Air and barium hydroxide on XXXIII

The compound XXXIII (10 mg.) was dissolved in water (1 ml.) and AR barium hydroxide (700 mg.) in water (8 ml.) was added. Air was passed through the solution for thirty hours and it was worked up in the usual way to give a gum with an ultra-violet spectrum characteristic of the bicyclic system, $\lambda_{max} = 325\ m\mu$, shifting to 335 and 396 $m\mu$ in base.

Air and ammonium hydroxide on XXXIII

The precursor XXXIII (10 mg.) was dissolved in 3N ammonium hydroxide solution (30 ml.) and air was passed through the solution for thirty hours. The solution was worked up in the usual way to give a gum with $\lambda_{max} = 325\ m\mu$, shifting to 335 and 396 $m\mu$ in base.

Air and sodium carbonate on XXXIII

The precursor XXXIII (10 mg.) was dissolved in a solution of AR sodium carbonate (80 mg.) in water (10 ml.). Air was passed through the solution and aliquots taken at 3, 24 and 48 hours were worked up in the usual way. The ultra-violet spectra of these aliquots were identical to the spectrum of starting material.

The reaction was repeated using XXXIII (25 mg.) and aerating for 24 hours. The gummy product (5 mg.) was paper chromatographed on the usual system (ascending) to give a large trailing spot of Rf 0.4 to 0.9.

Lead dioxide oxidation of XXXIII and LXI

The compound LXI (10 mg.) was dissolved in dry acetone (10 ml.) and stirred vigorously with lead dioxide (100 mg.). Aliquots were filtered off at 2, 5, 10, 15, 30, 60 and 150 minutes. None of these showed any ultra-violet absorption in the 250 to 400 mμ region.

The same results were obtained with the compound XXXIII.

Ferric chloride treatment of LXI

The compound LXI (10 mg.) was dissolved in ethanol (10 ml.) with AR ferric chloride hexahydrate (13 mg.) and aliquots were worked up in the usual way after 1, 5 and 24 hours. The ultra-violet spectra were identical to the spectrum of starting material and paper chromatography on unbuffered No. 1 paper (descending) using a benzene:acetic acid:water (125:72:3) system indicated that each aliquot contained only starting material, Rf 0.75.

Ferric chloride treatment of XXXIII

The compound XXXIII (10 mg.) was dissolved in ethanol (10 ml.) with AR ferric chloride hexahydrate (13 mg.) and aliquots were worked up in the usual way after 2, 5, 15 and 30 minutes and 1, 3 and 18 hours. The ultra-violet spectra showed a progressive shift from 325 mμ to longer wavelength, and the aliquot taken after 18 hours had $\lambda_{max} = 326$ mμ and 338 mμ, shifting to 396 mμ in base. Descending paper chromatography on buffered no. 1 paper using a benzene:acetic acid:water (125:72:3) system showed starting material, Rf 0.37, in all aliquots, and later aliquots had a blue fluorescent spot at Rf 0.5.

Manganese dioxide (four parts) treatment of LXI (one part)

The tricyclic compound LXI (10 mg.) was dissolved in acetone (15 ml.) and shaken with manganese dioxide (40 mg.). Aliquots were taken at 5, 10, 20, 40, 60 and 120 minutes, filtered through celite and the solvent was removed. The ultra-violet spectra of the aliquots all had $\lambda_{max} = 355\ m\mu$ shifting to $402\ m\mu$ in base which was the spectrum typical of starting material. Ascending paper chromatography on unbuffered No. 1 paper, using a benzene:acetic acid:water (127:72:3) system showed only starting material, Rf 0.2.

The experiment was repeated with LXI (20 mg.) for 40 minutes, and paper chromatography of the product with the usual system (see p.102) showed a yellow-blue spot, Rf 0.57.

Manganese dioxide treatment of XXXIII (4:1 by weight)

The precursor XXXIII (10 mg.) was shaken with manganese dioxide (40 mg.) in acetone (15 ml.) and aliquots were taken at 2, 5, 10, 15, 30 and 60 minutes. The ultra-violet absorption at 321 and $350\ m\mu$, shifting to 340 and $400\ m\mu$ in base, characteristic of starting material, was observed up to the 30 minute aliquot but the one hour aliquot showed no maximum in the 300 to $400\ m\mu$ region. Paper chromatography on unbuffered No. 1 paper using a benzene:acetic acid:water (125:72:3) system showed diminishing amounts of starting material with time.

Manganese dioxide treatment of LXI (1:1 by weight)

The compound LXI (15 mg.) was dissolved in acetone (25 ml.) and shaken with manganese dioxide (15 mg.). Aliquots were taken at 15, 30, 60 and 120 minutes and paper chromatography on the usual ascending system showed diminishing amounts of starting material, Rf 0.76, and increasing amounts of a yellow-green spot, Rf 0.50, with time.

Manganese dioxide treatment of XXXIII

The compound XXXIII (20 mg.) was dissolved in acetone (30 ml.)

and shaken with manganese dioxide (20 mg.). Aliquots were taken at 15, 30, 60, and 120 minutes, and paper chromatography on the usual system showed that these consisted mainly of starting material, Rf 0.83. The aliquot taken after 30 minutes showed a yellow spot of Rf 0.81.

The reaction was repeated with XXXIII (40 mg.) for 30 minutes and the product was crystallised from ethanol to give a solid (2 mg.), m.pt. = 184-191°C, with an ultra-violet spectrum identical to that of starting material.

Ferricyanide (3.3 moles) treatment of a sodium carbonate solution of LXI

The compound LXI (10 mg.) was dissolved in a solution of AR sodium carbonate (80 mg.) in distilled water (6 ml.) and 3.8 ml. of a 1% potassium ferricyanide solution was added, with stirring, under nitrogen. Stirring was continued, and aliquots were taken after 5, 10, 15, 30, 60 and 120 minutes, and worked up in the usual way. The ultra-violet spectra were identical to the spectrum of starting material but ascending paper chromatography on the usual system indicated that the starting material soon disappeared.

The reaction was repeated on LXI (10 mg.) for 30 minutes, and the gummy product showed a yellow fluorescent spot, Rf 0.55, on ascending paper chromatography with the usual system.

Ferricyanide (3.3M) treatment of a sodium bicarbonate solution of XXXIII

The precursor XXXIII (10 mg.) was dissolved in a solution of AR sodium bicarbonate (80 mg.) in distilled water (6 ml.) and 3.8 ml. of a 1% potassium ferricyanide solution was added with stirring under nitrogen. Aliquots were taken after 5, 15, 30 and 60 minutes and worked up in the usual way to give gums which were spotted on paper. Ascending paper chromatography on the usual system showed starting material as a pale blue spot, Rf 0.89, in the 5, 15 and 30 minute aliquots, and a yellow-green fluorescent spot, Rf 0.56, was observed in the 60 minute aliquot.

Isolation of the pyrone from oxidation of LXI

The tricyclic compound LXI (100 mg.) was dissolved in a solution of AR sodium carbonate (800 mg.) in distilled water (60 ml.), and 38 ml. of 1% potassium ferricyanide solution was added, with stirring, under nitrogen. The solution was stirred at room temperature for forty minutes and worked up in the usual way to give a solid which crystallised from ethanol as yellow needles (36 mg.) m.pt. = 284–286°C (found C, 62.42%; H, 5.6%. $C_{16}H_{12}O_6$.EtOH requires C, 62.42%; H, 5.24%). Sublimation at 220°C and 0.3 mm. pressure gave yellow needles, m.pt. = 286–288°C (found C, 64.07%; H, 4.13%. $C_{16}H_{12}O_6$ requires C, 64.00%; H, 4.03%). The infra-red spectrum had ν_{max} = 3500 (OH), 3250 (OH), 1750 and 1700 cm^{-1} (split CO_2H) and the ultra-violet spectrum had λ_{max} = 233 mμ (9,740) and 365 mμ (7,000), shifting to 239 mμ (18,400) and 404 mμ (13,370) in base. The n.m.r. spectrum is discussed in the theoretical part of this thesis.

Isolation of the pyrone from oxidation of XXXIII

The precursor XXXIII (100 mg.) was dissolved in a solution of AR sodium bicarbonate (800 mg.) in distilled water (60 ml.) and a 1% aqueous potassium ferricyanide solution (38 ml.) was added, with stirring, under nitrogen. Stirring was continued at room temperature for one hour and the reaction was worked up in the usual way to give a solid which crystallised from ethanol as yellow needles (5 mg.), m.pt.= 286-290°C, λ_{max} = 232 mμ (7,940) and 365 mμ (5,640), shifting to 240 mμ and 403 mμ in base. The infra-red spectrum of this compound was identical to that of the pyrone from the tricyclic compound LXI, and a mixed melting point of the two compounds was undepressed. The paper chromatographic characteristics of the two compounds on the usual system were identical, both appearing as a yellow-blue fluorescent spot, Rf 0.56.

Ferricyanide (2M.) treatment of a sodium carbonate solution of XXXIII

The precursor XXXIII (20 mg.) was dissolved in a solution of AR sodium bicarbonate (160 mg.) in distilled water (12 ml.) and a 1% solution of potassium ferricyanide (4.6 ml.) was added with stirring under nitrogen. Aliquots were taken at 5, 15, 30, and 60 minutes and worked up in the usual way. Paper chromatography with the usual system revealed starting material, Rf 0.75, in all samples, and the 60 minute aliquot showed a yellow-green spot, Rf 0.47, which was identical to a sample of the pyrone which was run on the same paper.

Ferricyanide (0.5M) treatment of a sodium carbonate solution of XXXIII

The precursor XXXIII (100 mg.) was dissolved in a solution of AR sodium carbonate (800 mg.) in distilled water (120 ml.) and 0.5% potassium ferricyanide solution (12 ml.) was added dropwise over fifteen minutes. The reaction was worked up in the usual way to give a gum (59 mg.) which crystallised from ethanol with m.pt. = 74-103°C. Both the solid and the gummy mother liquors showed considerable amounts of starting material, Rf 0.77, on paper chromatography on the usual system, and the gummy mother liquors also contained the pyrone, Rf 0.57.

Ferricyanide (2M) treatment of XXXIII at pH 7

The precursor XXXIII (30 mg.) was dissolved in ethanol (3 ml.) and water (18 ml.) and 1% potassium ferricyanide (6.9 ml.) was added, with stirring, under nitrogen. The pH of the solution was adjusted to pH 7 by addition of 10% sodium bicarbonate solution, and stirring was continued. Aliquots were taken at 5, 15, 30, 60 and 120 minutes and worked up in the usual way. Paper chromatography with the usual system showed decreasing amounts of starting material, Rf 0.78, and increasing amounts of pyrone, Rf 0.57.

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(2) Ruzicka and Meyer, Helv., 1921, 4, [illegible]

(3) Ruzicka and [illegible], Helv., 1922, 5, [illegible]

(4) Ruzicka and Stoll, Helv., 192[illegible], 5, [illegible]

(5) Ruzicka, [illegible], and [illegible], [illegible], [illegible], [illegible]

(6) Ruzicka, [illegible], [illegible], 341

(7) Ruzicka and Meyer, Helv., [illegible], 5, 315

(8) [illegible]

(9) [illegible]

(10) [illegible]

(11) [illegible]

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(12) [illegible], Helv., [illegible]

(13) [illegible], Helv., [illegible]

(14) [illegible]

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(16) [illegible]

(17) [illegible]

(18) [illegible]

(19) [illegible]

(20) [illegible]

(21) [illegible]

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