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A THESIS ENTITLED
"STRUCTURAL AND STEREOCHEMICAL STUDIES
IN THE DITERPENOID SERIES",

SUBMITTED TO THE

UNIVERSITY OF GLASGOW

FOR THE DEGREE OF DOCTOR OF PHILOSOPHY

IN THE FACULTY OF SCIENCE

BY

A. JOYCE RENFREW, B.Sc.

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ACKNOWLEDGMENTS

The author wishes to express her sincere thanks to Dr.K.H. Overton for his constant guidance and encouragement over the past three years. She is also grateful to Professor R.A. Raphael, F.R.S. for the opportunity to carry out this research and to Dr. J.D. Connolly for his interest and advice.

Thanks are due to Professor W. Klyne for measuring the optical rotatory dispersion curves and to Professor G. Snatzke for his circular dichroism measurements. The author is also appreciative of the assistance of Mrs. F. Lawrie for the measurement of solution infra-red spectra, Mr.J. Gall and Mr. J. Lennon for the recording of nuclear magnetic resonance spectra, Mr.J.M.L. Cameron, B.Sc., and his staff for micro-analyses and Miss J. Wilkie and Miss H. Humphries for mass spectrometric data.

Finally, the author wishes to record her thanks to the Scientific Research Council and to the Salters Institute of Industrial Chemistry for maintenance awards during the past three years.

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SUMMARY OF THESIS
ENTITLED
STRUCTURAL AND STEREOCHEMICAL STUDIES IN THE
DITERPENOID SERIES

Part 1. The absolute stereochemistry of the proton at C₈ in desoxyrosenono- and rosenono-lactone has never been defined unambiguously, although rosololactone has been shown to possess an 8 α hydrogen from X-ray analysis of dibromorosololactone. From direct correlation with rosololactone, by way of acetate pyrolysis and with the assistance of gas liquid - mass spectrometric analysis, the proton at C₈ in rosenono- and desoxyrosenono-lactone is assigned an α configuration, and hence isorosenono-lactone an 8 β configuration.

Part 2. Two metabolites, lactone α and lactone β , were isolated from the mould Trichothecium roseum, whose structure had not previously been determined. The structure of lactone α , C₂₀H₂₈O₄, m.p. 180°C, $[\alpha]_D$ -162°, is defined from chemical evidence and from a direct correlation with rosenono-lactone. Evidence towards the structure of lactone β , C₂₀H₂₈O₄, m.p. 221°C, $[\alpha]_D$ -124°, is presented.

Part 3. A hydrocarbon, C₂₀H₃₂, $[\alpha]_D$ -117°, obtained from rosenonolactone, was found to be antipodal with the hydrocarbon formed from the acid isomerisation product of diol Y. Thus, the structure and absolute stereochemistry of diol Y is defined.

Part. 4. By the formation of tricyclic derivatives from eperuic and labdanolic acids, evidence is presented to support an α configuration for the methyl groups at C₁₃ in the two series. This assignment is confirmed by X-ray analysis of the p-bromo-phenacyl ester from labdanolic acid.

THE STRUCTURAL RELATIONSHIPS AND BIOSYNTHESIS OF THE TRICYCLIC DITERPENOIDS

In the hundred years which have elapsed since the word "isoprene" was introduced into chemical literature by Williams,⁽¹⁾ the structure of a large number of terpenoids has been elucidated and, from consideration of this wealth of information, there gradually emerged the realisation that these compounds were constructed on a definite architectural plan. At the beginning of this century study of the monoterpenes brought forth the theory that these were compounds with a carbon skeleton consisting of two isoprene units attached in a "head to tail" sequence but, until 1921, the idea that the higher terpenoids were structurally related was not generally realised. About this time Ruzicka's interest in the higher terpenoids developed, and the probable role of isoprene polymers in the generation of the sesquiterpenes was accepted. With the knowledge that lanosterol was an intermediate between squalene and cholesterol^(3, 4) this basic biogenetic hypothesis was extended to include not only mono-, sesqui- and di-terpenes but also the structures of lanosterol and the pentacyclic triterpenoids. It appeared that all of these compounds were derived in a similar manner from aliphatic precursors, and this led to the proposition by Ruzicka⁽²⁾ in 1953, of the Biogenetic Isoprene Rule. According to this rule terpenoids are compounds formed by combination of isoprene (C₅) units into aliphatic substances such as geraniol (C₁₀),

farnesol (C15), geranyl geraniol (C20) and squalene (C30) which subsequently cyclise and, where appropriate, rearrange by acceptable mechanisms to give the individual members of the mono-, sesqui-, di-, and tri-terpenoids respectively.

Consideration of the biosynthesis of the terpenoids according to this view falls into three distinct stages. First, there is the problem of origin of the isoprene units. Secondly, there is the manner in which these units condense to the aliphatic precursors postulated in the Isoprene Rule. Finally, there is the question of cyclisation, rearrangement and subsequent transformation of these precursors to the actual terpenoid.

The importance of acetic acid, in the form of acetyl co-enzyme A, as the precursor in all natural product synthesis, has been established for some time now. By means of acetate labelled with C¹³ or C¹⁴ it has been possible to show that acetic acid is a carbon source for the biosynthesis of all steroids and terpenoids so far investigated, in particular geraniol,⁽⁵⁾ squalene,⁽⁸⁾ rosenonolactone^(6, 7) and gibberellic acid.⁽⁹⁾ Cholesterol has probably been investigated more thoroughly than most natural products of this type and, largely as a result of the exhaustive studies of Cornforth and Popjak,^(10, 11) the origin of all the carbon atoms in cholesterol (II), which had been derived from acetate (I), is as shown.

Although it was now obvious that acetate molecules condense to give five-carbon fragments, it was not until Folkers⁽¹²⁾ isolated mevalonic acid (IIIa) that a true understanding of the mode of synthesis of the isoprene unit was derived. This acid can be obtained from acetyl co-enzyme A (1a) by a sequence of Claisen-like condensations, Figure I.

Mevalonic acid was shown to play a major role as a precursor in the biosynthesis of polyisoprenoids, by its ability to replace acetate in the biosynthesis of cholesterol,⁽¹³⁾ the conversion being almost quantitative in certain biological systems. The key position of the acid in terpenoid biogenesis was further established by the studies of Birch and Arigoni. These workers and their associates demonstrated the intermediacy of mevalonate by the incorporation of 2-C¹⁴-MVA into the triterpenoids soyasapogenol A (IV),⁽¹⁴⁾ lupeol (V), betulin (VI) and betulinic acid (VIa),⁽¹⁶⁾ and into the diterpenoids rosenonolactone (VII),^(6, 7) pleuromutilin (VIII)⁽¹⁵⁾ and gibberellic acid (IX).⁽⁹⁾

Mevalonic acid loses one of its carbon atoms on incorporation into the terpenoids and steroids. Tavormina⁽¹³⁾ demonstrated this by showing that 2-C¹⁴-MVA was incorporated into labelled cholesterol, whereas 1-C¹⁴-MVA gave inactive cholesterol.

In experiments concerning the conversion of mevalonic acid into squalene the necessity of adenosine triphosphate (ATP) was established, giving a clue to the first step in the production of the isoprene unit, that is the formation of mevalonate-5-phosphate. Further incubation⁽¹⁷⁾ of this phosphomevalonic acid led to the formation of the corresponding C₅-pyrophosphate (X), and stoichiometric quantities of adenosine-diphosphate were formed during the reaction. From mevalonic-5-pyrophosphate there originates the much sought-after biological isoprene unit, which has been identified as Δ^3 -isopentenyl pyrophosphate (XI) by both Bloch⁽¹⁷⁾ and Lynen⁽¹⁸⁾ and their respective co-workers. By a series of most elegant experiments using 1-C¹⁴-MVA, 2-C¹⁴-MVA and ATP³² alone and in combination, Bloch proved the chemical constitution of the intermediate. Lynen isolated the intermediate by blocking the enzyme preparation with iodo-acetamide and also showed that 2-C¹⁴-MVA gave rise to Δ^3 -isopentenyl pyrophosphate with C¹⁴ activity solely in the methylene group. The conversion of mevalonate-5-pyrophosphate to Δ^3 -isopentenyl pyrophosphate (IsPP) requires adenosine triphosphate⁽¹⁹⁾ and during this transformation it has been shown⁽¹⁸⁾ that carbon dioxide and adenosine diphosphate are produced at the same rate as Δ^3 -isopentenyl pyrophosphate. When the oxygen of the hydroxyl group is labelled as O¹⁸ in MVA, conversion of this to Δ^3 -isopentenyl pyrophosphate produces inorganic phosphate containing O, ¹⁸ confirming that the 3-hydroxyl group

is activated as its phosphate ester before expulsion. As no uptake of deuterium is observed in the production of Δ^3 -isopentenyl pyrophosphate the decarboxylation at C_1 and the dehydration at C_3 must be concerted. This series of reactions is shown in Figure 2.

Before condensation of the two C5 units can commence, it is necessary for Δ^3 -isopentenyl pyrophosphate to isomerise to dimethyl allyl pyrophosphate (XII). There is little doubt that both these C5 units are the "active isoprenes", and that both are necessary for the formation of the terpenoids. Lynen⁽²⁰⁾ and Bloch⁽²¹⁾ set up biological systems in which only one or other of these isoprene units was present and showed that it failed to produce the C15 carbon chain of farnesyl pyrophosphate (XIV). Furthermore, Bloch⁽²²⁾ found that during the synthesis of squalene in a medium containing D_2O , three or four deuterium atoms were incorporated into the isoprene chain. Two of these were positioned on the terminal isopropyl groups and originated from the isomerisation of Δ^3 -isopentenyl pyrophosphate to dimethyl allyl pyrophosphate. The other one or two, located at the centre of the squalene chain, were considered by Bloch to result from a reductive mechanism.

Lynen⁽²⁰⁾ and Bloch⁽²²⁾ showed that the formation of farnesyl pyrophosphate involves initially an allylic carbonium ion, derived by ionisation of the carbon-oxygen bond of dimethyl allyl pyrophosphate, and

thereafter alkylation of the reactive double bond of Δ^3 -isopentenyl pyrophosphate. Subsequent loss of a proton affords geranyl pyrophosphate (XIII), the immediate precursor of the mono-terpenes. The geranyl pyrophosphate so formed is itself an allylic pyrophosphate and can similarly alkylate another molecule of Δ^3 -isopentenyl pyrophosphate to give farnesyl pyrophosphate (XIV), the probable precursor of the sesqui-terpenoids. A continuation of this process leads to the C₂₀ geranyl-geranyl pyrophosphate (XV) from which the diterpenoids can be derived and from further additions it is possible to account for the ubiquinones and sesterterpenes. This scheme of addition is represented in Figure 3.

It must be emphasised that there is no evidence for the existence of an intermediate carbonium ion; the elimination of the pyrophosphate fragment, the alkylation of the double bond and the loss of the proton may well occur by a concerted mechanism.

The scheme proposed by Ruzicka⁽²⁾ for the cyclisation of squalene can be extended to include the cyclisation of geranyl-geranyl pyrophosphate to give the diterpenoids. Little evidence exists at present for the mode of formation of these C₂₀ compounds, but all experimental results so far confirm the premise that diterpenoids conform to the Biogenetic Isoprene Rule.

Either geranyl-geranyl pyrophosphate (XV), or its isomer geranyl-linaloyl pyrophosphate (XVI), is the direct precursor of the diterpenoids, and concerted cyclisation initiated by a proton leads to the bicyclic compound (XVII). Subsequent additions, eliminations and rearrangements, proceeding in a stereospecific trans diaxial manner afford the individual diterpenoids. Thus, the stereochemistry of most diterpenoids accords with their formation from geranyl-geranyl pyrophosphate (XV) in the chair-chair conformation (XIX), leading to the intermediate bicyclic alcohol (XVIII) with a trans-anti backbone. There was however a small group assigned a trans-syn backbone, and these could have been formed by cyclisation of geranyl-geranyl pyrophosphate (XV) in a chair-boat conformation (XX) to give the intermediate trans-syn alcohol. (XXI).

Diterpenoids in this group included eperuic acid (XXII), rimuene (XXIII), rosenonolactone (VII), gibberellic acid (IX) and cafestol (XXIV). Scott and his collaborators⁽²³⁾ have shown that from a combined X-ray and circular dichroism study the previously assigned configuration at C₉ in cafestol is in error, and have pointed out that rosenonolactone and gibberellic acid do in fact conform to a trans-anti precursor (XVIII), subsequent skeletal rearrangements resulting in the trans-syn backbone. Eperuic acid has also been shown⁽²⁴⁾ to have the normal trans-anti backbone, although it clearly arises from the antipode of bicyclic alcohol (XVIII).

Rimuene, whose structure has caused much speculation, has finally been shown to possess the same trans-anti stereochemistry as the rest of the diterpenoids.

The biosynthetic studies of Birch^(15, 25) and Arigoni^(26, 27) on the mould metabolites rosenonolactone (VII), gibberellic acid (IX), and pleuromutilin (VIII), support in every way the Biogenetic Isoprene Rule and also the theory that diterpenoids conform to a "trans-anti precursor rule" by having the labdane alcohol (XVIII) or its antipode as a biogenetic intermediate. Figure 4 illustrates the classes into which the diterpenoids can be divided on a structural basis, these differences being introduced by transformations and subsequent rearrangements of the basic labdane skeleton (XVIII).

There are, however, three small groups of diterpenoids which do not have the labdane alcohol (XVIII) as a precursor. Here, the geranyl-geranyl pyrophosphate (XV) takes up the conformation (XXV), and on cyclisation affords the monocyclic diterpenoid cembrene (XXVI) and its congeners,⁽²⁹⁾ the bicyclic diterpenoids related to verticillol⁽³⁰⁾ (XXVII) and the tricyclic diterpenoids of the taxane series, which includes taxinin (XXVIII)⁽²⁸⁾ whose structure has recently been defined by X-ray analysis.⁽³¹⁾

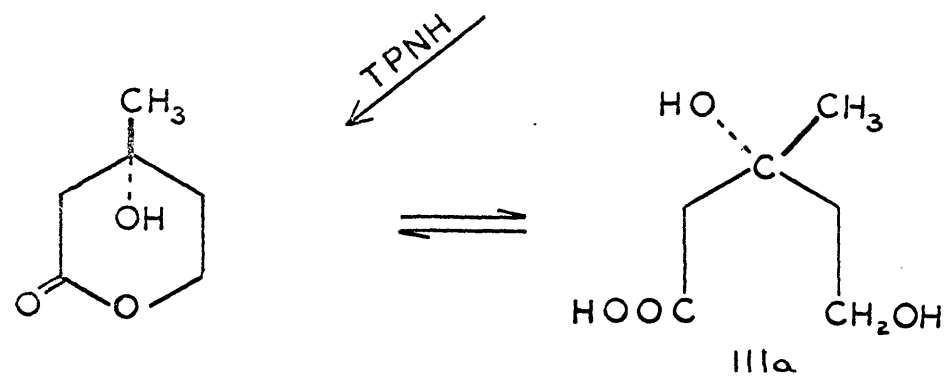
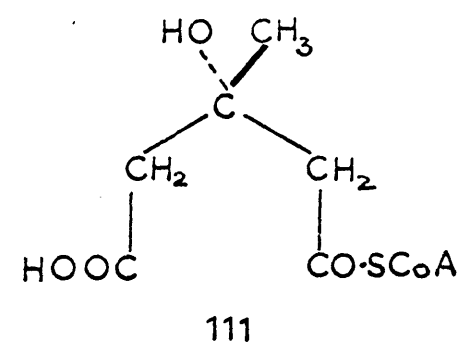
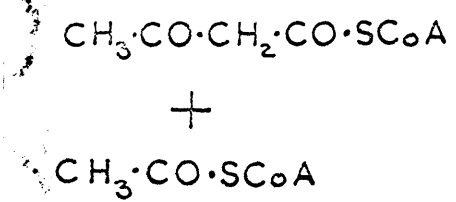
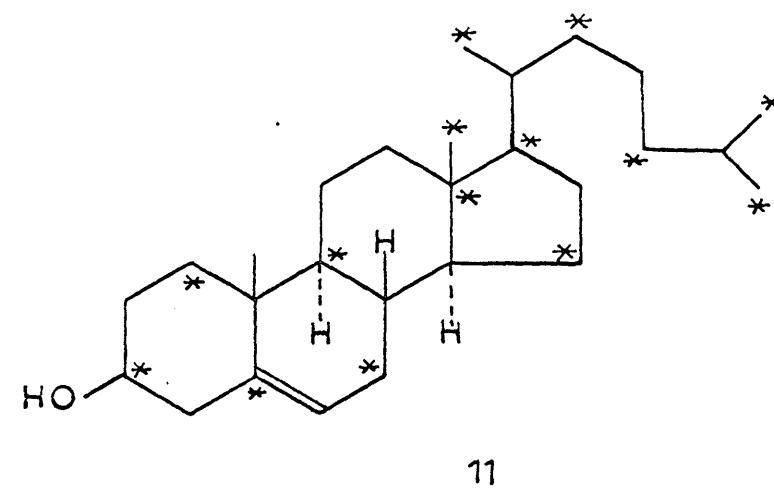
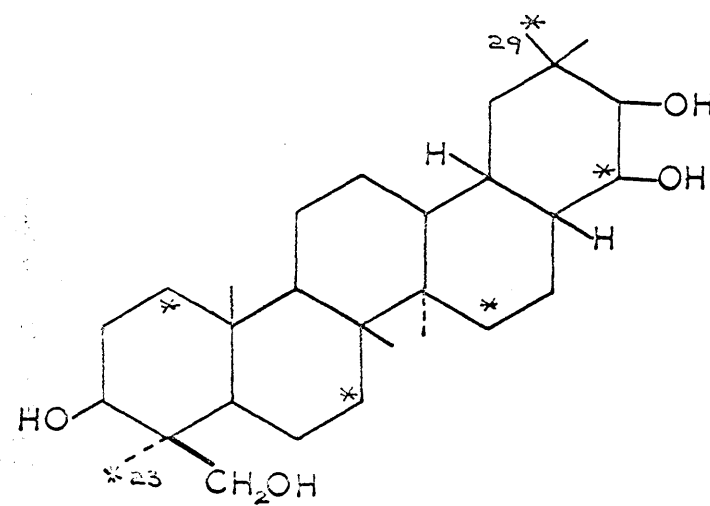
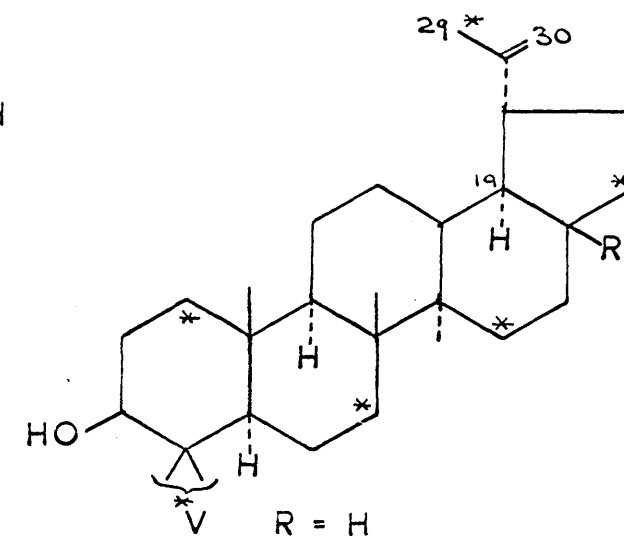


FIGURE 1



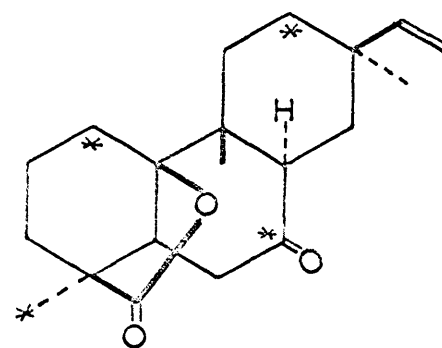
IV



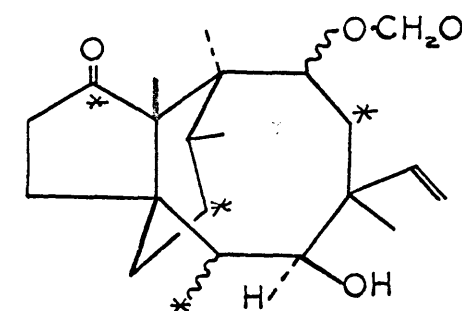
R = H

VI R = -CH₂OH

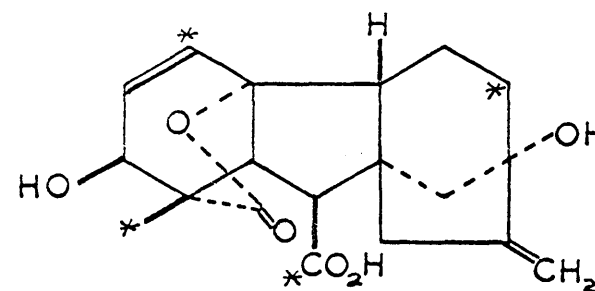
VI_a R = -COOH



VII



VIII



IX

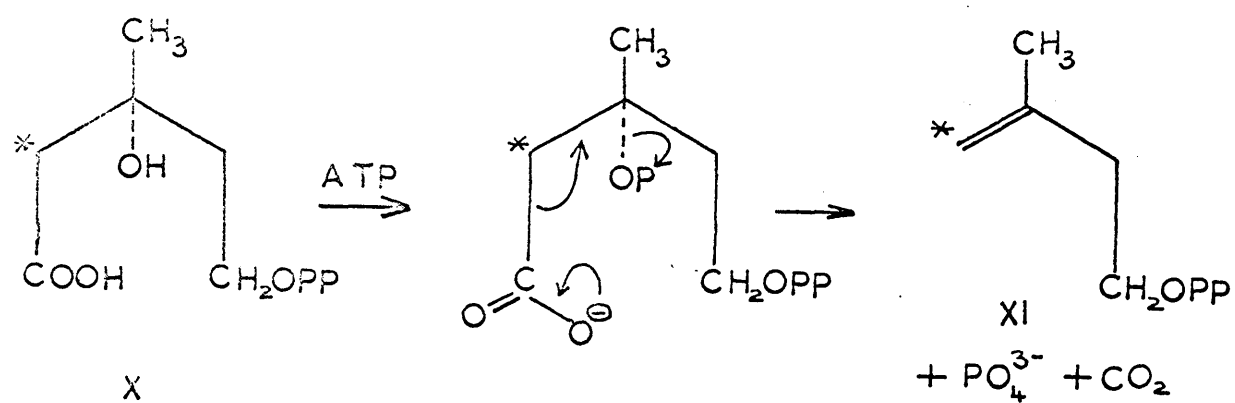


FIGURE 2

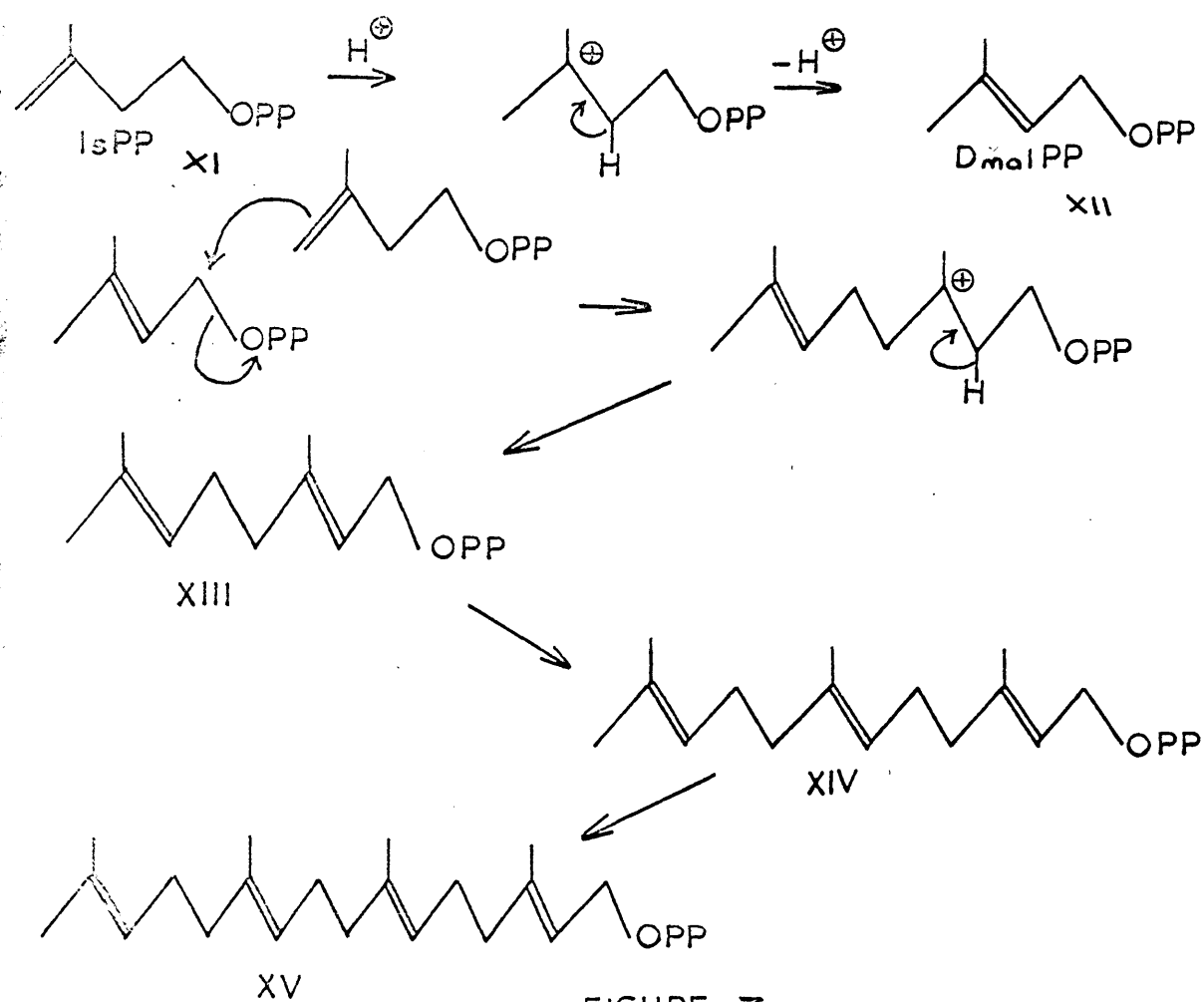
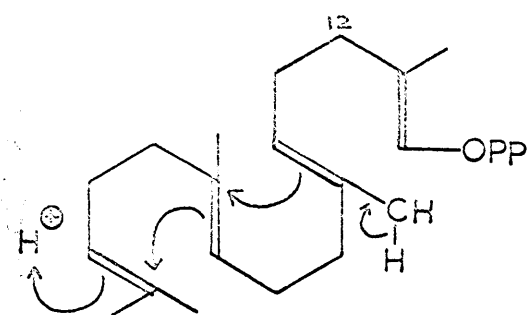
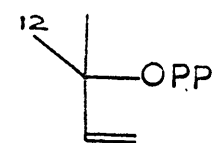


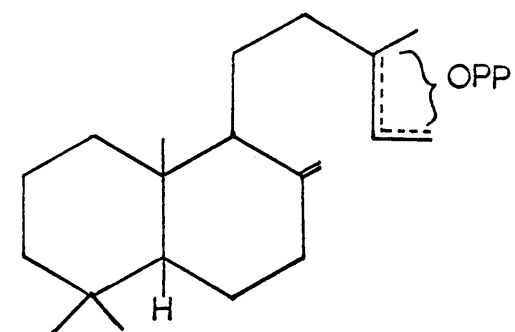
FIGURE 3



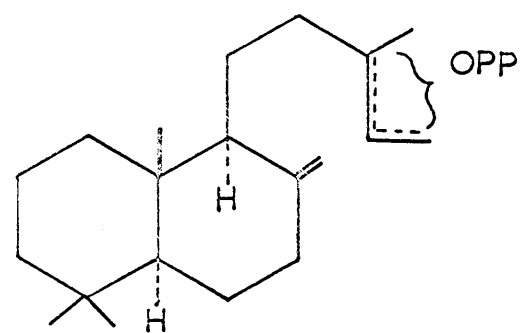
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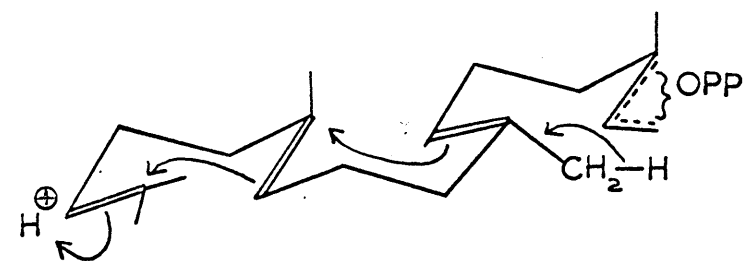
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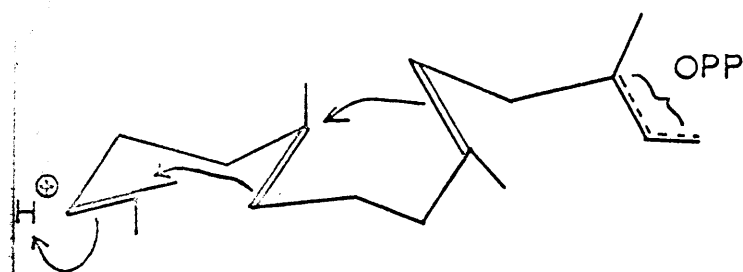
XVII



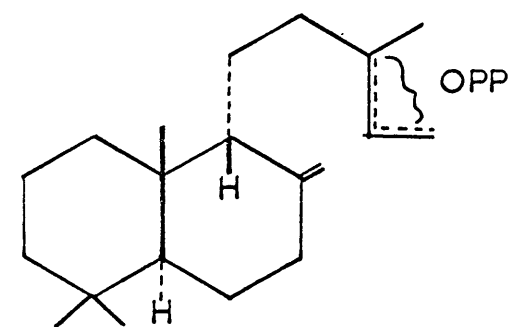
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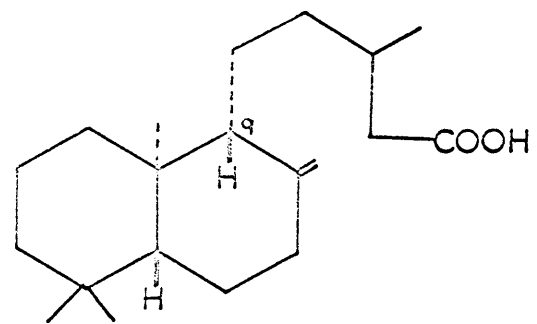
XIX



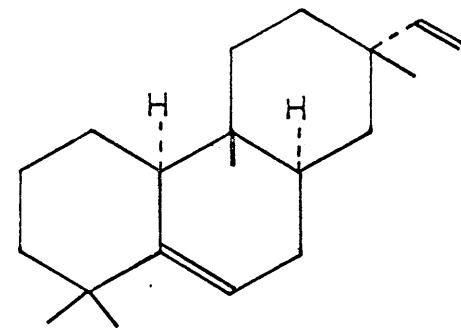
XX



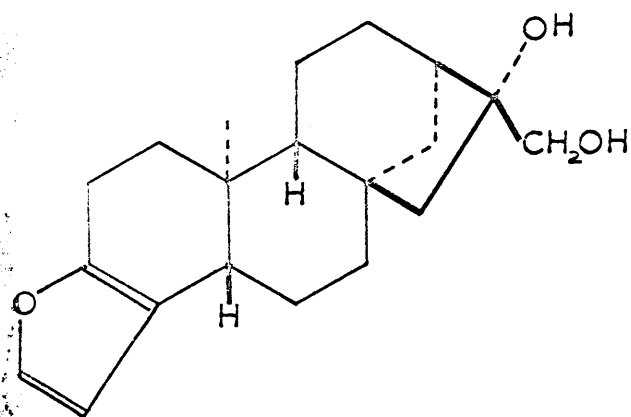
XXI



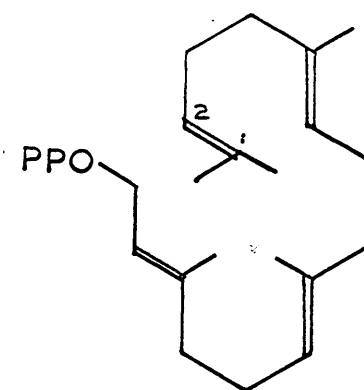
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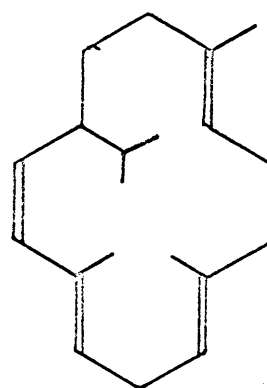
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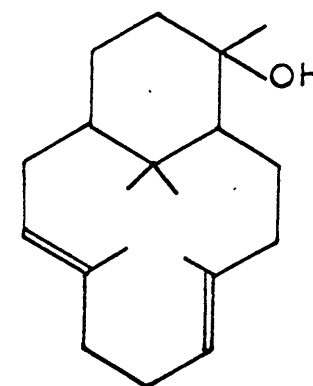
XXIV



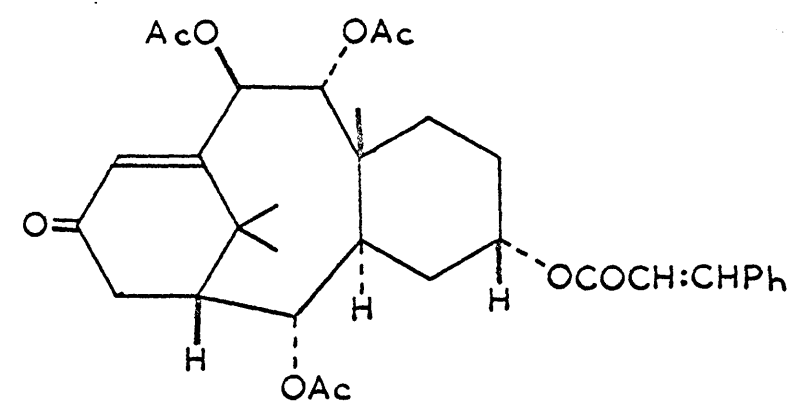
XXV



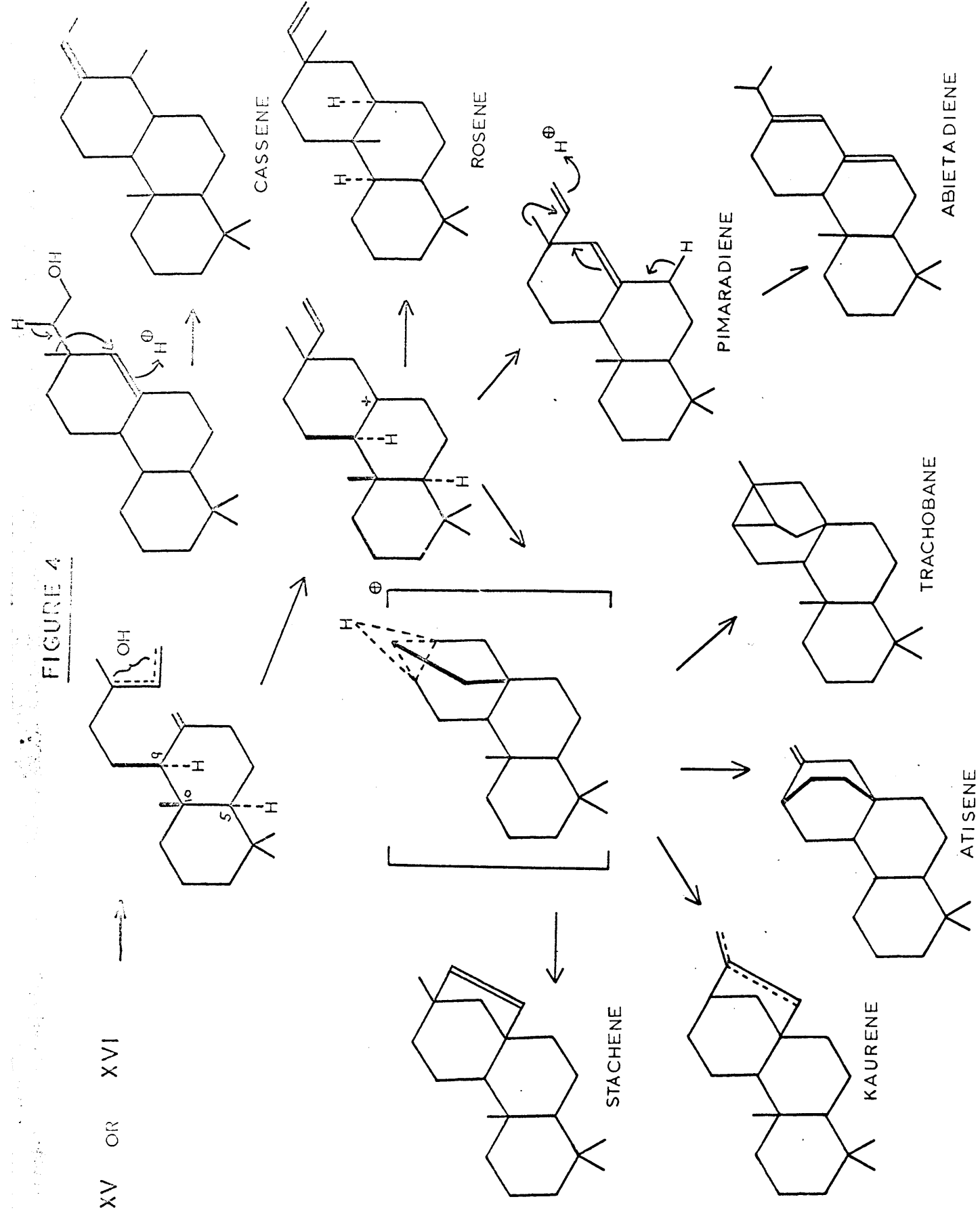
XXVI



XXVII



XXVIII



THE METABOLITES OF TRICHOTHECEUM ROSEUM LINK

The metabolites of the mould T. roseum were first examined by Freeman and Morrison^(32, 33, 34) and by Michael⁽³⁵⁾ who isolated a series of related diterpenoids termed rosein I, -II and -III, and a sesquiterpenoid trichothecin which was finally assigned the structure (XXIX) by Godtfredsen and Vangedal.⁽³⁶⁾ Rosein I and -II were assigned the names rosenonolactone and rosololactone respectively by the Liverpool group,⁽³⁷⁾ who deduced their structures and absolute stereochemistries.⁽³⁸⁻⁴¹⁾ No further work has been published on rosein III, a closely related metabolite to rosenono- and rosololactone, but evidence towards its structure will form a portion of this thesis.

Rosenonolactone^(37, 38, 40) m.p. 214°C, $[\alpha]_D - 107.5^\circ$, $C_{20}H_{28}O_3$, had bands at 1786 cm^{-1} and 1724 cm^{-1} in the infra-red, characteristic of a δ -lactone and a cyclo-hexanone. On catalytic reduction rosenonolactone absorbed only one mole of hydrogen, and on ozonolysis gave formaldehyde and a nor-acid, $C_{19}H_{26}O_4$. From this evidence it was deduced that the compound was a tricyclic lactone possessing a vinylidene group.

On dehydrogenation of the lactone with selenium 1 : 7 dimethyl-phenanthrene together with 9-hydroxy-1:7-dimethyl-phenanthrene (XXX)

were formed and on this basis rosenonolactone was given the partial structure (XXXI) with the carbonyl group placed at C₇. Dihydro-rosenonolactone (XXXIIa) was reacted with ethane dithiol and the resulting thioketal desulphurised with Raney nickel to give dihydrodesoxyrosenonolactone (XXXIII). Treatment of this with ethanolic hydrochloric acid yielded an acid (XXXIV), C₂₀H₃₀O₂, which was assumed to be a βγ unsaturated acid, as it lost carbon dioxide at its melting point, and had no vinylic protons in the infra-red.

Dihydrorosenonolactone (XXXIIa) when oxidised with potassium permanganate yielded a keto-acid (XXXV), C₂₀H₃₀O₅, and subsequent treatment with base gave a keto di-acid, C₁₀H₁₄O₅ (XXXVI) and a cyclohexanone, C₁₀H₁₈O, (XXXVII). These structures were proved by synthesis,⁽⁴²⁾ although their stereochemistry was undefined. The lactonic carbonyl group of rosenonolactone was attached at a quaternary position, since reduction to a methyl group and subsequent dehydrogenation with selenium, resulted once more in the formation of 1 : 7 dimethyl-phenanthrene. Hence, in the partial structure (XXXI), the lactonic carbonyl group was positioned at C₄.

Lithium aluminium hydride reduction of rosenonolactone gave a triol, which was not an α-glycol and which formed a diacetate. From this it was assumed that the triol had one tertiary hydroxyl group,

this group being at C₁₀ and being the termination of the γ -lactone.

Rosenonolactone was thus assigned the structure (XXXII) from this evidence, without any predictions as to its stereochemistry.

Rosololactone, m.p. 186°C, $[\alpha]_D + 6.3^\circ$, C₂₀H₃₀O₃, had bands (CCl₄) in the infra-red at 1780 cm⁻¹ (γ -lactone) and 3600 cm⁻¹ (hydroxyl). An interesting phenomenon was observed⁽⁴³⁾ in the solid state infra-red spectrum of rosololactone and also to a much lesser degree in the solution spectrum. Here, two distinct carbonyl absorptions were obtained at 1763 and 1710 cm⁻¹ accounted for by intermolecular hydrogen bonding.

Selenium dehydrogenation of this lactone gave 1 : 7 dimethyl-phenanthrene and ozonolysis formed a nor-acid, C₁₉H₂₈O₅. Treatment of dihydrorosololactone with sulphuric acid gave a diene acid (XXXVIIa) $\lambda_{\max}^{238 \text{ m}\mu}$) which yielded a $\beta\gamma$ unsaturated acid on hydrogenation, isomeric with the acid (XXXIV) from dihydrodesoxyrosenonolactone.

Oxidation of dihydrorosololactone gave a ketone, which on treatment with acid or base yielded an enone acid, C₂₀H₃₀O₃, $\lambda_{\max}^{248 \text{ m}\mu}$, $\epsilon_{\log} 4.05$, and 314 m μ , $\epsilon_{\log} 1.83$, formed by opening of the lactone ring. This suggested that the hydroxyl group was situated β to the attachment of the alkyl oxygen group of the lactone and therefore structures (XXXVIII) or (XXXIX) were proposed for rosololactone.

Desoxyrosenonolactone, m.p. 115-116°C, $[\alpha]_D + 57^\circ$, $C_{20}H_{30}O_2$, was isolated from T. roseum by Arigoni,⁽⁴⁴⁾ who assigned to the dihydro derivative the structure (XXXIII), by correlating it with the compound derived from desulphurisation of dihydrorosenonolactone-7-ethylene mercaptal.

The stereochemistry of rosenonolactone and the closely related diterpenoids is of paramount importance in defining the biogenetic precursor⁽⁴²⁾ of these metabolites. The degradation of the keto-acid (XXXV) from rosenonolactone with alkali afforded, as previously stated, a keto-acid (XXXVI), which epimerised from the less stable cis configuration to the more stable trans configuration on treatment with base. Thus, the cis-acid (XXXVIa) must be the initial product derived from ring A of dihydrorosenonolactone. The absolute stereochemistry of (XXXVIa) has been assigned as shown, since the O.R.D. curve of the epimer (XXXVIb) was comparable to that of (-)trans-2-methyl-2-carboxy-6-ketocyclohexyl-propionic acid (XL), whose absolute stereochemistry is known.⁽⁶²⁾ This established the absolute stereochemistry at positions 4, 5 and 10 in rosenonolactone, as in (XLI).

Relactonisation of the rosenoic acid (XXXIV) gave two isomeric γ -lactones, allo- and neo-hydroxy-rosonoic lactone. Neither of these

lactones was identical with or antipodal with desoxyrosenonolactone, or with the γ and δ lactones derived under identical conditions from either dihydropimaric or dihydroisopimaric acid. Whalley⁽⁴⁴⁾ concluded from these results that the lactone ring and the methyl group at C₉ were cis to one another and thus assigned a syn-orientation to positions C₉ and C₁₀.

Treatment of rosenonolactone with mild acid or base converted it into a mixture of unchanged starting material and isorosenonolactone. From ultra-violet and infra-red absorption spectra the iso compound still possessed an isolated carbonyl group and a γ -lactone. This reaction was shown to be reversible. Dehydrogenation of the iso-lactone with selenium gave 1 : 7 dimethyl-phenanthrene along with 9-hydroxy-1:7-dimethyl-phenanthrene, the same products as were obtained from rosenonolactone. From this it was deduced that both lactones have the same basic carbon skeleton (XLII), differing only in their stereochemistry at C₈, the asymmetric centre adjacent to the carbonyl group. Lithium aluminium hydride reduction of rosenonolactone yielded a triol with a relatively unhindered C₇ position, as shown by hydrolysis of the diacetate of this triol, which readily yielded the parent triol. Reduction of isorosenonolactone afforded a mixture of triols epimeric at C₇. This was rationalised by the assumption that the carbonyl group at C₇ is less accessible than in

rosenonolactone. The diacetates of these two triols on hydrolysis yielded in one instance the original triol and in the more hindered case the monoacetate. On this evidence and with reference to models Whalley postulated that rosenonolactone had a B/C trans ring junction with a trans-syn-trans configuration (XLIII), while conversely, iso-rosonolactone had a B/C cis ring junction and was represented as the trans-syn-cis isomer (XLIIIa). The steric hindrance experienced by the carbonyl group at C₇ in the iso series can be explained by the C₁₃ substituents which shield this position.

Thus, the absolute configuration of rosenonolactone was defined by Whalley⁽⁴⁰⁾ at positions 4, 5, 8, and 10, only position 13 remaining in doubt.

The biosynthesis of rosenonolactone has been shown to occur by the scheme (XIX) → (XVIII) → (XLIII) from the tracer studies of Birch⁽²⁵⁾ and Arigoni⁽⁴²⁾. When 1-C¹⁴ acetic acid and 2-C¹⁴ mevalonate were fed to T. roseum, rosenonolactone was obtained labelled as in (XLV) and (VII) respectively. The results clearly show that the molecule was built up from four mevalonate units as shown from the labelling pattern obtained. The absence of activity in the carbon dioxide derived from the lactonic carbonyl showed that the C₄ α methyl group was derived specifically from the C₂ position of

mevalonate, in agreement with similar results obtained in the
triterpene field⁽⁴⁶⁾ and gibberellic acid.⁽²⁵⁾

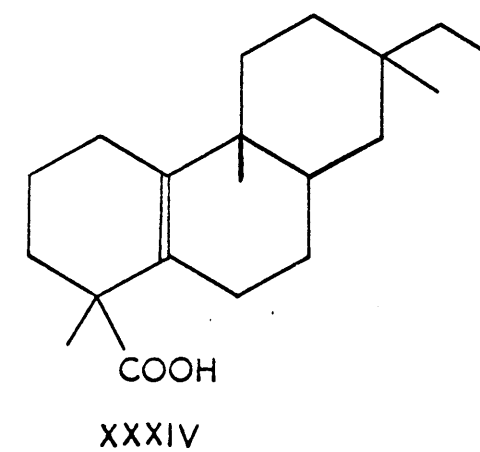
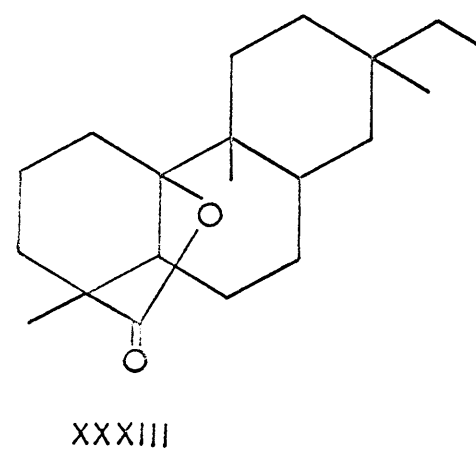
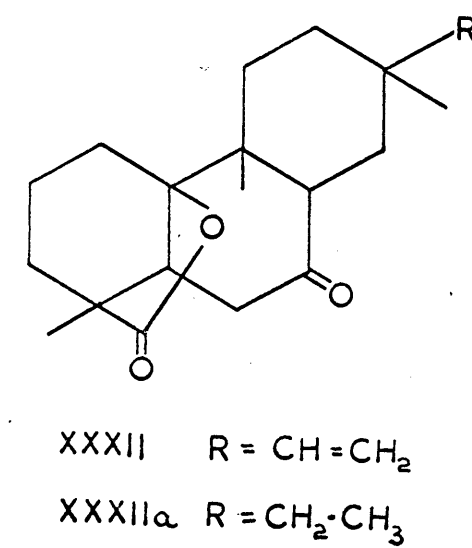
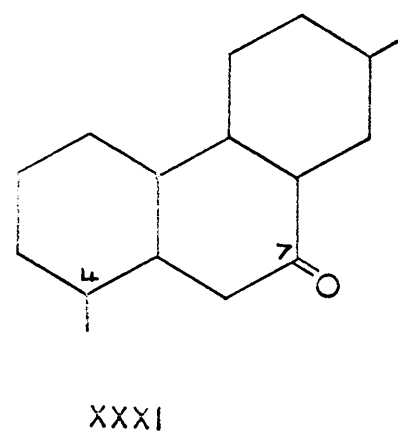
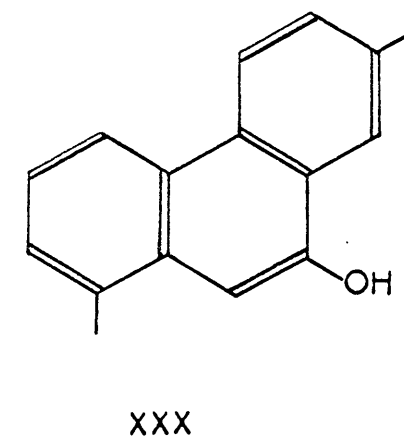
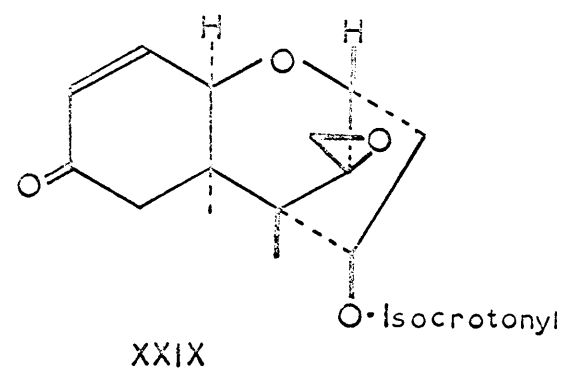
THE INTERRELATION OF ROSENONO-, ROSOLO-,
ISOROSENONO- AND DESOXYROSENONO-LACTONE

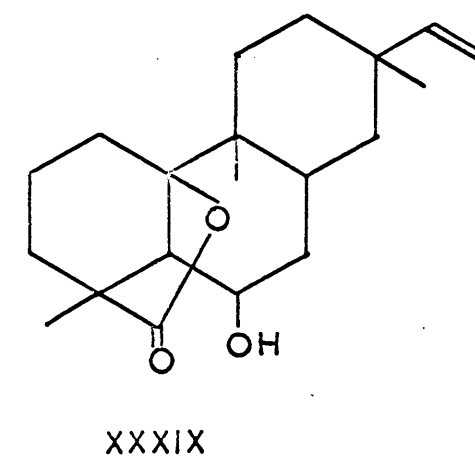
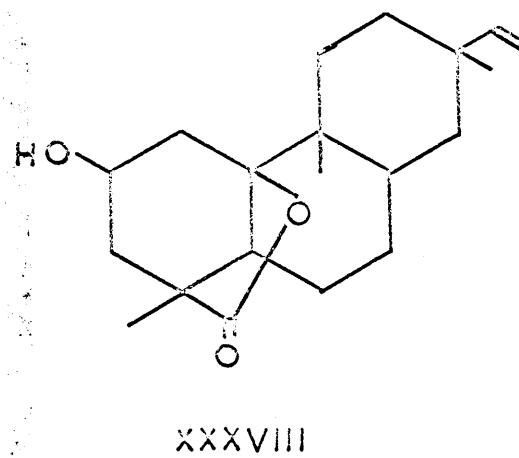
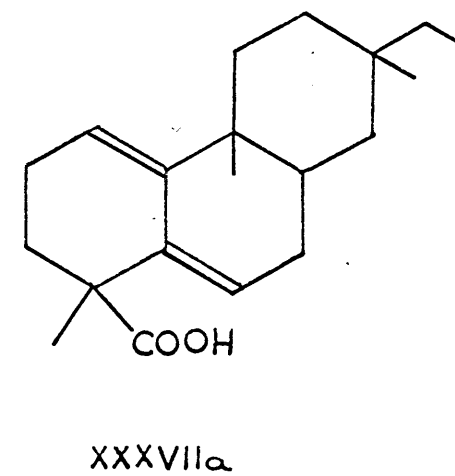
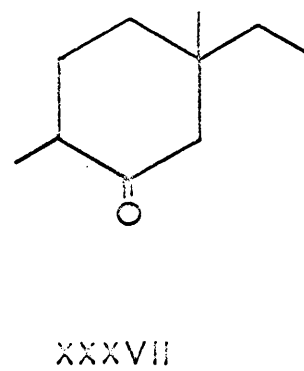
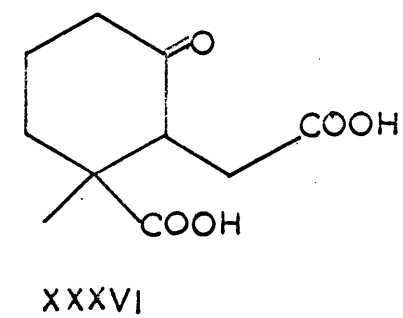
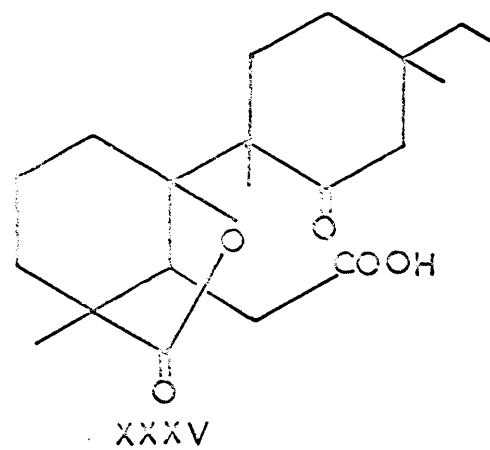
Whalley⁽⁴⁰⁾ defined the absolute configuration of rosenonolactone at positions C_5 , C_8 , C_9 and C_{10} , the asymmetric centre C_{13} remaining undetermined. This problem was solved by Scott and his associates⁽⁴⁷⁾ who derived the absolute stereochemistry of dibromorosololactone (XLVI) by X-ray diffraction studies and also showed that the hydroxyl group is in the C_6 β (axial) position. The X-ray analysis provided the complete structural solution for rosololactone (XLVIII). A direct correlation between rosololactone and rosenonolactone was obtained by formation of the same diosphenol (XLIX) from each lactone. Thus, rosenonolactone has the same absolute stereochemistry as rosololactone at positions C_4 , C_5 , C_9 , C_{10} and C_{13} and is assigned the structure (XLVII). The δ configuration attributed to rosenonolactone was based on the relationship between it and isorosenonolactone, the former having the less hindered C_7 carbonyl group and therefore possessing a trans B/C ring junction. This evidence does not establish conclusively the stereochemistry at C_8 in rosenonolactone.

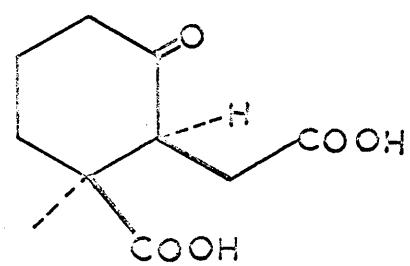
Likewise, the stereochemistry of desoxyrosenonolactone (L) at position 8 remains in some doubt. The dihydro derivative has been shown to be identical with the product from desulphurisation of the

thioketal of dihydrosenenonolactone and has been assigned the α configuration at C₈ ⁽⁴⁴⁾. However, the same thioketal was formed from isosenonolactone, epimerisation at C₈ being attributed to the interaction between the C₇ and the C₁₃ substituents, when C₈ has a β hydrogen atom. This evidence does not prove conclusively the absolute configuration of desoxysenenonolactone at position 8.

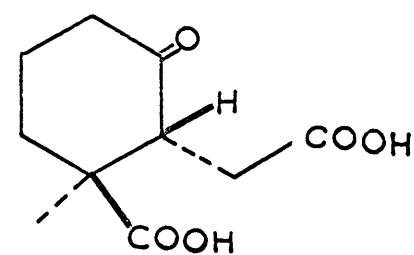
Consequently, although the absolute stereochemistry of rosenono-, isosenono-, and desoxysenenono- lactone has been established at positions C₅, C₉, C₁₀ and C₁₃, the configuration at C₈ in these compounds remains to be defined unambiguously.



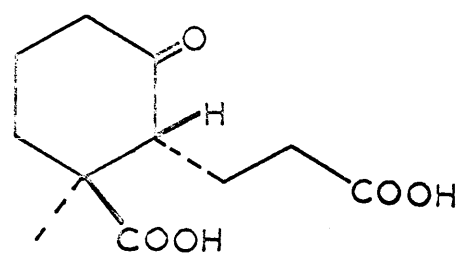




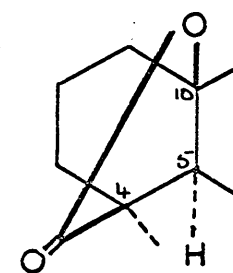
XXXVIa



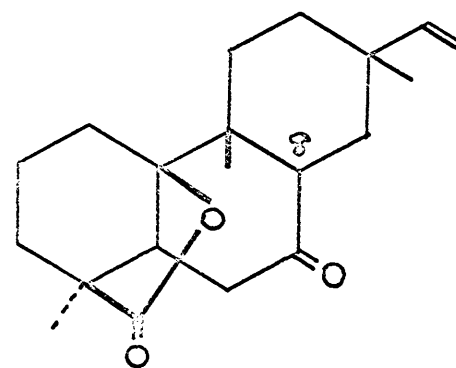
XXXVIb



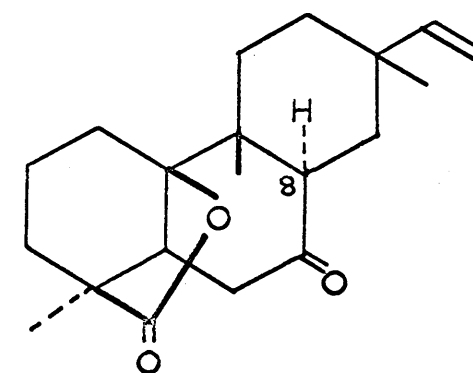
XL



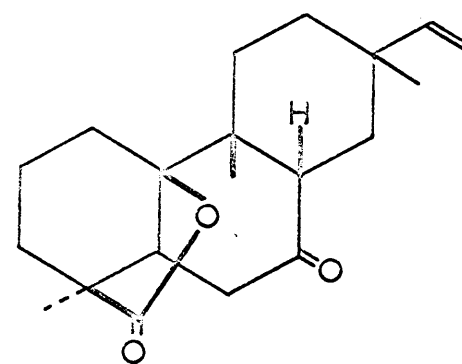
XLI



XLII

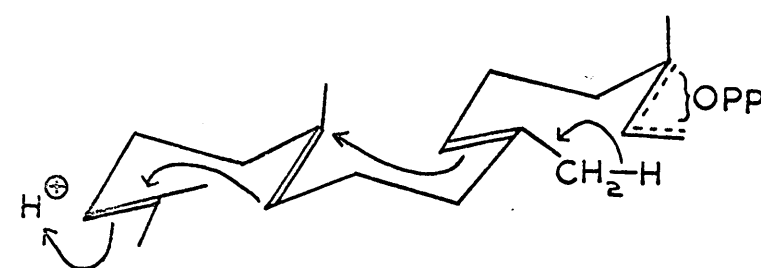


XLIII

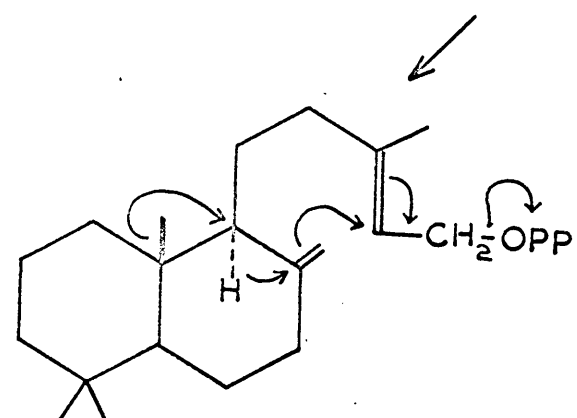


XLIIIa

CH_3COOH



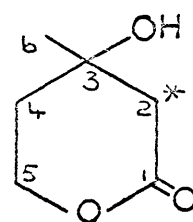
XIX



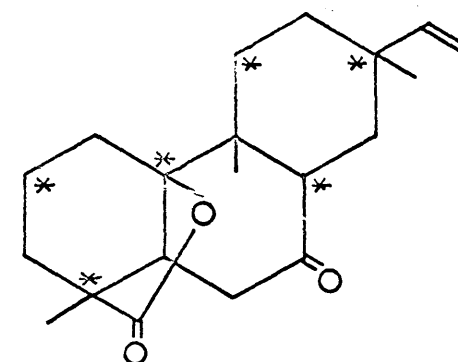
XVIII



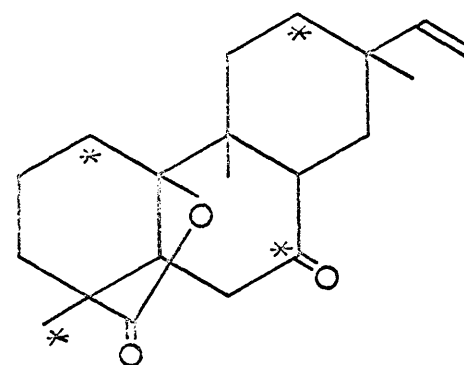
XLIII



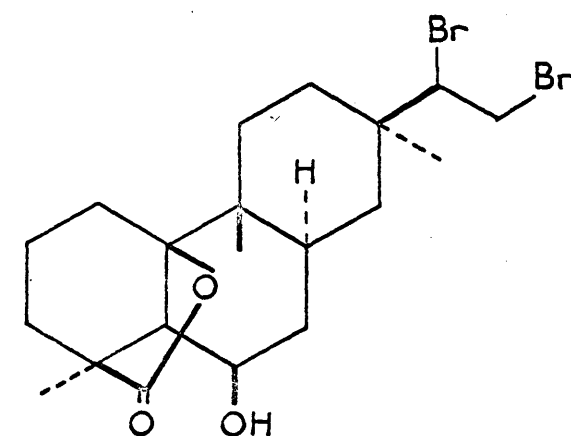
XLIV



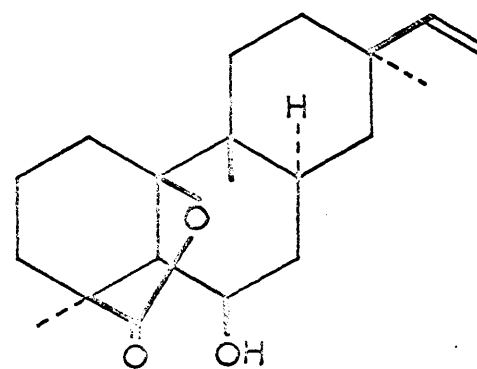
XLV



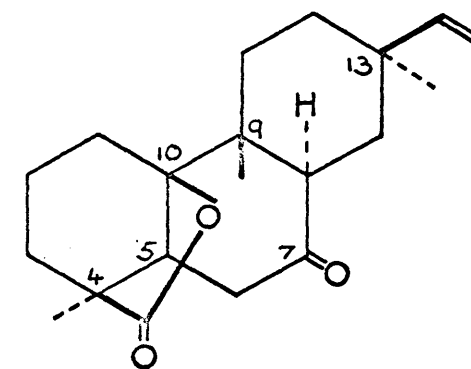
VII



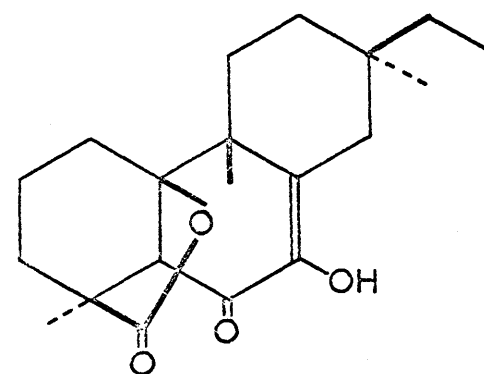
XLVI



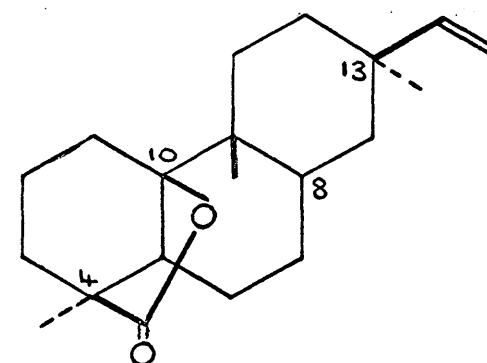
XLVIII



XLVII



XLIX



L

DISCUSSION

To establish unambiguously the configuration at C_8 in rosenonolactone (and hence in isorosenonolactone) and in desoxyrosenonolactone it was necessary to relate these compounds directly to rosololactone whose absolute stereochemistry has been confirmed⁽⁴⁷⁾ by X-ray analysis. Epimerisation at C_8 had to be guarded against in the course of these correlations since this would render the results inconclusive.

The experimental interrelation of desoxyrosenono- rosenono- and rosololactone was expected to be a relatively simple task. However, the various approaches discussed in the sequel revealed examples of unexpected reactivity or inertness which we feel must be a property of the peculiar conformation and environment of ring B in these compounds.

The initial intention was to remove the oxygen function at C_6 in rosololactone and at C_7 in rosenonolactone or its C_8 epimer. All attempts to form the thioketal^(48, 49, 38) of rosenono-, rosono-, and isorosenono-lactone failed to produce the desired product, the lactone ring being cleaved under the conditions used. Lack of success was also encountered on attempted formation of the tosylhydrazones⁽⁵¹⁾ of rosono-, rosenono- and isorosenono-lactone, the only products formed

being the dihydro lactones as seen by m.p., mixed m.p. with the authentic dihydro derivatives. In the hope of obtaining the $\Delta^{6(7)}$ -desoxy compounds, the three hydroxy-lactones were dehydrated with thionyl chloride in pyridine at 20°C. Dehydration of rosololactone afforded the oily $\Delta^{5(6)}$ -desoxyrosenonolactone (LI), $C_{20}H_{28}O_2$, as the sole product of the reaction, shown by t.l.c. (silver nitrate-silica gel) and g.l.c. (1% SE 30; 2% 20M Peg; 5% QF1). The structure (LI) was supported by the one proton multiplet at $\tau 4.7$; H_6 ; in the n.m.r. spectrum. Hydrogenation of this Δ^5 -compound with 10% palladium charcoal in ethanol yielded only an acid whereas hydrogenation in acetic acid over 10% palladium charcoal or platinum oxide yielded the same acid along with the dihydro - $\Delta^{5(6)}$ -desoxy lactone (LII), $C_{20}H_{30}O_2$. The $\Delta^{5(6)}$ -dihydrodesoxy-lactone (LII) was the only product formed on hydrogenation of (LI) in ethyl acetate with 10% palladium charcoal or 1% palladium on calcium carbonate as catalyst. Hence, this route for the production of desoxyrosenonolactone from rosololactone proved unsuccessful.

Reduction of rosenonolactone with sodium borohydride in methanol afforded the alcohol, rosenololactone (LIII), while reduction of isorosenonolactone with sodium borohydride yielded the same alcohol (LIII), (m.p., m.m.p., i.r., and n.m.r.), plus isorosenololactone (LIV). The common alcohol (LIII) must be formed from isorosenonolactone

by epimerisation at C₈ prior to reduction. The broad multiplet at T 6.1 ($w^{\frac{1}{2}} = 24$ c/sec.) ($\underline{\text{CH}}\text{-OH}$) in the n.m.r. spectrum of (LIII) does not define conclusively the stereochemistry at C₇, as in the 7 β -OH, 8 α -H, and the 7 α -OH, 8 α -H configurations, the value of the coupling of H₇ with H₈ and the two protons at C₆ is very similar as estimated from models. However, as the α face of rosenonolactone is the less hindered it is considered more likely that hydride attack will take place from this side to afford a 7 β -hydroxyl. Thus, rosenololactone is assumed to have the 7 β -OH, 8 α -H configuration as shown in (LIII).

The product (LIV) obtained only from isorosenonolactone can have the configuration 7 α or 7 β -OH, 8 β -H, or 7 α -OH, 8 α -H. From the multiplet at T 6.0 ($w^{\frac{1}{2}} = 10$ c/sec.) ($\underline{\text{CH}}\text{-OH}$) in the n.m.r. spectrum of (LIV), it is possible to eliminate the configurations 7 α -OH, 8 α -H, and 7 β -OH, 8 β -H, and define the stereochemistry of the alcohol (LIV) as 7 α -OH, 8 β -H. The 7 β -OH, 8 β -H structure is eliminated because this involves two large diaxial couplings between H₇(α) and H₈(β) and between H₆(β) and H₇(α). In the configuration 7 α -OH, 8 α -H, the large diaxial coupling between H₇(β) and H₈(α) and the relatively large coplanar coupling between H₆(β) and H₇(β), eliminates this as a possible

structure for (LIV). However, in the configuration 7 α -OH, 8 β -H, the coupling of $H_7(\beta)$ with $H_8(\beta)$ and the protons at C_6 complies with half height value of the coupling constant ($w^{\frac{1}{2}} = 10$ c/sec.). Thus, if rosenololactone has the 7 β -OH, 8 α -H configuration, then isorosenololactone (LIV) has the 7 α -OH, 8 β -H configuration. This, in fact, is reasonable since after epimerisation at C_8 the β face is more accessible to hydride attack.

Catalytic reduction of rosenonolactone yielded
Catalytic reduction of rosenonolactone yielded

pound

dihydrorosenololactone (LIIIa) (7 β -OH, 8 α -H), the same compound being obtained by hydrogenation of rosenololactone (LII) (m.p., m.m.p., i.r., n.m.r.). Likewise, catalytic reduction of isorosenonolactone afforded dihydroisorosenololactone (LIVa) (7 α -OH, 8 β -H), hydrogenation of isorosenololactone (LIV) giving the same compound (m.p., m.m.p., i.r., n.m.r.).

Dehydration of dihydrorosenololactone with thionyl chloride afforded two products, as seen from t.l.c. (silica gel-silver nitrate) and g.l.c. (1% SE 30; 2% 2OM Peg), which proved inseparable by chromatography over silver nitrate-silica gel. One of these enolactones was found to epoxidise, the other remaining unchanged (as seen by g.l.c.) under the conditions used. The structure (LV) (mass spec. m.wt. 318) m.p. 144-145, was ascribed to the epoxylactone from the one proton multiplet centred at τ 7.1 $\left(\begin{array}{c} \diagup \quad \diagdown \\ \text{O} \\ \diagdown \quad \diagup \\ -\text{CH} \text{ --- } \text{CR}_2 \end{array} \right)$ and therefore

one of the products of dehydration was the $\Delta^{7(8)}$ -dihydrodesoxyrosenonolactone (LVI). To the product of dehydration (mass spec. m.wt. 302) which failed to epoxidise, was attributed the structure (LVII) from the absence of olefinic protons in the n.m.r. and from the multiplet centred at $\tau 6.0$ (1H) assigned to the C_6 proton. The mechanism of formation of the product may be postulated as (LVIII) \rightarrow (LIX) \rightarrow (LVII).

Dehydration of dihydroisosenololactone afforded one product, $\Delta^{7(8)}$ -dihydrodesoxyrosenonolactone (LVI), $C_{20}H_{30}O_2$, m.p. $134-135^\circ$, so assigned from the one proton multiplet centred at $\tau 4.7$ and from its identical behaviour on g.l.c. (1% SE 30; 2% 20M Peg) with one of the compounds (LVI), obtained from dehydration of dihydrorosenololactone.

In the alcohols (LIII) and (LIV) the 7-OH and the 8-H are situated trans diaxially and consequently trans elimination occurs in both compounds to afford the $\Delta^{7(8)}$ -dihydrodesoxyrosenonolactone. It is difficult to explain why dehydration of isosenololactone affords only one compound whereas elimination of rosenololactone affords two, though the more hindered nature of the α -hydroxyl in isosenololactone may impose greater stereospecificity. It is surprising that no $\Delta^{6(7)}$ -dihydrodesoxyrosenonolactone is obtained on dehydration, especially from isosenololactone where the 6 β -H and the 7 α -OH are well set up for trans diaxial elimination.

As dehydration of the hydroxy-lactones with thionyl chloride failed to yield the desired $\Delta^{6(7)}$ -ene-lactones, borate pyrolysis⁽⁵²⁾ of the alcohols (XLVIII), (LIII) and (LIV) was attempted in the hope that cis-elimination of the hydroxyl groups would occur to yield the $\Delta^{6(7)}$ -compounds.

Dihydrosololactone and boric acid were pyrolysed at 350°C, under nitrogen, yielding a product (LX) (mass spec. m.wt. 302), which absorbed in the infra-red (CHCl_3) at 1770 cm^{-1} (γ -lactone) and 1660 cm^{-1} (double bond) and had no intense absorption in the ultra-violet above 220 $\text{m}\mu$. The structure (LX) was assigned on the basis of its n.m.r. spectrum and those of the hydrogenation product (LXI), a crystalline saturated lactone, m.p. 116°, $\text{U}_{\text{max}}^{\text{CHCl}_3}$ 1770 cm^{-1} (γ -lactone) (mass spec. m.wt. 304) and the epoxide (LXII) (mass spec. m.wt. 318).

In the spectrum of (LX) the multiplet at T4.2 (1H) is attributed to H_A coupling vicinally with H_B and H_C and allylicly with H_X . The broad multiplet at T5.2 (1H) arises from H_Y coupling with H_X , H_M and H_N . The proton at C_5 (H_X) is deshielded by the carbonyl group in the lactone ring and appears as a multiplet at T7.3 from its vicinal coupling with H_Y and its allylic coupling with H_A . On hydrogenation of (LX) the n.m.r. spectrum shows loss of the multiplet at T4.2 (H_A),

but the multiplets at T5.2 (H_Y) and T7.6 (H_X) remain. By double irradiation, H_X and H_Y were shown to be vicinally coupled and the observed coupling constant ($J = 6$ c/sec) is in accordance with the dihedral angle $\vartheta_{XY} = 30^\circ$. H_X couples further with H_Z ($J = 10$ c/sec) demonstrating that hydrogenation took place from the β face of the molecule, the dihedral angle ϑ_{XZ} being 170° , in agreement with the value of J . H_Y couples with H_N ($J = 3$ c/sec) and with H_M ($J = 8$ c/sec) in agreement with the dihedral angles $\vartheta_{YN} = 130^\circ$, $\vartheta_{YM} = 10^\circ$.

From the n.m.r. spectrum of the epoxide (LXII), epoxidation was inferred to have taken place from the β face. The multiplet, T5.15, (1H), corresponds to H_Y , which couples vicinally with H_X ($J = 6$ c/sec; $\vartheta_{YX} = 30^\circ$), with H_N ($J_{YN} = 3$ c/sec; $\vartheta_{YN} = 130^\circ$) and with H_M ($J_{YM} = 9$ c/sec; $\vartheta_{YM} = 10^\circ$). H_X couples only with H_Y and is seen as a doublet, T7.4 ($J_{XY} = 6$ c/sec; $\vartheta_{XY} = 30^\circ$). H_A attached to the epoxide ring, makes a dihedral angle of 60° with H_B and H_C and from the modified Karplus equation for epoxides ($J_{AB} = 5.1 \cos^2 \vartheta_{AB}$), ⁽⁵³⁾ the calculated values for the coupling constant $J_{AB} = J_{AC} = 5.1 \cos^2 60^\circ = 1.25$ c/sec, is in agreement with the observed broadened singlet at T6.9, assigned to H_A .

Thus, the desired $\Delta^{6(7)}$ -ene-lactone was not formed from borate pyrolysis of dihydrorosenololactone. Unsuccessful results were also obtained in the pyrolysis of the borate esters of dihydrorosenolo- and dihydroisosenololactone, the lactone ring being cleaved in both cases.

Attention was finally directed to acetate pyrolysis. The acetates of dihydrorosenololactone (LXIII) (mass spec. m.wt. 362), m.p. 157-158°, $U_{\max}^{\text{CHCl}_3}$ 1770, 1720, 1240 cm^{-1} ; dihydrorosenololactone (LXIV), (mass spec. m.wt. 362), m.p. 168°C, $U_{\max}^{\text{CHCl}_3}$ 1770, 1720, 1240 cm^{-1} , and dihydroisorosenololactone (LXV), (mass spec. m.wt. 362), m.p. 165°, $U_{\max}^{\text{CHCl}_3}$ 1770, 1720, 1240 cm^{-1} , were prepared and these were pyrolysed at 450°/15 mm.Hg. in an atmosphere of nitrogen affording in each case the desired $\Delta^{6(7)}$ ene-lactone, (mass spec. m.wt. 302) as the sole product, but in low yield. Infra-red absorption ($U_{\max}^{\text{CHCl}_3}$ 1770, 1660, and 670 cm^{-1}) of the pyrolysis product of the acetate of dihydroisorosenololactone confirmed that the acetate residue had been replaced by a double bond. These three products had very similar cracking patterns in their mass spectra and had the same retention time on g.l.c. (1% SE 30; 2% 20 M Peg; 1% CHDMS). Hydrogenolysis of the $\Delta^{6(7)}$ ene-lactones was found to occur on hydrogenation in ethyl acetate over 10% palladium charcoal as seen from the formation of acidic products presumably via the Δ^5 isomer. This was avoided using acetic acid as solvent and platinum oxide as catalyst. Only one product (mass spec. m.wt. 304) was formed on hydrogenation of the $\Delta^{6(7)}$ compound from dihydroisorosenololactone. It had a very similar cracking pattern to that of dihydrodesoxyrosenonolactone, but differed in retention time from it on g.l.c. (2% 20M Peg; 1% SE 30; 5% SE 30). On hydrogenation of the $\Delta^{6(7)}$ compounds from dihydrorosenolo- and dihydrorosenolo-lactone, the same three products

were obtained in each case, as seen from g.l.c. (same retention time and peaks superimposable) and combined gas chromatographic-mass spectrometric (g.c.m.s.) analyses of the two mixtures. Peak 1; m.wt. 304; Peak 2; m.wt. 304; Peak 3; m.wt. 304. The fragmentation pattern of peak 1 was identical with that of dihydrodesoxyrosenonolactone and the cracking pattern of peak 2 was identical with that of the hydrogenation product of the $\Delta^{6(7)}$ -compound from dihydroisorosenololactone, i.e. dihydrodesoxyisorosenonolactone. On addition of dihydrodesoxyrosenonolactone to either of the mixtures, peak 1 was enhanced and on addition of dihydrodesoxyisorosenonolactone to the mixture, peak 2 was enhanced. The compound in peak 3 (mass spec. m.wt. 304) had certain similarities in its cracking pattern to that of dihydrodesoxyrosenonolactone and its C_8 epimer and was considered to be either the C_5 epimer of dihydrodesoxyrosenonolactone (LXVI) formed by hydrogenation of the $^{5(6)}$ intermediate from the β face of the molecule or the isomeric (LXVII) in which the γ -lactone is now attached at C_6 instead of C_{10} , with subsequent hydrogenation from the α -face.

Thus, from these results of gas liquid chromatography and mass spectrometric analyses it has been possible to assign the 8α configuration to rosenono- and desoxyrosenono-lactone and the 8β configuration to isorosenonolactone from a direct comparison with

rosololactone. An explanation as to why the 8-normal series, but not the 8-iso series should rearrange on hydrogenation, would necessitate careful investigation of the interaction of the lactones with the catalyst surface.

A second direct correlation between rosenono- and desoxyrosenono-lactone was provided by formation of the dihydro ether (LXVIII) from both. Dihydrorosenonolactone was reduced with lithium aluminium hydride in tetrahydrofuran to the dihydrotriol (LXIX), $C_{20}H_{36}O_3$, m.p. $140-141^{\circ}C$, $[\alpha]_D + 105^{\circ}$. Reaction of the triol with toluene-p-sulphonyl chloride afforded the toluene-p-sulphonate (LXX) as the major product, along with the alcohol (LXXI) in minor amount. Reduction of the mixture, without prior separation, with lithium aluminium hydride in tetrahydrofuran, yielded the dihydro ether (LXVIII), $C_{20}H_{34}O$, as the major product. Reduction of dihydrodesoxyrosenonolactone with lithium aluminium hydride in tetrahydrofuran gave the dihydrodiol (LXXII), $C_{20}H_{36}O_2$, m.p. $136^{\circ}C$, $[\alpha]_D - 35^{\circ}$, and reaction of this with toluene-p-sulphonyl chloride gave the dihydro ether (LXVIII), identical in its ir., n.m.r., and mass spectra and in its behaviour on t.l.c. and g.l.c. (1% SE 30; 1% QF1; 2% 20M Peg;) with the dihydro ether from dihydrorosenonolactone.

In the course of parallel experiments with dihydroisorosenololactone it was observed that reaction with toluene-p-sulphonyl chloride failed to produce the desired toluene-p-sulphonate but yielded instead the $\Delta^{7(8)}$ -compound, (LVI), $C_{20}H_{30}O_2$, m.p. 134-135°, by immediate elimination of the toluene-p-sulphonate residue. This was confirmed by the multiplet in the n.m.r. spectrum at T4.7 (1H) attributed to the olefinic proton at C₇ and from the identical behaviour of this compound on g.l.c. (2% 20M Peg; 1% SE 30;) with one of the dehydration products from dihydrorosenololactone. Because of this undesired elimination the route taken to relate rosenono- and desoxyrosenono-lactone was not available for isorosenonolactone. However, an attempt was made to prepare the dihydro ether (LXVIII) from rosololactone. Dihydrorosololactone was reduced with lithium aluminium hydride in tetrahydrofuran to afford the triol (LXXIII). Reaction of this dihydrotriol with toluene-p-sulphonyl chloride in pyridine yielded two isomeric hydroxy-ethers as seen by g.c.m.s. (1% SE 30), (mass spec. m.wt. 306), to which we attribute the structures (LXXIV) and (LXXV). These compounds failed to separate on chromatography, but dehydration with thionyl chloride yielded two products that were separable by thin layer chromatography. One of these (mass spec. m.wt. 288) was assigned the structure (LXXVI) from the quartet centred at T4.6 (1H) arising from the coupling of H₆ with the protons

at C₇, and from the AB quartet centred at T6.1 and 6.65 (J = 8 c/sec) signifying the presence of the ether group. The other dehydration product (mass spec. m.wt. 288) was ascribed the structure (LXXVII) from the quartet at T5.5 (1H) attributed to the coupling of H₆ with the methylene group at C₇, from the AB quartet centred at T6.15 and 6.6 (J = 8 c/sec), and from the absence of olefinic protons in the n.m.r. Hydrogenation of the ene-ether (LXXVI) failed to yield the desired saturated ether (LXXVIII), affording only the unsaturated primary alcohol (LXXVIII), m.p. 115° C, (mass spec. m.wt. 290), $\text{U}^{\text{CHCl}_3}_{\text{max}} 3600 \text{ cm.}^{-1}$, AB quartet at T6.48 and 6.80 (J = 12 c/sec).

Oxidation of the mixture of hydroxy ethers (LXXIV) and (LXXV) with Sarret reagent afforded the keto-ether (LXXIX) (mass spec. m.wt. 304), $\text{U}^{\text{CHCl}_3}_{\text{max}} 1720 \text{ cm.}^{-1}$, and the hydroxy-ether (LXXV) (same retention time on g.l.c.). Attempts to form the tosyl-hydrazone of this keto-ether prior to reductive removal, were unsuccessful. Consequently all efforts to relate the dihydro-ether (LXVIII) from rosenonolactone with the ether from rosololactone failed.

Endeavours to prepare the toluene-p-sulphonate of rosololactone were unsuccessful, the hydroxyl group failing to react because of its hindered nature.

Replacement of the hydroxyl group in rosololactone by iodine⁽⁵⁰⁾⁽⁴⁶⁾ with a view to subsequent reductive removal of the iodine failed, because on attempted formation of the 6-iodo compound the lactone ring was cleaved.

Thus, all efforts to define the absolute stereochemistry at C₈ in the lactones of the rosane series met with difficulties which were not envisaged when this work was undertaken. Cleavage or rearrangement of the lactone ring presented a problem in correlating the oxygenated lactones with desoxyrosenonolactone and the boat conformation of ring B in rosolo- and rosenono-lactone probably contributed to the failure of certain of these reactions. However, the results obtained from pyrolysis of the acetates (LXIII), (LXIV) and (LXV) define unambiguously the stereochemistry of rosenono- (XLVII) and desoxyrosenono-lactone (L) at C₈, and provide a good illustration of the potential of the combined use of gas chromatography and mass spectrometry in problems involving the identification and comparison of small quantities of non-crystalline materials present in a mixture.

EXPERIMENTAL

All melting points were determined on a Kofler hot-stage apparatus and were uncorrected.

Ultra-violet spectra (u.v.) were obtained on a Unicam S.P.800 recording spectrometer. Infra-red solution spectra (i.r.) were recorded linearly in cm.^{-1} as percentage transmission by Mrs. F. Lawrie on a Unicam S.P. 100 double-beam spectrophotometer. Other infra-red spectra were recorded on a Perkin-Elmer 237 spectrophotometer, (qualitative).

Proton magnetic resonance spectra (n.m.r.) were determined on the Perkin Elmer R.10, 60 megacycle and the Varian H.A. 100 megacycle spectrometers by Mr. J. Lennon and Mr. J. Gall, tetramethyl-silane being used as an internal reference in carbon tetrachloride solutions of the samples.

Mass spectral molecular weights were obtained with an A.E.I. M.S. 9 mass spectrometer by Mr. F. Preston and Miss J. Wilkie.

Gas-liquid chromatography was performed on Pye Argon and Perkin Elmer F.11 chromatographs.

Gas-liquid mass spectral analyses (g.c.m.s.) were carried out on the L.K.B. spectrometer by Miss H. Humphries.

Micro analyses were by Mr. J.M.L. Cameron, B.Sc., and his staff.

Merck Kieselgel - G was used in thin layer (t.l.c.) and preparative thin layer chromatography (p.t.l.c.) Detection was achieved by means of ceric ammonium nitrate : sulphuric acid oxidation or iodine vapour adsorption in t.l.c. and by means of water in p.t.l.c.

Woelm alumina, deactivated to the appropriate Brockmann grade, ⁽⁸⁶⁾ was used for column chromatography.

Unless otherwise stated "light petroleum" refers to petroleum ether of b.p. 60-80°.

All solutions were dried over sodium sulphate (anhydrous).

The following abbreviations are used in reporting n.m.r. data.

s.	singlet	d.	doublet
t.	triplet	q.	quartet
m.	multiplet	H.	proton

Our thanks are due to Drs. N.J. McCorkindale and S.A. Hutchinson for growing T.roseum.

Attempted preparation of the Thioacetal of Rosenonolactone

1. Rosenonolactone (10 mg)⁽⁴⁸⁾ was dissolved in glacial acetic acid (1 ml) and excess ethane dithiol (0.2 ml) and boron trifluoride etherate (0.05 ml) added. The reaction was left at 20°C for 3 hours and worked up by addition of aqueous sodium bicarbonate solution and extraction into ethyl acetate. The combined extracts were washed well with water, dried and the solvent removed to yield an oil (7 mg) composed of starting material plus a less polar component. P.t.l.c. of the reaction product and extraction of the less polar band gave a compound which no longer had a γ -lactone (absence of band at 1780 cm.⁻¹ in i.r.). Thus, the desired thioacetal of rosenonolactone had not been formed.

2. Rosenonolactone (5 mg) was dissolved in ethane dithiol⁽⁴⁹⁾ (0.5 ml) and boron trifluoride etherate (0.05 ml) added. The reaction was left at 20°C for periods of 3, 6 and 12 hours and for 3 days and in each case a portion of the solution worked up as described previously. All failed to yield the thioacetal of rosenonolactone, the only product being a non-polar compound without a γ -lactone.

Attempted preparation of the Tosylhydrazone of Rosenonolactone

Rosenonolactone (7 mg) was refluxed in ethanol (3 ml) with tosylhydrazine⁽⁵¹⁾ (5 mg) for 2 hours. The reaction was worked

up by removal of the solvent and t.l.c. of the product showed a less polar compound had been formed. P.t.l.c. with ethyl acetate: light petroleum (3 : 7) as eluant yielded only dihydrorosenonolactone, m.p. 158-159°C; m.m.p. 158-159°C; (lit. value⁽³⁸⁾ 138°C); $U_{\max}^{\text{CHCl}_3}$ 1770 cm.⁻¹ (γ -lactone), 1720 cm.⁻¹ (cyclohexanone), absence of olefinic absorption (1640 cm.⁻¹).

Conversion of Rosenonolactone to the Dihydro Ether (LXVIII)

Hydrogenation of rosenonolactone (100 mg) was effected in ethanol (10 ml) over 10% palladium charcoal. Work up by filtration through cellulose powder and removal of the solvent yielded dihydro-rosenonolactone, m.p. 157-158°C.

The dihydrorosenonolactone (95 mg) was refluxed in dry tetrahydrofuran (10 ml) with lithium aluminium hydride (150 mg) for 5 hours and the reaction worked up by addition of a saturated solution of sodium sulphate until all the excess hydride was destroyed. Filtration and removal of the solvent afforded the crude triol (LXIX), which crystallised from pentane after purification by p.t.l.c., m.p. 140-141°C; $[\alpha]_D^{25} + 105^\circ$, (c = 0.8, chloroform); $U_{\max}^{\text{CHCl}_3}$ 3550 cm.⁻¹ (broad band). (Found: C, 74.3; H, 10.9. $\text{C}_{20}\text{H}_{36}\text{O}_3$ requires, C, 74.0; H, 11.2%).

The dihydrotriol (LXIX) (55 mg) was dissolved in dry pyridine (2 ml) and excess toluene-p-sulphonyl chloride (200 mg) added and the solution left at 20°C for 12 hours. Work up by addition of water and extraction into ether afforded the ether toluene-p-sulphonate (LXX) (60 mg). Reduction, without prior purification, with lithium aluminium hydride (140 mg) in dry tetrahydrofuran (20 ml) as solvent, gave the desired dihydro ether (LXVIII) as the major product, along with the alcohol (LXXI) as the minor product. Separation by p.t.l.c. using ethyl acetate: light petroleum (1 : 9) as eluant afforded the fully saturated ether (LXVIII) (25 mg) and the ether alcohol (LXXI) (5 mg). The dihydro ether (LXVIII) failed to crystallise. $\nu_{\text{max}}^{\text{CHCl}_3}$ 1040, 910 cm^{-1} ; 2H singlet at τ 6.25 ($-\text{CH}_2-\text{O}-$). (Found: C, 82.7; H, 11.7. $\text{C}_{20}\text{H}_{34}\text{O}$ requires C, 82.7; H, 11.8%). (LXVIII) was found to be identical with the dihydroether from desoxyrosenonolactone from its i.r. and n.m.r. spectra and from its behaviour on g.l.c. (1% SE 30; 2% 20M Peg; 1% QF1).

For a fuller account of the above series of reactions see page: 100

Reduction of Rosenonolactone with Sodium Borohydride

Rosenonolactone (20 mg) was dissolved in methanol (2 ml), sodium borohydride (30 mg) added, and the solution left at 20°C for 1.5 hours. Water was added and the aqueous solution extracted into ethyl acetate,

the extracts washed with water, then dried, and the solvent removed to yield the alcohol (LIII), m.p. 220°C , (lit.value⁽³⁸⁾ 222°C after softening at 218°C) ; $[\alpha]_{\text{D}} + 38^{\circ}$, (C = 0.42, chloroform); 1H multiplet centred at T6.1 (CH_2OH); $\text{U}_{\text{max}}^{\text{CCl}_4}$ 3625, 1777, 1640 cm^{-1} .

Hydrogenation of rosenololactone (LIII) (10 mg) in ethanol (2 ml) over 10% palladium charcoal yielded dihydrorosenololactone (LIIIIa), m.p. $192\text{--}193^{\circ}\text{C}$. ; 1H multiplet centred at T6.1 (CH_2OH); $\text{U}_{\text{max}}^{\text{CCl}_4}$ 3620, 1780 cm^{-1} .

Reduction of Rosenonolactone with Adams Catalyst

Rosenonolactone (30 mg) was hydrogenated in acetic acid (3 ml) over platinum oxide (50 mg) for 2 days. Thereafter, filtration through cellulose powder, and removal of the acetic acid in vacuo, afforded the dihydro alcohol found to be identical with (LIIIIa) obtained above, m.p. 193°C , m.m.p. $192\text{--}193^{\circ}\text{C}$; $\text{U}_{\text{max}}^{\text{CCl}_4}$ 3620, 1780 cm^{-1} ; 1H multiplet in the n.m.r. centred at T6.1.

Acetate of Dihydrorosenololactone (LXIV)

Rosenololactone (50 mg) was added to "analar" acetic anhydride (3 ml) with fused sodium acetate (50 mg), and the solution refluxed for 3 hours. Water was added, the aqueous solution extracted into ethyl acetate, the extracts washed with sodium bicarbonate, with water, and then dried. Removal of the solvent afforded the desired acetate

(LXIVa) (40 mg), m.p. 186°C , (lit. value⁽³⁸⁾ 186°C); $U_{\text{max}}^{\text{CHCl}_3}$ 1770, 1730, 1250 cm^{-1} ; (Mass spec. m.wt. 360; $\text{C}_{22}\text{H}_{32}\text{O}_4$ requires 360).

The acetate of rosenololactone (LXIVa) (40 mg) was hydrogenated in ethanol with 10% palladium charcoal as catalyst to yield the dihydro-acetate (LXIV), m.p. 168°C ; 3H singlet at 7.95 ($\text{CH}_3\text{COO-}$); 1H multiplet centred at $\tau 4.9$ ($-\text{CH}-\text{OAc}$). (Mass spec. m.wt. 362. $\text{C}_{22}\text{H}_{34}\text{O}_4$ requires 362.).

Pyrolysis of the Dihydro-acetate (LXIV)

The dihydro-acetate (LXIV) (25 mg) was pyrolysed at 450° in an atmosphere of nitrogen at 15mm.Hg. pressure. T.l.c. of the product showed a component of desired polarity, and p.t.l.c. yielded Δ^6 -dihydrodesoxyrosenonolactone (0.5 mg). G.l.c. showed one product (2% 20M Peg; 1% SE 30; 1% CHDMS).

Hydrogenation of the pyrolysed acetate in ethyl acetate with 10% palladium charcoal yielded only an acid as seen from t.l.c.

Hydrogenation of the pyrolysed acetate in acetic acid with platinum oxide as catalyst afforded three compounds, separable by g.c.m.s. (1% SE 30). Peak 1. M.wt. 304; $\text{C}_{20}\text{H}_{32}\text{O}_2$ requires 304. Fragmentation pattern and retention time on g.l.c. (1% SE 30; 2% 20M Peg), identical with dihydrodesoxyrosenonolactone (XXXIII).

Peak 2. M.wt. 304; $C_{20}H_{32}O_2$ requires 304. Fragmentation pattern and retention time on g.l.c. (1% SE 30; 2% 20M Peg), identical with dihydrodesoxyisosenonolactone.

Peak 3. M.wt. 304; $C_{20}H_{32}O_2$ requires 304. Assigned structure (LXVI) or (LXVII).

Attempted Borate Pyrolysis of Rosenololactone

Rosenololactone (LIII) (15 mg) and boric acid⁽⁵²⁾ (5 mg) were heated under nitrogen at 350°C for 3 hours. The reaction was allowed to cool to 20°C, water added and the aqueous solution extracted into ethyl acetate. The extracts were dried, and the solvent removed to yield products (8 mg) shown by t.l.c. to contain unchanged starting material and a less polar component. P.t.l.c. of the crude product and extraction of the less polar band failed to yield the desired ene-lactone ($U_{max}^{CHCl_3}$ 1690 cm^{-1} ; absorption absent at 1770 cm^{-1} , γ -lactone).

Dehydration of Dihydrorosenololactone

Dihydrorosenololactone (LIIIIa) (20 mg) was dissolved in dry pyridine (1 ml), thionyl chloride (0.05 ml) added and the reaction left at 20°C for 20 mins. Work up by addition of water, extraction into ether, washing the extracts with water, drying and removing the solvent afforded two products (LVI) and (LVII), as shown by g.l.c. (1% SE 30; 2% 20M Peg.), which proved inseparable by p.t.l.c. over silver nitr ate-

silica gel. One of the components of the mixture had the same retention time on g.l.c. as $\Delta^{7(8)}$ -dihydrodesoxyrosenonolactone.

Epoxidation of (LVI) and (LVII)

M-chloroperbenzoic acid (10 mg) was added to (LVI) and (LVII) (15 mg) in chloroform (1 ml) and the solution left at 20°C for 12 hours. Work up by addition of calcium hydroxide⁽⁸²⁾ and filtration, furnished two products, (LVII) and (LV), separable by p.t.l.c. using ethyl acetate: light petroleum (1 : 9) as eluant. The less polar product with the same retention time on g.l.c. as (LVII) formed above, was an oil and had signals in the n.m.r. at T6.0 (m, 1H); $U_{\max}^{\text{CHCl}_3}$ 1780, 1680 cm^{-1} (Mass spec. m.wt. 302. $\text{C}_{20}\text{H}_{30}\text{O}_2$ requires 302).

The more polar product (LV) crystallised from light petroleum, m.p. 144-145°C; signals in the n.m.r. at T7.1 (1H, q); $U_{\max}^{\text{CHCl}_3}$ 1780 cm^{-1} . (Mass spec. m.wt. 318. $\text{C}_{20}\text{H}_{30}\text{O}_3$ requires 318).

Desoxyrosenonolactone (L)

Desoxyrosenonolactone (40 mg) was crystallised from light petroleum, m.p. 115°C; $[\alpha]_D + 56^\circ$; (lit. value⁽⁴⁴⁾ m.p. 115°-116°C; $[\alpha]_D + 57^\circ$)

Dihydrodesoxyrosenonolactone (XXXXIII)

Desoxyrosenonolactone (40 mg) was hydrogenated in ethanol (5 ml) over 10% palladium charcoal. The normal work up yielded the

dihydro compound (XXXIII), which crystallised from light petroleum, m.p. 100°C ; $[\alpha]_{\text{D}} + 50^{\circ}$, ($c = 0.4$, chloroform). (Found: C, 78.6; H, 10.4. $\text{C}_{20}\text{H}_{30}\text{O}_2$ requires C, 78.9; H, 10.6%).

Diol from Dihydrodesoxyrosenonolactone (LXXII)

Dihydrodesoxyrosenonolactone (XXXIII) (40 mg) was added to anhydrous ether (20 ml) with excess lithium aluminium hydride (80 mg) and the solution refluxed for 5 hours. Work up as previously described afforded the diol (LXXII) (30 mg), m.p. 136°C ; $[\alpha]_{\text{D}} - 35^{\circ}$; ($c = 0.6$, chloroform); $\text{U}_{\text{max}}^{\text{CHCl}_3} 3600 \text{ cm}^{-1}$ (broad); AB quartet at T6.3 and 6.78 ($J = 12 \text{ c/sec}$): (Found C, 77.7; H, 10.9. $\text{C}_{20}\text{H}_{36}\text{O}_2$ requires C, 78.3; H, 11.2%).

Dihydro Ether (LXVIII)

The dihydro diol (LXXII) (20 mg) was dissolved in pyridine (1 ml), excess toluene-*p*-sulphonyl chloride (150 mg) added and the solution left at 20°C for 12 hours. Normal work up afforded the dihydro ether (LXVIII), found to be identical with the dihydro ether derived from rosenonolactone from i.t., n.m.r., and its behaviour on t.l.c. and g.l.c. $\text{U}_{\text{max}}^{\text{CHCl}_3} 1040, 910 \text{ cm}^{-1}$. 2H singlet at T6.25 ($-\text{CH}_2-\text{O}-$) (Found: C, 82.7; H, 11.7. $\text{C}_{20}\text{H}_{34}\text{O}$ requires C, 82.7; H, 11.8%).

Isorosenonolactone (XLIIIa)

Rosenonolactone (200 mg) in ethanol (20 ml) and 10% ethanolic potassium hydroxide (20 ml), was left at 20°C for 2 hours. Water was added and the aqueous solution extracted thoroughly into ethyl acetate, the extracts washed with water, dried and the solvent removed to yield a mixture of rosenonolactone (XLVII) and isorosenonolactone (XLIIIa) (190 mg). P.t.l.c. using chloroform: benzene (2 : 3) as eluant and with repeated elution of the chromatoplates, afforded (XLVII) (90 mg), m.p. 214°C, m.m.p. 214°C; $[\alpha]_D - 107.5^\circ$, and (XLIIIa) which crystallised from ether, m.p. 144°C; $[\alpha]_D + 32^\circ$, (c = 0.6, chloroform); (lit.value, ⁽³⁸⁾ m.p. 144°C; $[\alpha]_D + 20^\circ$); $\nu_{\text{max}}^{\text{CCl}_4}$ 1783, 1717, 1640 cm^{-1} ; n.m.r. signals at τ 8.8 (3H, s); 8.92 (3H, s) and 9.2 (3H, s).

Attempted preparation of the Thioetal of Isorosenonolactone

1. Isorosenonolactone (10 mg) was dissolved in acetic acid (1 ml) and boron trifluoride etherate (0.05 ml) and excess ethane dithiol (0.1 ml)⁽⁴⁸⁾ added. The reaction was left at 25°C for 6 hours and worked up as previously described. From t.l.c. a less polar product had been formed which, when isolated by p.t.l.c. was shown to have no absorption at 1780 cm^{-1} (γ -lactone).

2. A slow stream of hydrogen chloride⁽⁴⁸⁾ was bubbled into a solution of isorosenonolactone (10 mg) in ethane dithiol (0.2 ml) at 0°C for 1 hour. The solution was left at 0°C for 3 hours and half of it removed, the remainder being left for a further 12 hours at 0°C. In both cases the less polar products formed, failed to possess a γ -lactone as seen from infra-red.

Attempted preparation of the toluene-p-sulphonate of Dihydroisorosenololactone

Dihydroisorosenololactone (LIVa) (40 mg) was dissolved in dry pyridine (1 ml), excess toluene-p-sulphonyl chloride (150 mg) added, and the solution left for 14 hours at 20°C. Work up in the usual way afforded one product (LVI) as seen from t.l.c. and g.l.c. (1% SE 30; 2% 20M Peg). (LVI) crystallised from light petroleum, m.p. 135°C; 1H quartet at T4.7. (Mass spec. m.wt. 302. $C_{20}H_{30}O_2$ requires 302). (Found: C, 79.3; H, 9.7. $C_{20}H_{30}O_2$ requires C, 79.4; H, 10.0%).

Isorosenololactone (LIV)

Isorosenonolactone (XLIIIa) (60 mg) was reduced with sodium borohydride (40 mg) and worked up as previously reported. T.l.c. showed that two products had been formed and purification by p.t.l.c. afforded rosenololactone (LIII) (10 mg), m.p. 220°C, m.m.p. 220°C, and isorosenololactone (LIV) (30 mg), m.p. 131°C (lit.value⁽³⁸⁾ 181°C); $\nu_{max}^{CCl_4}$ 3600, 1770, 1640 cm^{-1} ; 1H multiplet at T6.0. (Mass spec.

m.wt. 318. $C_{20}H_{30}O_3$ requires 318).

Dihydroisorosenolactone (LIVa)

Isorosenolactone (LIV) (20 mg) was hydrogenated in ethanol (5 ml) with 10% palladium charcoal as catalyst. The normal work up afforded dihydroisorosenolactone (LIVa) (17 mg), which crystallised from light petroleum, m.p. 165°C , (lit.value⁽³⁸⁾ 166°C). ^1H multiplet at T6.0.

Dihydroisorosenolactone, prepared by reduction of isorosenonolactone in acetic acid with platinum oxide as catalyst, was found to be identical (m.p., m.m.p., i.r., n.m.r.) to that formed as described above.

Attempted preparation of the Tosylhydrazone of Isorosenonolactone

Isorosenonolactone (10 mg) was refluxed in ethanol (2 ml) with tosylhydrazine⁽⁵¹⁾ (10 mg) for 2 hours and the solvent removed in vacuo. T.l.c. of the product showed a slight change of polarity with that of starting material (XLIIIa). Purification by p.t.l.c. afforded a compound identical to that of dihydroisorosenonolactone, m.p. $152-153^{\circ}\text{C}$; m.m.p. $152-153^{\circ}\text{C}$; $U_{\text{max}}^{\text{nujol}}$ $1770, 1720\text{ cm}^{-1}$.

Continued reflux overnight failed to produce the desired tosylhydrazone.

Acetate of Dihydroisorosenololactone (LXV)

Dihydroisorosenololactone (LIVa) (35 mg) was dissolved in pyridine (1 ml), acetic anhydride (1 ml) added, and the solution left at 20°C for 12 hours. Removal of the solvent in vacuo, and purification by p.t.l.c. yielded the desired acetate (LXV) (30 mg), which crystallised from light petroleum, m.p. 165°C; $\nu_{\text{max}}^{\text{CHCl}_3}$ 1770, 1740, 1240 cm^{-1} ; ^1H singlet at τ 4.95, ($\text{CH}\cdot\text{OAC}$); ^3H singlet at τ 7.9, ($\text{CH}_3\cdot\text{COO}-$). (Mass spec. m.wt. 362. $\text{C}_{22}\text{H}_{34}\text{O}_4$ requires 362).

Pyrolysis of the Acetate of Dihydroisorosenololactone

The acetate (LXV) was pyrolysed at 450°C under nitrogen at a pressure of 15 mm. Hg. The product (1 mg) was found to be one component by t.l.c. and g.l.c. (2% 20M Peg; 1% SE 30; 1% CHDMS). $\nu_{\text{max}}^{\text{CHCl}_3}$ 1770, 1660, 670 cm^{-1} .

Hydrogenation of the pyrolysed acetate in acetic acid over platinum oxide afforded one compound as shown by t.l.c. and g.l.c. (2% 20M Peg; 1% SE 30). The retention time of this compound on g.l.c. differed from that of dihydrodesoxyrosenonolactone, but enhanced Peak 2 of the hydrogenated pyrolysed acetates of dihydrorosenolo- and dihydrorosolo-lactone. The fragmentation pattern differed slightly from that of dihydrodesoxyrosenonolactone. (Mass spec. m.wt. 304. $\text{C}_{20}\text{H}_{34}\text{O}_2$ requires 304).

Attempted Borate Pyrolysis of Isorosenolactone

Isorosenolactone (LIV) (10 mg) and boric acid (4 mg) were heated under nitrogen at 350°C for 1 hour. The reaction was allowed to cool to 20°C, water added and the aqueous solution extracted with ethyl acetate. The extracts were dried, and the solvent removed to yield two products (5 mg) found by t.l.c. to be less polar than (LIV). Infra-red of the crude product showed no absorption at 1770 cm.⁻¹ (δ-lactone).

Dehydration of Dihydroisorosenolactone

Dihydroisorosenolactone (LIVa) (10 mg), pyridine (1 ml) and thionyl chloride (0.05 ml) were left at 20°C for 20 mins. Work up as previously described yielded one product, as seen from g.l.c. (1% SE 30; 2% 20M Peg.) of identical retention time with (LVI), obtained from attempted tosylation of dihydroisorosenolactone and from dehydration of dihydrorosenolactone. M.p. 135°C, m.m.p. 135°C; $U_{\text{max}}^{\text{nujol}}$ 1770, 1640 cm.⁻¹. Signals in the n.m.r. at T4.7 (1H, q), 8.5 (3H, s), 8.6 (3H, s), 9.25 (3H, s), 9.1 (3H, d).

Rosonolactone (LXXXI)

Rosolactone (50 mg) was dissolved in acetone (2 ml) and Jones oxidant (0.05 ml) added with cooling. The reaction was kept at 0°C for 30 mins. and worked up by addition of water and extraction into

ethyl acetate. Removal of the solvent and purification by p.t.l.c. afforded the keto-lactone (LXXXI) (40 mg), which crystallised from ether-light petroleum, m.p. 128°C , (lit.value⁽³⁸⁾ 126°C); $U_{\text{max}}^{\text{nujol}}$ 1770, 1720, 1650 cm^{-1} .

Attempted preparation of the Thioketal of Rosonolactone

All methods previously attempted to afford the thioketals of rosonono- and isorosenono-lactone, were repeated with rosonolactone, but all failed to product the desired thioketal.

Attempted preparation of the Tosylhydrazone of Rosonolactone

Rosonolactone (LXXXI) (10 mg) was refluxed in ethanol (2 ml) with tosylhydrazine⁽⁵¹⁾ (10 mg) for 3 hours. The usual work up yielded only dihydrorosenolactone (7 mg), m.p. 140°C , m.m.p. 140°C , (lit.value⁽³⁸⁾ 138°C). Further reflux overnight failed to produce the required tosylhydrazone.

Dehydration of Rosololactone

Rosololactone (20 mg) was dissolved in dry pyridine and thionyl chloride (0.05 ml) added. The reaction was kept at 20°C for 20 mins., cooled to 0°C and water added cautiously. The aqueous solution was extracted into ethyl acetate, the extracts dried, and the solvent removed to afford an oil (LI) (14 mg). Purification by p.t.l.c. with ethyl acetate: light petroleum (1 : 9) gave one product as seen from t.l.c. on silica gel-

silver nitrate and g.l.c. (1% SE30; 2% 20M Peg; 1% QF1). 1H quartet at T4.7 ($-\underline{\text{CH}}=\text{CR}_2$). (Found: C, 79.8; H, 9.5. $\text{C}_{20}\text{H}_{28}\text{O}_2$ requires C, 79.9; H, 9.4%).

Hydrogenation of (LI)

The Δ^5 -desoxyrosenonolactone (LI) (15 mg), was hydrogenated in ethanol (5 ml) over 10% palladium charcoal. The normal work up yielded only an acid and no trace of starting material or the desired saturated lactone, as seen from t.l.c. An acid plus (LII) was obtained when acetic acid was the solvent with platinum oxide or palladium charcoal as catalyst. The dihydro- $\Delta^{5(6)}$ -desoxyrosenonolactone (LII) had the same retention time on g.l.c. (1% SE30; 2% 20M Peg; 5% SE 30) as the compound obtained from dehydration of dihydrorosenolactone with thionyl chloride in pyridine.

Hydrogenation of (LI) in ethyl acetate over platinum oxide, palladium charcoal or 1% palladium on calcium carbonate⁽⁸¹⁾ afforded the dihydro compound (LII) in all cases, the bond at $\text{C}_5 - \text{C}_6$ remaining unsaturated. The $\Delta^{5(6)}$ -dihydrodesoxyrosenonolactone (LII) failed to crystallise. 1H quartet at T4.7 ($-\underline{\text{CH}}=\text{CR}_2$). (Found: C, 79.2; H, 9.9. $\text{C}_{20}\text{H}_{30}\text{O}_2$ requires C, 79.4; H, 10.0%).

Attempts to convert Dihydrorosololactone into the ether LXXVIII

Dihydrorosololactone (40 mg) was prepared by hydrogenation of rosololactone (40 mg) in ethanol (5 ml) over 10% palladium charcoal, m.p. 140°C , (lit.value⁽³⁸⁾ 138°C). The dihydrorosololactone was added to dry tetrahydrofuran (10 ml) with lithium aluminium hydride (50 mg) and the solution refluxed for 5 hours. Work up as previously described afforded the dihydrotriol (LXXIII) (30 mg), an oil, $\text{U}_{\text{max}}^{\text{CHCl}_3}$ 3550 cm^{-1} (broad). (Mass spec. m.wt. 324. $\text{C}_{20}\text{H}_{36}\text{O}_3$ requires 324).

Action of toluene-p-sulphonyl chloride on the Triol (LXXIII)

The triol (LXXIII) (35 mg) was dissolved in dry pyridine (1 ml), toluene-p-sulphonyl chloride (150 mg) added and the solution left at 20°C for 48 hours. Work up in the normal fashion yielded two isomeric compounds (LXXIV) and (LXXV) (30 mg), as seen by g.c.m.s. (1% SE 30). These compounds failed to separate by p.t.l.c. $\text{U}_{\text{max}}^{\text{CHCl}_3}$ 3550 cm^{-1} (absence of toluene-p-sulphonate absorption). (Mass spec. m.wt. of LXXIV = LXXV = 306. $\text{C}_{20}\text{H}_{34}\text{O}_2$ requires 306).

Dehydration of the Hydroxy-Ethers (LXXIV) and (LXXV)

The hydroxy-ethers (30 mg) were dissolved in pyridine (1 ml) and thionyl chloride (0.04 ml) added, and the solution left at 20°C for 20 mins. Work up as previously described afforded two

products, separable by p.t.l.c. with ethyl acetate: light petroleum (1:32) as eluant, the plate being eluted twice to increase the separation of the two components. The less polar product (LXXVI), an oil (13 mg), was shown to be one component by t.l.c. over silica gel-silver nitrate and by g.l.c. (1% SE 30; 2% 20M Peg). $\nu_{\text{max}}^{\text{CHCl}_3}$ 1640, 820 cm^{-1} ; 1H quartet at T4.6, ($\text{CH} = \text{CR}_2$), AB quartet T6.1 and 6.65 ($J = 8 \text{ c/sec}$), ($-\text{CH}_2-\text{O}-$). (Mass spec. m.wt. 288. $\text{C}_{20}\text{H}_{32}\text{O}$ requires 288).

The more polar ene-ether (LXXVII) was also shown to be one component by t.l.c. over silica gel-silver nitrate and by g.l.c. (1% SE30; 2% 20M Peg). $\nu_{\text{max}}^{\text{CHCl}_3}$ 1680 cm^{-1} ; 1H quartet at T5.4 ($-\text{CH}-\text{O}-$); AB quartet at T6.15 and 6.6 ($J = 8 \text{ c/sec}$)($-\text{CH}_2-\text{O}-$). (Mass spec. m.wt. 288. $\text{C}_{20}\text{H}_{32}\text{O}$ requires 288).

Hydrogenation of the Ene-Ether (LXXVI)

The $\Delta^{5(6)}$ ether (LXXVI) was hydrogenated in the following systems

1. Ethyl acetate over 10% palladium charcoal.
2. Ethyl acetate over platinum oxide.
3. Ethyl acetate over 1% palladium calcium carbonate. (81)

In all cases no change was observed in (LXXVI) as seen by t.l.c. and g.l.c.

Hydrogenation of the ene-ether (LXXVI) was repeated in the following systems:

1. Acetic acid over platinum oxide.
2. Ethanol over 10% palladium charcoal.

In these systems hydrogenolysis took place, the unsaturated primary alcohol (LXXVIII) being formed, which crystallised from light petroleum, m.p. 115°C ; $U_{\text{max}}^{\text{CHCl}_3}$ 3600 cm^{-1} ; AB quartet at $\tau 6.48$ and 6.80 ($J = 12\text{ c/sec}$) ($-\text{CH}_2-\text{OH}$). (Mass spec. m.wt. 290. $\text{C}_{20}\text{H}_{34}\text{O}$ requires 290).

Preparation of the 6-Keto-Ether (LXXIX).

The hydroxy-ethers (LXXIV) and (LXXV) (20 mg) were oxidised, without prior separation, with chromium trioxide (20 mg) in dry pyridine (1 ml) at 20°C for 12 hours. Work up by addition of water and extraction into ethyl acetate afforded two products, unchanged (LXXV) (as seen from g.l.c. 1% SE30; 2% 20M Peg) and the desired 6-keto-ether (LXXIX) obtained by p.t.l.c. with ethyl acetate: light petroleum (1 : 9) as eluant. The 6-keto-ether (LXXIX), failed to crystallise. $U_{\text{max}}^{\text{CHCl}_3}$ 1720 cm^{-1} . (Mass spec. m.wt. 304. $\text{C}_{20}\text{H}_{32}\text{O}_2$ requires 304).

Attempted preparation of the Tosylhydrazone of the
6-Keto Ether (LXXIX)

The 6-keto ether (6 mg) was refluxed in ethanol (2 ml) with tosylhydrazine (5 mg) for 2 hours. Work up as previously stated gave only starting material and failed to produce the desired tosylhydrazone.

Attempted preparation of toluene-p-sulphonate of Rosololactone

Rosololactone (10 mg) was dissolved in pyridine (1 ml) and toluene-p-sulphonyl chloride (20 mg) added. The solution was left at 20°C for 48 hours and worked up in the usual manner to yield only starting material as seen by i.r., t.l.c., m.p., and mixed m.p..

Attempted replacement of the Hydroxyl group of Rosololactone
with Iodine

1. Triphenyl phosphite: methyl iodide⁽⁵⁰⁾ was prepared by refluxing triphenyl phosphite (3 ml) with methyl iodide (1 ml) for 36 hours. This solution (0.05 ml) was left with rosololactone (5 mg) for 48 hours and thereafter worked up by addition of ethyl acetate, the solution washed well with sodium thiosulphate and then with water, and the solvent removed to afford a product which contained no lactone ring (no absorbtion in the i.r. at 1780 cm.⁻¹) and hence the desired 6-iodo-desoxyrosenonolactone had not been formed.

2. Rosololactone (10 mg) was refluxed with triphenyl phosphite⁽⁴⁶⁾ (0.2 ml) and methyl iodide (0.1 ml) for 36 hours. The reaction, on work up as described above, afforded a product which again was found to have no γ -lactone absorption in the infra-red.

Pyrolysis of the Borate Ester of Dihydrorosololactone

Dihydrorosololactone (20 mg) was heated at 300°C under nitrogen with boric acid (5 mg)⁽⁵²⁾ for 1 hour. The product was taken up in ethyl acetate, washed well with water, the extracts dried and the solvent removed to yield one product (LX) (t.l.c. and g.l.c. 1% SE 30; 1% CHDMS; 2% 20M Peg). $U_{\max}^{CCl_4}$ 1774, 1660 cm^{-1} , no intense absorption above 220m ; 1H multiplets at T4.2, 5.2 and 7.5 (Mass spec. m.wt. 302. $\text{C}_{20}\text{H}_{30}\text{O}_2$ requires 302).

Hydrogenation of the pyrolysis product (LX)

Hydrogenation of (LX) (6 mg) in ethanol with either 10% palladium charcoal or platinum oxide as catalyst, gave only more polar products as seen from t.l.c.

In ethyl acetate over 10% palladium charcoal or platinum oxide, (LX) remained unchanged as seen from g.l.c. and t.l.c.

Effective hydrogenation was obtained in acetic acid over platinum oxide, the product (LXI) crystallising from light petroleum,

m.p. 116°C ; $\text{U}_{\text{max}}^{\text{CCl}_4}$ 1770 cm.^{-1} ; 1H multiplets in the n.m.r. at T5.2 and T7.3. (Mass spec. m.wt. 318. $\text{C}_{20}\text{H}_{30}\text{O}_3$ requires 318).

Epoxidation of the pyrolysis product (LX)

The unsaturated lactone (LX) (20 mg) in chloroform (1 ml) with m-chloroperbenzoic acid (10 mg) was left at 20°C for 12 hours. Work up as described previously⁽⁸²⁾, afforded the epoxide (LXII) (10 mg), which crystallised from light petroleum, m.p. 124°C ; $\text{U}_{\text{max}}^{\text{CCl}_4}$ 1775 cm.^{-1} ; 1H multiplet at T5.15; 1H doublet at T7.4 ($J = 6\text{ c/sec}$); 1H broad singlet at T6.9. (Mass spec. m.wt. 318. $\text{C}_{20}\text{H}_{30}\text{O}_3$ requires 318.)

Acetate of Dihydrosololactone (LXIII)

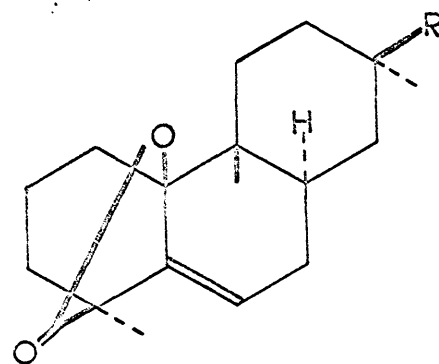
Rosololactone (60 mg) was refluxed with acetic anhydride (2 ml) and fused sodium acetate (30 mg) for $2\frac{1}{2}$ hours. On the normal work up, the product (LXIIIa) was obtained, which crystallised from ether: light petroleum as needles, m.p. 168°C , (lit.value⁽³⁸⁾ 167°C); $\text{U}_{\text{max}}^{\text{CHCl}_3}$ $1770, 1720, 1640, 1240\text{ cm.}^{-1}$.

Hydrogenation of (LXIIIa) in ethanol (5 ml) with 10% palladium charcoal as catalyst yielded the dihydro-acetate (LXIII), which crystallised from ether, m.p. $157\text{--}158^{\circ}\text{C}$; $\text{U}_{\text{max}}^{\text{CHCl}_3}$ $1770, 1720, 1240\text{ cm.}^{-1}$; 1H multiplet at T4.9; ($-\text{CH}_2\text{--OAC}$); 3H singlet at T8.0 ($\text{CH}_3\text{--COO}$). (Mass spec. m.wt. 362. $\text{C}_{22}\text{H}_{34}\text{O}_4$ requires 362).

Pyrolysis of the Acetate of Dihydrorosenolactone (LXIII)

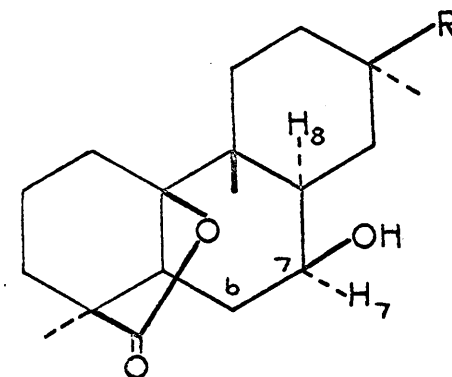
The acetate (LXIII) was pyrolysed at 450°C under nitrogen at 15mm. Hg. pressure, and afforded in very low yield $\Delta^{6(7)}$ -dihydro-desoxyrosenonolactone, the same compound as obtained from pyrolysis of the acetate (LXIV) from dihydrorosenolactone, as seen from t.l.c. on silica gel-silver nitrate and g.l.c. (1% SE30; 2% 20M Peg; 1% CHDMS).

Hydrogenation of the pyrolysed product in acetic acid over platinum oxide, yielded three products as seen from g.l.c. (2% 20M Peg; 1% SE30). These products were separable by g.c.m.s. into three components, identical in molecular weight and mass spectral fragmentation pattern with the three products obtained by a similar route from dihydrorosenolactone.



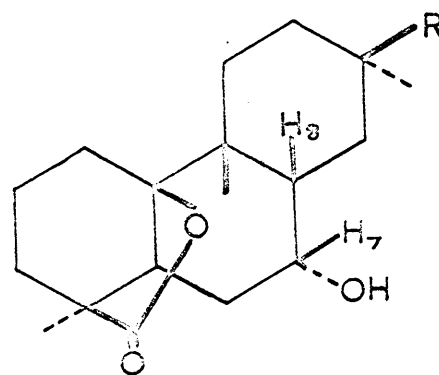
LI $R = \text{CH}=\text{CH}_2$

LII $R = \text{CH}_2\text{CH}_3$



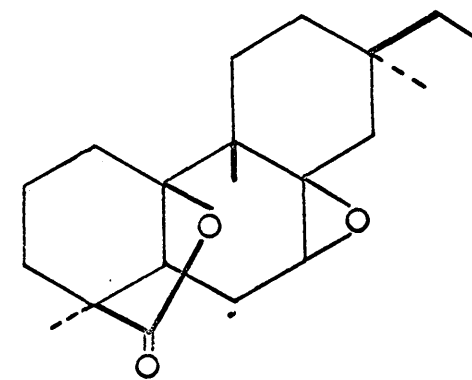
LIII $R = \text{CH}=\text{CH}_2$

LIII α $R = \text{CH}_2\text{CH}_3$

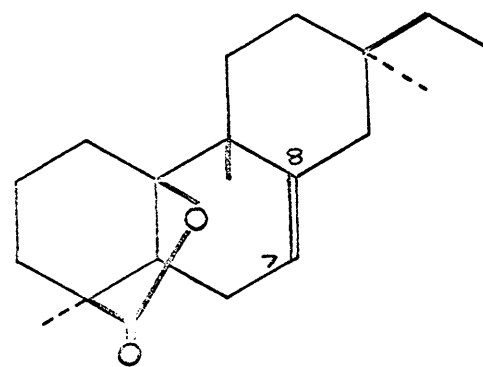


LIV $R = \text{CH}=\text{CH}_2$

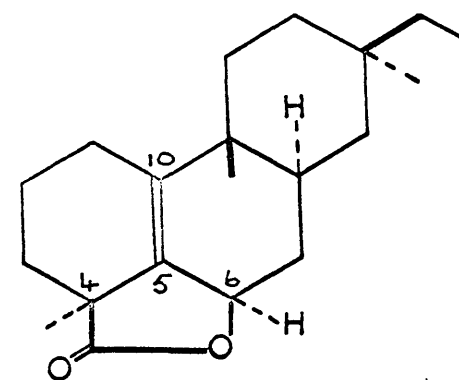
LIV α $R = \text{CH}_2\text{CH}_3$



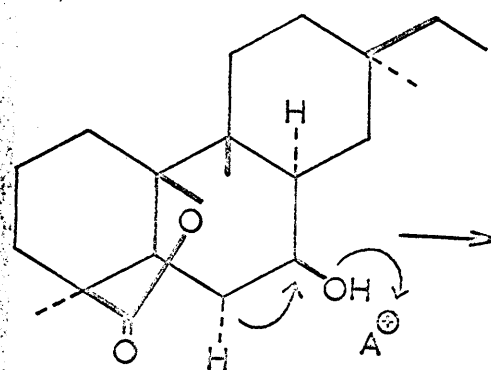
LV



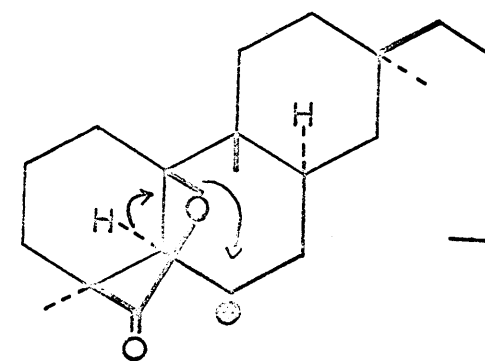
LVI



LVII

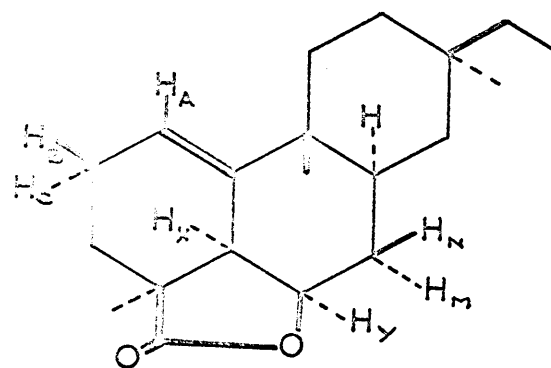


LVIII

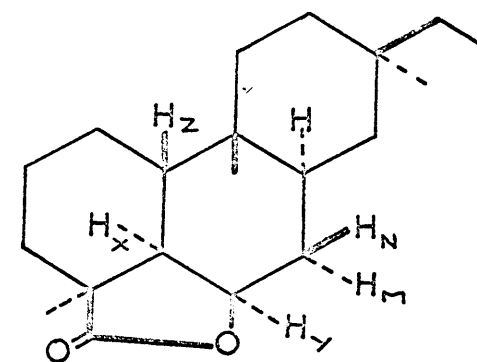


LIX

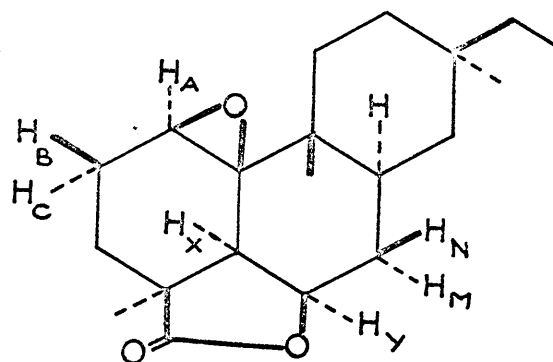
LVII



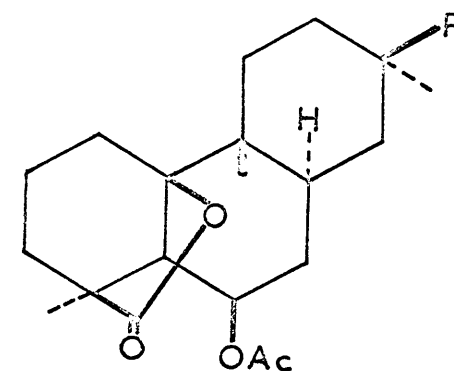
LX



LXI

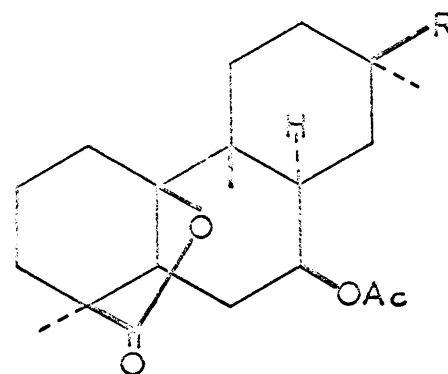


LXII

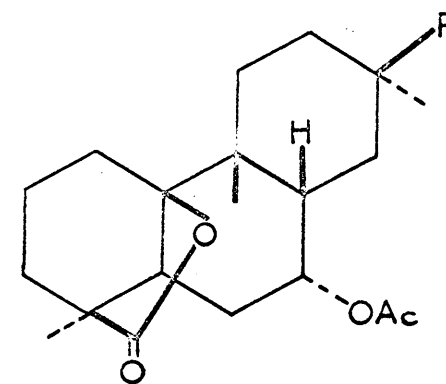


LXIII R = CH₂·CH₃

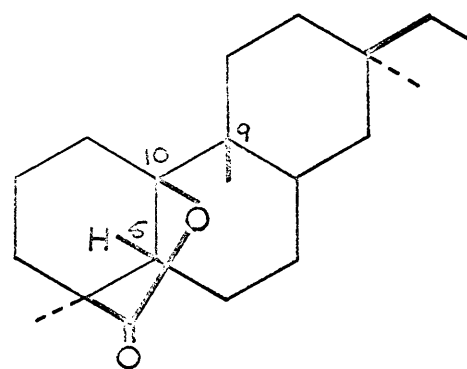
LXIII_a R = CH=CH₂



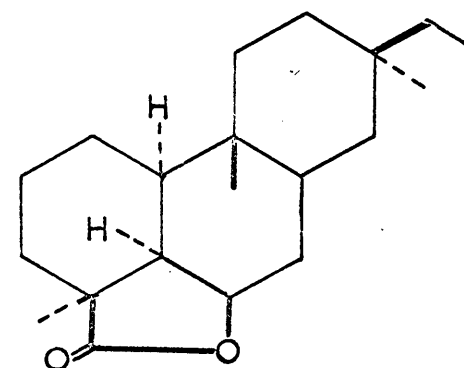
LXIV $R = \text{CH}_2 \cdot \text{CH}_3$
 LXIV α $R = \text{CH}=\text{CH}_2$



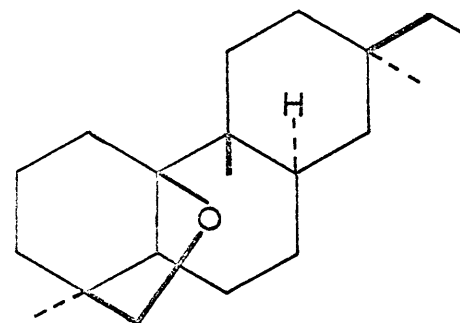
LXV $R = \text{CH}_2 \cdot \text{CH}_3$
 LXV α $R = \text{CH}=\text{CH}_2$



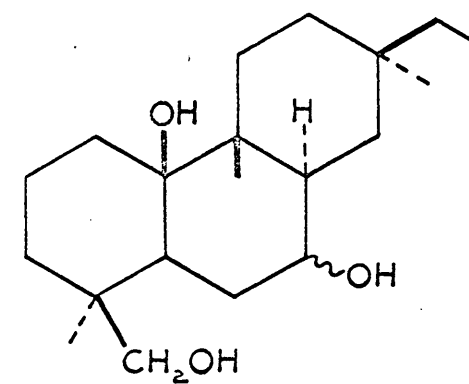
LXVI



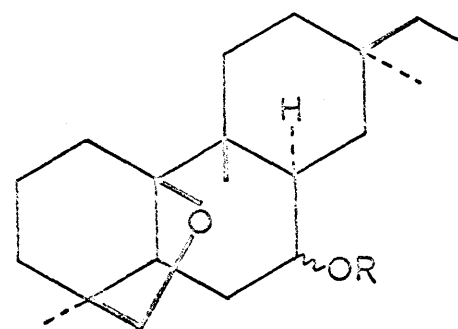
LXVII



LXVIII

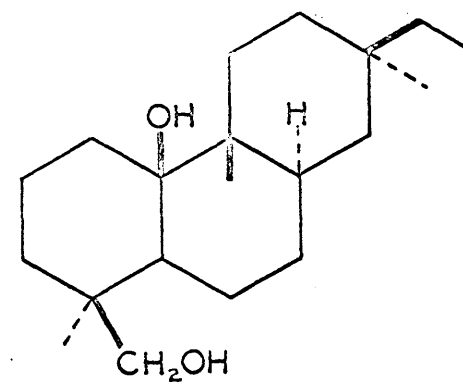


LXIX

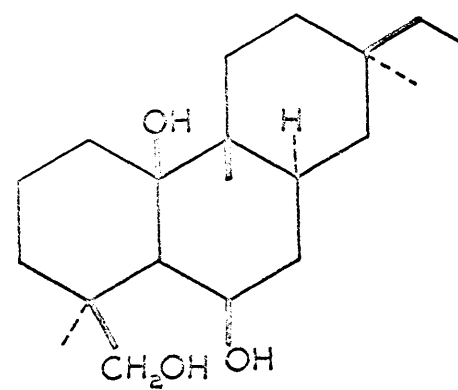


LXX R = Ts

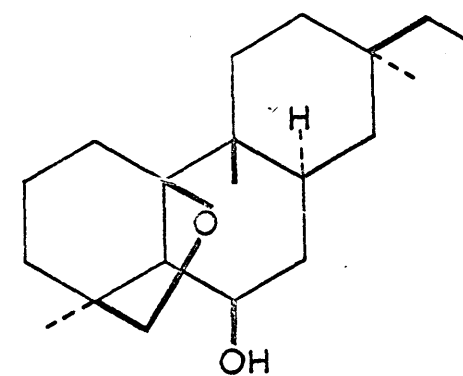
LXXI R = H



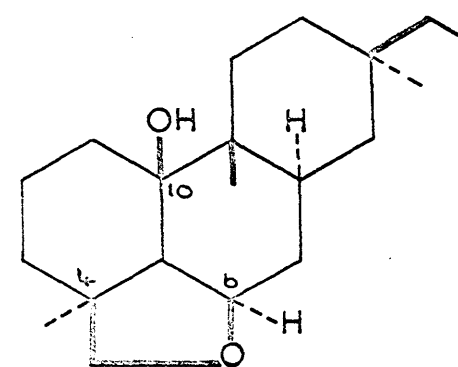
LXXII



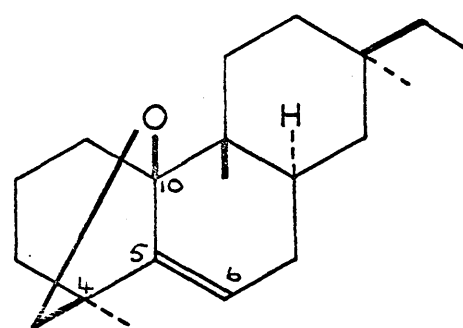
LXXIII



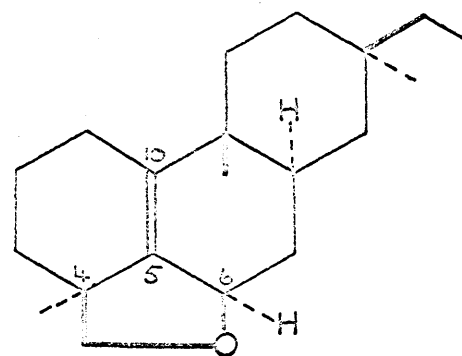
LXXIV



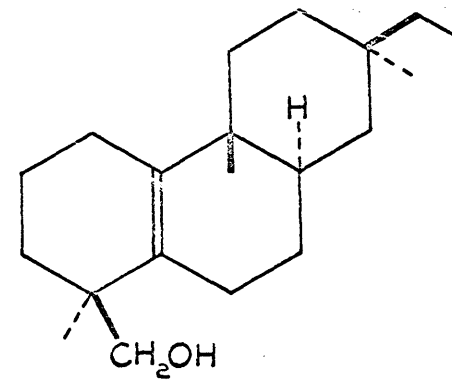
LXXV



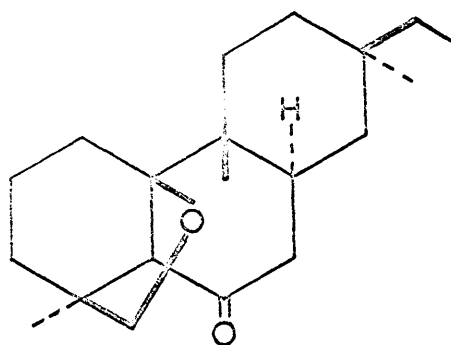
LXXVI



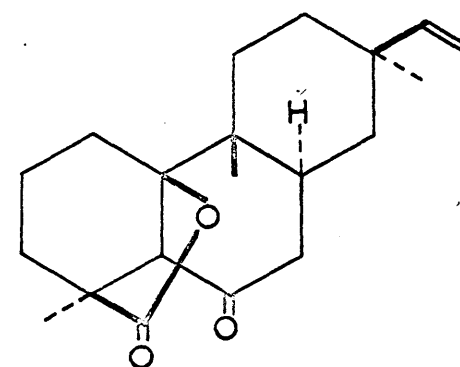
LXXVII



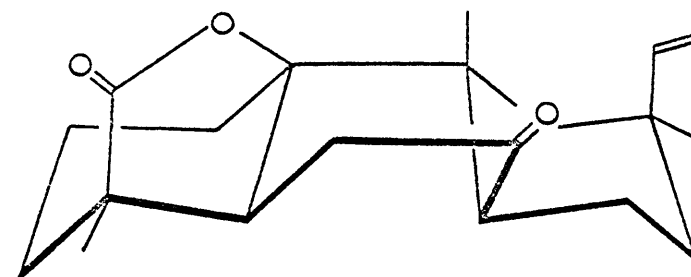
LXXVIII



LXXIX

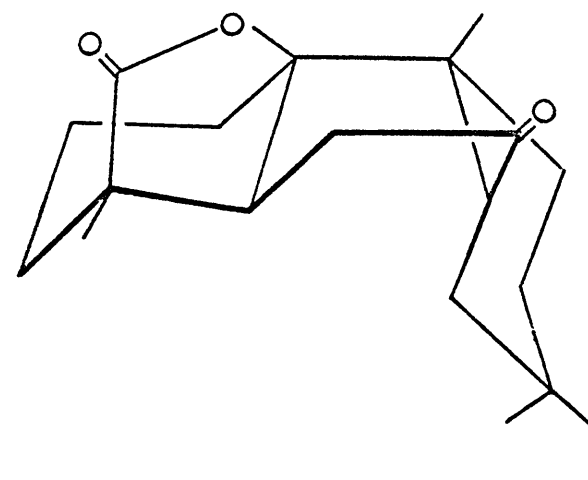


LXXXI



XLVII

ROSENONOLACTONE



XLIIIa

ISOROSENONOLACTONE

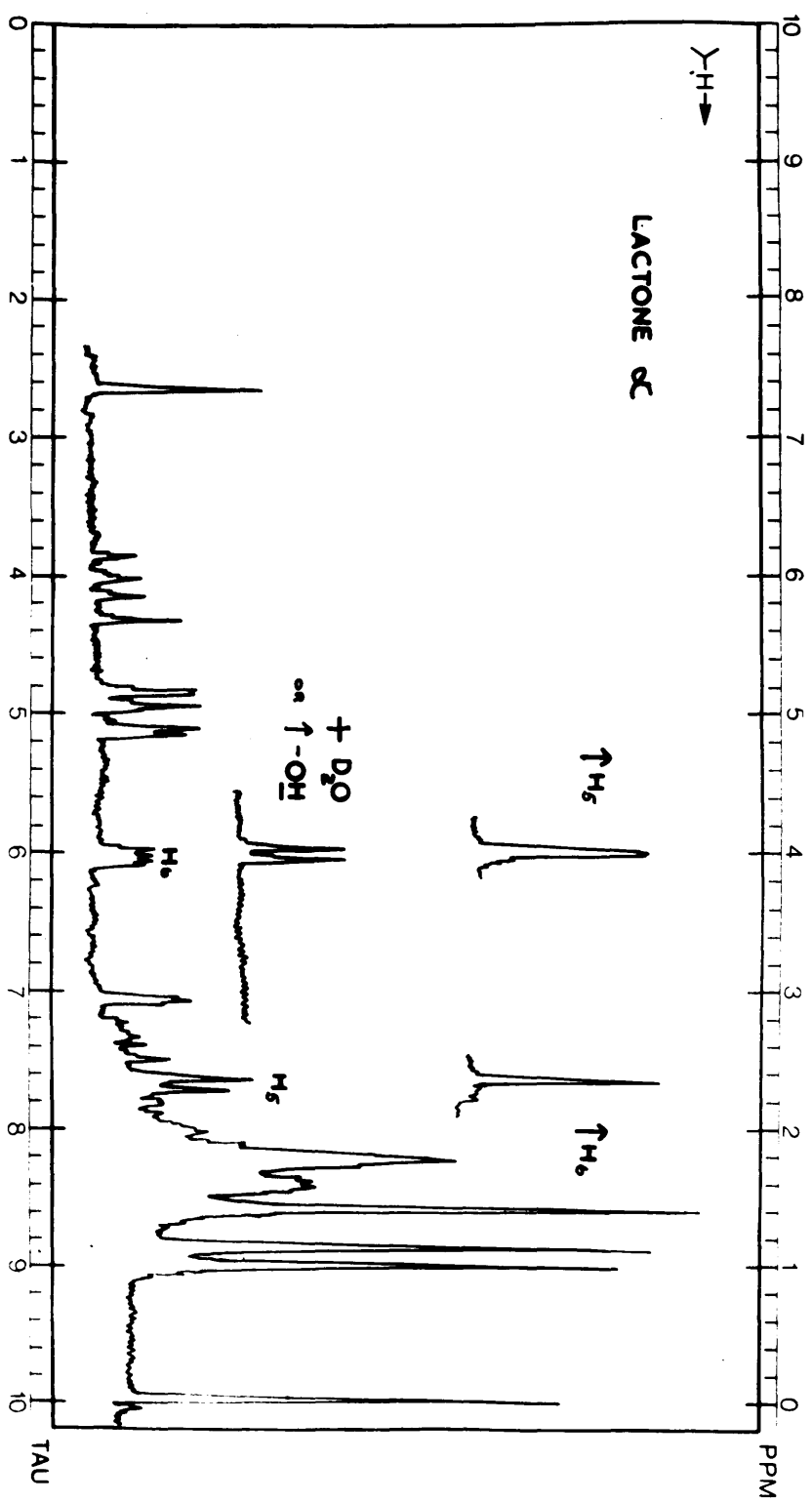


FIGURE 6

LACTONES α AND β .

In the course of our work on rosenonolactone, two metabolites were isolated from the mould T.roseum, lactone α , which had not been previously reported, and lactone β , which had been isolated by Freeman and Morrison⁽³²⁾⁽³⁴⁾ and named rosein III, though these workers made no attempt to determine its structure. Since commencing this work on lactone β , we have learnt that Birch and Rickards at Manchester have also isolated this metabolite from T.roseum and have kindly informed us⁽⁸⁷⁾ of a tentative structure (XCIX).

Lactone α , (LXXX), $C_{20}H_{28}O_4$, m.p. $180^{\circ}C$, $[\alpha]_D -162^{\circ}$, was obtained from T.roseum in 4% yield (see experimental), and had bands in the infra-red at $U_{max}^{(CCl_4)}$ 3590 (bonded hydroxyl), 1788 (γ -lactone), 1722 (cyclohexanone) and 1640 cm^{-1} (vinyl double bond), the band at 3590 cm^{-1} remaining unchanged on dilution. The O.R.D. curve of lactone α was very similar to the curve from rosenonolactone (XLVII) and differed radically from that of rosenolactone (LXXXI) (Figure 5). From this evidence it was surmised that lactone α (LXXX) was a hydroxylated rosenonolactone.

This was supported by the n.m.r. spectrum (Figure 6) which showed the vinyl double bond at C_{13} and the three quaternary methyl groups (T8.64, 8.92 and 9.05), the low methyl signal at T8.64 being assigned to the methyl group at C_9 , as it is deshielded by the β hydroxyl at C_6 .

The quartet at T6.1 (1 H, $W = \frac{1}{2} 10 \text{ c/sec}$) was ascribed to the carbinol proton (CH-OH), this quartet collapsing to a doublet ($J = 6 \text{ c/sec}$) on D_2O exchange. Upon irradiation at T7.5 (H_5), the quartet collapsed to a broadened singlet. Thus, the hydroxyl group can have only one neighbouring proton and must be positioned at C_6 or C_{14} , and must be axial (β). Either of these assignments would allow the hydroxyl group to bond with the carbonyl group at C_7 , while at C_{14} the hydroxyl group would have an opportunity of bonding with the vinyl double bond at C_{13} . Dihydrolactone α (LXXXII), $\text{C}_{20}\text{H}_{30}\text{O}_4$, m.p. 174°C , formed by hydrogenation of lactone α (LXXX) absorbed in the infra-red at 3590 (bonded hydroxyl), 1787 (γ -lactone) and 1722 cm^{-1} (cyclohexanone) and hence the hydroxyl group must be bonded to the carbonyl group and not to the olefinic double bond.

One method of differentiating between the C_6 and the C_{14} positions for the assignment of the hydroxyl group was to form the diketone. On oxidation, either an α or a β diketone would be formed and should be easily distinguishable by their spectroscopic properties. Jones and Sarrett reagents and ruthenium tetroxide were used as oxidising agents but all failed to produce the desired diketone, the initial product being unstable.

Another approach considered, was to remove the keto-group at C_7 and identify the resulting product as either rosololactone, with the hydroxyl group at C_6 or as a new alcohol, having the hydroxyl group at C_{14} . Wolff Kishner reduction was unsuitable as lactone α rearranged under basic conditions and attempted formation of the thioketal, prior to reductive removal, was also unsatisfactory, as lactone α was unstable to the conditions necessary for the reaction.

By preparation of a cyclic derivative from the diol (LXXXIII) of lactone α , it was hoped that the n.m.r. spectrum of this compound might show the relationship between the hydroxyl and carbonyl groups. The α face of the molecule is less hindered and it was expected that sodium borohydride would attack from this side to afford the cis diol (LXXXIII). However, it was possible for the sodium borohydride to complex with the hydroxyl group and therefore attack from the β face. Consequently, two diols epimeric at C_7 , were formed in approximately equal amounts on reduction. Because of the small quantities of lactone α

at our disposal it was essential to obtain a good yield in all the reactions undertaken. Fortunately, on catalytic hydrogenation, attack took place as anticipated, solely from the less hindered (α) side to afford the cis-dihydrodiol (LXXXIV), $C_{20}H_{32}O_4$, m.p. $215-216^\circ C$, $[\alpha]_D^{25} + 25^\circ$, identical with one of the hydrogenated products from sodium borohydride reduction (shown by formation of identical acetonides).

Absorbtion in the infra-red at $U_{\max}^{CCl_4}$ 3642, 3615, 3550 and $3470.\text{cm.}^{-1}$, accounted for the free, inter- and intra-molecular hydrogen bonding of the hydroxyl groups, and the absorbtion at 1777 cm.^{-1} signified that the γ -lactone was still present. The $(\underline{\text{CH}}-\text{OH})_2$ protons were centred at T5.9 (multiplet, $W^{\frac{1}{2}} = 12\text{ c/sec}$) and T6.3 (multiplet, $W^{\frac{1}{2}} = 16\text{ c/sec}$). On D_2O exchange, the multiplet at T5.9 collapsed to a quartet, ($J = 3$ and 10 c/sec), but the multiplet at T6.3 remained complex. The spectrum does not establish unambiguously the position of the two hydroxyl groups, but it inferred that one was at C_6 and the other at C_7 from the complexity of the multiplets at T5.9 and 6.3.

By reaction of the dihydrodiol (LXXXIV) with anhydrous copper sulphate and acetone, the acetonide (LXXXV), $\text{C}_{23}\text{H}_{36}\text{O}_4$, m.p. $167-168^\circ\text{C}$, $[\alpha]_{\text{D}} + 36.4^\circ$, was formed and from the analysis of the single and double resonance spectra of this compound, a unique solution (LXXX) for the structure of lactone α was obtained.

The $-\underline{\text{CH}}-\text{OR}$ protons were centred at T5.65 (quartet, $J = 4$ and 8 c/sec) and T6.0 (quartet, $J = 3$ and 8 c/sec). From this splitting pattern and with the assistance of models, it can be seen that one hydroxyl group must be situated at C_7 and the other at C_6 and not at C_{14} , and ring B in lactone α must be a boat with the protons at C_5 and C_8 , α and axial. With the acetonide group β orientated, the protons at C_6 and

C_7 are coplanar, confirmed by double irradiation at T8.26, both quartets collapsing to doublets with coupling constants ($J = 8$ c/sec) in accordance with the dihedral angle between H_B and H_C , $\vartheta_{BC} = 0^\circ$. Thus H_A and H_D must both resonate at T8.26 to account for the collapse of both quartets to doublets. Assuming that the stereochemistry of lactone α is the same as in rosenonolactone (LXVII) at C_4 , C_9 , C_{10} and C_{13} , the structure and stereochemistry of lactone α is as shown in (LXXX).

To support this assumption, a direct correlation was established between lactone α and rosenonolactone, by way of the diosphenol (LXXXVI). Treatment of dihydrolactone α (LXXXII) with bismuth oxide and acetic acid afforded an acid, identified as the derived methyl ester (LXXXVI). This ester, $C_{21}H_{28}O_4$, m.p. $121^\circ C$, absorbed in the infra-red, $U_{max}^{CCl_4}$ 3500 (bonded hydroxyl), 1735 (ester), 1660 cm^{-1} (cross conjugated dienone), and in the ultra-violet λ_{max} 257 $m\mu$, \sum 5590 and 300 $m\mu$, \sum 3696, the expected bathochromic shift being observed λ_{max} 356 $m\mu$, \sum 3096, on addition of sodium hydroxide. The diosphenol ester (LXXXVI) gave a characteristic intense purple colouration with alcoholic ferric chloride solution. The singlet at T3.5 ($-O-H$) disappearing on D_2O exchange, the singlet at T6.33 (3H) ($-COO-CH_3$) and the three quaternary methyl groups appearing as singlets at T8.5, 8.7 and 9.2, confirmed the structure of the diosphenol ester as (LXXXVI).

Acetylation of (LXXXVI) yielded the desired acetate (LXXXVII), which had bands in the infra-red at 1765 (acetate), 1730 (methyl ester) and 1650 cm.^{-1} (dienone) and in the ultra-violet at $\lambda_{\text{max}}\ 250\text{ m}\mu$, $\Sigma\ 11,800$, the hypsochromic shift being anticipated on acetylation of the diosphenol ester (LXXXVI). In the n.m.r. spectrum a singlet at $\tau 7.75$ (3H) ($\text{CH}_3\text{-COO-}$) appeared in addition to those resonances in the diosphenol ester spectrum.

The α -furfurylidene dihydrorosenonolactone⁽⁵⁴⁾ (LXXXVIII), m.p. $191\text{-}192^\circ\text{C}$, (lit.value⁽⁵⁴⁾ 191°C), was prepared from dihydrorosenonolactone and this had the expected bands at 1770, 1680 and 1600 cm.^{-1} in the infra-red, and at $\lambda_{\text{max}}\ 322\text{ m}\mu$, $\Sigma\ 23,000$ in the ultra-violet. Ozonolysis of (LXXXVIII) in acetic acid at 20°C afforded, on reductive work up, the diosphenol (LXXXIX), m.p. 174°C , (lit.value⁽⁵⁴⁾ $173\text{-}176^\circ\text{C}$), which absorbed in the infra-red at 1766 (δ -lactone), 1670 (enone), 1640 (vinyl double bond) and 3400 cm.^{-1} (hydroxyl). A black-green colouration was observed with alcoholic ferric chloride solution and the ultra-violet maximum at $\lambda_{\text{max}}\ 287\text{ m}\mu$, $\Sigma\ 11,150$, shifted to $\lambda_{\text{max}}\ 355\text{ m}\mu$ on addition of sodium hydroxide. Refluxing this diosphenol (LXXXIX) in acetic acid caused the lactone ring to open, yielding an unsaturated acid, which on methylation afforded a compound identical with (LXXXVI) obtained from lactone α , m.p. 121°C , m.m.p.

121°C, infra-red ($U_{\max}^{CCl_4}$ 3500, 1735 and 1660 cm.^{-1}), ultra-violet (λ_{\max} 257 $m\mu$, and 300 $m\mu$) and n.m.r. (T6.33 (3H, s), 8.7 (3H, s), 8.8 3H, s) and 9.2 (3H, s).). This direct correlation proved conclusively that the absolute configuration of the new metabolite lactone α , is as shown in (LXXX).

During the course of our work on lactone α , a method was considered for relating it to rosenonolactone, by formation of the mesylate and thereafter reductive removal of this group with lithium aluminium hydride. Treatment of lactone α with methane sulphonyl chloride in pyridine failed to yield the desired product, but formed instead a compound (XC), $C_{20}H_{28}O_4$, m.p. 156-157°C, $[\alpha]_D + 4^\circ$, isomeric with lactone α . A compound was isolated from the mould T.roseum in extremely small yield (8 mgs. from 4 gms. of broth extract), which had identical m.p., n.m.r., i.r. and t.l.c. retention time with (XC), but it is considered that this may be an artifact formed upon work up of the mould. The structure of (XC) was uniquely defined by a detailed study of its n.m.r. and infra-red spectra.

In the infra-red, bands at 3560 and 1782 cm.^{-1} denoted the presence of a bonded hydroxyl group and a δ -lactone, but showed the absence of a keto group. The three quaternary methyl groups (T8.76 (3H); T9.04 (6H),) were observed in the n.m.r. spectrum and the protons appearing

as doublets at T5.33 ($J = 6$ c/sec) and T7.48 ($J = 6$ c/sec) were mutually coupled since on irradiation at T5.33, the doublet at T7.48 collapsed to a singlet. As these protons couple with each other and with nothing else, they must be flanked on either side by tetra-substituted carbon atoms. Consequently, the doublets at T5.33 and T7.48 were assigned to H_6 and H_5 respectively, H_5 being at lower field than the rest of the methine protons, as it is deshielded by the lactone ring. The hydroxyl group failed to oxidise and no change was observed in the n.m.r. spectrum on D_2O exchange, in agreement with the assignment to the hydroxyl group of a tertiary position.

These results might have been interpreted by the formation of a symmetrical dimer (XCI), from the condensation of two molecules of lactone α , but from osmometric and mass spectrometric molecular weight determinations, (XC) was found to be monomeric.

The hemiketal (XC) failed to form when lactone α was refluxed in pyridine alone, but in ethanolic potassium hydroxide, lactone α was isomerised to (XC), (m.p., m.m.p., i.r., u.v., and n.m.r.).

From the close proximity of the oxygen functions at C_6 and C_7 and of the γ -lactone ring (as seen from the 3D diagram of lactone (LXXX)) it is possible that (XC) is formed in ethanolic potassium hydroxide by the C_6 -OH attacking the lactonic carbonyl group to afford a new

γ -lactone and thereafter the oxygen function at C_{10} could attack the ketonic carbonyl group at C_7 to give the hemiketal (XC). In methane sulphonyl chloride and pyridine, a possible mechanism for the formation of (XC) from lactone α could be initial polarisation of the electrons from the ketonic carbonyl group at C_7 , and subsequent attack of this by the oxygen function at C_{10} , the hydroxyl group at C_6 having already formed a new γ -lactone with the lactonic carbonyl group.

In an attempt to open the hemiketal ring, (XC) was treated with aqueous acid and base and also with thionyl chloride in pyridine, but no change was observed.

Reduction of lactone α with lithium aluminium hydride in anhydrous ether yielded an oily tetrol (XCII), (mass spec. m.wt. 338), considered to be epimeric with the tetrol (XCIII) (mass spec. m.wt. 338), obtained by reduction of (XC), by comparison of their n.m.r. spectra. The poly-ol (XCII) absorbed in the infra-red at $\nu_{\max}^{CCl_4}$ 3619, 3530 and 3352 cm^{-1} , attributed to the non-bonded and bonded hydroxyl groups, the bond at 3352 cm^{-1} decreasing in intensity on dilution and therefore arising partially from intermolecular hydrogen bonding. It was anticipated that hydride attack would take place from the α face of lactone α , as it is the less hindered, to yield a β -OH at C_7 . This

was borne out by estimation of the coupling constants of the carbinol protons in the n.m.r. spectrum. The protons of the primary hydroxyl group appear as an AB quartet centred at T5.45 and 6.7 ($J = 11$ c/sec) (geminal coupling). They are mutually coupled, since irradiation at T5.45 causes the doublet at T6.7 to collapse to a singlet, and are non-equivalent as free rotation of the C_4-C_{19} bond is prevented by hydrogen bonding. Consequently, one of the protons of the C_{19} methylene group is deshielded by the β hydroxyl groups at C_{10} and C_6 . The doublet centred at T5.6 ($J = 9$ c/sec) is assigned to H_6 ($CH-OH$), and the quartet at T6.2 ($J = 4$ and 9 c/sec) to H_7 ($CH-OH$). Upon irradiation at T5.6, the quartet at T6.2 collapses to a doublet ($J = 4$ c/sec, residual coupling to H_8), while in the reverse experiment the doublet at T5.6 collapses to a singlet. Irradiation at H_8 , T8.65, causes the quartet at T6.2 to collapse to a doublet ($J = 9$ c/sec, residual coupling to H_6). The observed coupling constants of H_6 and H_7 with their neighbours are met by a twist boat conformation for ring B, caused by the hydrogen bonding between the hydroxyl groups at C_6 , C_{10} and C_{19} and by the 1 : 3 diaxial interaction between the hydroxyl at C_7 and the methyl group at C_9 . This rotates C_6 up, giving a dihedral angle of 90° between H_5 and H_6 ($J_{5,6} = 0$ c/sec).

The tetrol (XCIII) from (XC) has bands at $U_{\max}^{CCl_4}$ 3616, 3552, 3438 and 3340 cm.^{-1} in the infra-red, the bands at 3438 and 3340 cm.^{-1} decreasing in intensity on dilution. The methylene protons of the primary

hydroxyl group appear as an AB quartet centred at T5.5 and T6.7 ($J = 11$ c/sec) and are mutually coupled as irradiation at T5.5 reduces the doublet at T6.7 to a singlet. Deshielding by the β hydroxyl groups at C₆ and C₁₀ causes the difference in chemical shift of the two geminal protons. The broad singlet at T5.84 ($J = 3$ c/sec) and the quartet at T6.18 ($J = 3$ and 12 c/sec) are assigned to the carbinol protons at C₆ and C₇ respectively. Upon double irradiation at T6.18 (H₇) the broad singlet at T5.84 (H₆) sharpens to a clean singlet, while in the reverse experiment the quartet at T6.18 reduces to a doublet ($J = 12$ c/sec, residual coupling with H₈). Upon irradiation at T8.2 (H₈), the quartet at T6.18 (H₇) collapses to a broad singlet ($J = 3$ c/sec, residual coupling to H₆). From the observed coupling constants of H₆ and H₇ with their neighbouring protons and with the knowledge that ring B has a twist boat conformation the hydroxyl group at C₇ is assigned the α configuration. This is to be expected from consideration of hydride attack on the hemiketal (XC). The formation of these epimeric tetrols from (LXXX) and (XC) is further proof that no major structural rearrangement has taken place by action of base on lactone α (LXXX) to afford the product (XC).

Acetylation of these tetrols (XCII) and (XCIII) yielded two diacetates from each compound, the primary and either the C₆ or the C₇ hydroxyl group acetylating. These diacetates of (XCII) and (XCIII) proved

inseparable by chromatography.

In the hemiketal (XC), the hydroxyl group should acetylate more readily than a normal tertiary hydroxyl group. In acetic anhydride and pyridine, (XC) formed the acetate (XCa), (mass spec. m.wt. 374), m.p. 170°C , as shown by infra-red absorption (CCl_4), 1783 (γ -lactone), 1750 (acetate) and 1240 cm^{-1} (acetate) and by the n.m.r. spectrum, where the $-\text{COOCH}_3$ protons appear as a singlet, $\tau 7.90$ (3H). From double irradiation experiments, the doublet at $\tau 7.45$ ($J = 6\text{ c/sec}$) is seen to be coupled to the doublet at $\tau 4.65$ ($J = 6\text{ c/sec}$) and these may be assigned to H_5 and H_6 respectively, $\vartheta_{5,6} = 20^{\circ}$. On acetylation, H_6 is shifted downfield by $\tau 0.68$, compared with H_6 in (XC), which is a large downfield shift for a proton in this environment. However, it can be shown with the assistance of models that, in the preferred conformation of the acetate group, H_6 is in the deshielding region of the acetate carbonyl group and this could account for its large downfield shift.

The hydroxyl group of lactone α was originally considered to be at C_6 or C_{14} , and at one stage in our work, C_{14} was the more strongly favoured position. Because of the small quantities of lactone α available, an attempt was made to synthesise this supposed C_{14} alcohol from rosenonolactone. One proposed method was to brominate dihydro-rosenonolactone at C_8 , dehydrobrominate to afford the $\Delta^{8(14)}$ -dihydro-

rosenonolactone, and thereafter attach a hydroxyl group at C₁₄ to yield the desired hydroxyrosenonolactone. Bromination of dihydrorosenonolactone with bromine in acetic acid gave three products. Only one of these was obtained pure by chromatography, as the crystalline C₆ mono-bromide (XCIV), C₂₀H₂₉O₃ Br, m.p. 141°C, $[\alpha]_D - 70^\circ$. This mono-bromide had bands in the infra-red at 1770 (γ -lactone) and 1725 cm.⁻¹ (cyclohexanone) and in the ultra-violet $\lambda_{\max} 308 \text{ m}\mu$, $\sum 62$. The ketonic frequency is thus 9 cm.⁻¹ higher and the ultra-violet maximum 13 m μ higher than the corresponding absorption maxima of dihydrorosenonolactone. From these results it is impossible to deduce the configuration of the C₆ halogen, due to the fact that ring B has a rigid boat conformation, in which the plane of the carbonyl almost bisects the angle between the C₆ hydrogen and the C₆ bromine. In the n.m.r. spectrum of the mono-bromide, the doublet centred at T5.9 (1H, J = 12 c/sec) (-CH Br-) shows that the configuration of the bromine atom at C₆ is α , the dihedral angle between H₅ and H₆ being 180°, in accordance with the value of the coupling constant.

Dehydrobromination of (XCIV) with lithium bromide and lithium carbonate in dimethyl formamide⁽⁵⁵⁾, afforded the unsaturated ketone (XCV), C₂₀H₂₈O₃, m.p. 167°C, $[\alpha]_D^{+20}$. This structural assignment was confirmed by absorption in the infra-red at 1779 (γ -lactone) and 1679 cm.⁻¹ (enone) and in the ultra-violet λ_{\max}

235 m μ , \sum 10,884. The olefinic proton at C₆ appeared as a singlet in the n.m.r. at T4.2.

Failure to form the C₈ mono-bromide as an intermediate in the synthesis of the C₁₄ alcohol, led to the use of rosenonolactone as a model compound for the reactions of lactone α . If the hydroxyl group in lactone α were at C₁₄ and in an axial (β) configuration, by addition of an easily eliminated oxygen function at C₁₇, it should have been possible to form a dihydrofuran derivative (XCVI), by attacking C₁₇ with this hydroxyl group at C₁₄.

Epoxidation of rosenonolactone yielded the desired compound (XCVII), C₂₀H₂₈O₄, m.p. 176°C, confirmed by the presence of the one protons multiplets at T7.4, 7.6 and 7.8. Attempted opening of this epoxide ring with freshly distilled boron trifluoride etherate⁽⁸⁴⁾, afforded an acid, and variations in temperature and concentration failed to yield the expected aldehyde. The epoxide of lactone α was prepared on a pilot scale, in the hope that, on opening this epoxide ring, attack by the C₁₄-OH might stabilize the system and yield the desired dihydrofuran derivative. However, the action of boron trifluoride on the epoxide of lactone α afforded only acidic products.

The lack of success of the above route led to an attempt to form the vicinal glycol of lactone α , with a view to subsequent ring closure

using toluene-p-sulphonyl chloride, to afford either the dihydrofuran (XCVI) or the 16-hydroxy derivative (XCVIa). Osmylation of rosenonolactone gave the diol (XCVIII), $C_{20}H_{30}O_5$, m.p. $188^{\circ}C$, $[\alpha]_D -110^{\circ}$, but all endeavours to obtain the corresponding glycol from lactone α failed, as decomposition of the metabolite occurred under the conditions used.

At this stage in the work evidence first emerged, which made the location of the hydroxyl group at C_6 probable.

Lactone β (mass spec. m.wt. 332), m.p. $221^{\circ}C$, $[\alpha]_D -124^{\circ}$, isolated from the mould T.roseum in 1% yield (see experimental), had bands in the infra-red (CCl_4) at 3610 (bonded hydroxyl), 1782 (γ -lactone) and 1722 cm^{-1} (cyclohexanone) and its O.R.D. curve was again almost superimposable (Figure 7) on that of rosenonolactone. In the n.m.r. spectrum, the three quaternary methyl groups (T8.9, 9.0 and 9.1) and the vinyl double bond at C_{13} , characteristics of the rosane skeleton, were observed. From this information, lactone β appeared to be another hydroxylated rosenonolactone, isomeric with lactone α . The multiplet at T5.9(1H) was assigned to the carbinol proton ($-CH-OH$), D_2O exchange resolving this multiplet into a quartet ($J = 3$ and 5 c/sec). From this splitting pattern, the hydroxyl group was considered to have two neighbouring protons and thus, if lactone β has a rosane skeleton, the hydroxyl group can be placed at C_1 , C_3 , C_{11} or C_{12} .

Oxidation of lactone β with Jones reagent yielded a dione, (mass spec. m.wt. 330), m.p. 198°C , $[\alpha]_{\text{D}} -100^{\circ}$, and $U_{\text{max}}^{\text{CCl}_4} 1767$ (δ -lactone) and 1710 cm.^{-1} (cyclohexanone). From the integration curve of the n.m.r. spectrum of the dione, two additional protons were observed between T7.0 and 8.0 the expected region in which the protons ($-\text{CH}_2-\text{CO}$) would resonate.

Treatment of this dione with methanolic potassium hydroxide yielded three products, only one of which was obtained pure by chromatography. This compound (mass spec. m.wt. 362), m.p. 119°C , absorbed in the infra-red at 1735 (ester) and 1714 cm.^{-1} (cyclohexanone) and from estimation of the extinction coefficients of these absorptions, it was found that one ester group and two keto groups were present in the molecule. In the n.m.r. spectrum, a three proton singlet at T6.4 was assigned to the protons ($-\text{COO CH}_3$), but the unresolvable multiplets between T6.7 and 8.6 remained unexplained. From these results, it can be seen that the lactone ring has been cleaved to afford an acid, which was esterified under the conditions of the reaction. To account for the addition of mass 32 to the dione on base cleavage, a mass 17 (OH) must be added, in addition to the mass 15, attributed to the methyl group of the ester. Unfortunately, no hydroxyl group was observed in the infra-red spectrum. The action of base on the dione might have been explained as in Figure 8, by assigning the hydroxyl group in lactone β to C_{11} . However,

this would result in the molecule possessing three keto groups, and from the infra-red spectrum, only two appear to be present.

Dehydration of dihydrolactone β with phosphorous oxychloride in pyridine yielded an unsaturated ketone (mass spec. m.wt. 316), m.p. 194°C , which had bands in the infra-red (CCl_4) at 1763 (γ -lactone), 1707 (cyclohexanone) and 1645 cm^{-1} (double bond), and had no intense absorption in the ultra-violet above $220\text{ m}\mu$. Two one proton singlets were seen in the n.m.r. spectrum at $\tau 4.64$ and 5.1 , in addition to a complex multiplet centred at $\tau 6.75$ (2H), the three expected quaternary methyl groups $\tau 8.88$ (3H), 9.02 (6H), and the secondary methyl group ($\tau 9.05$). Hydrogenolysis occurred during hydrogenation of this enone in methanol over 10% palladium charcoal, an acid being formed as shown by t.l.c. However, hydrogenolysis was avoided by reduction over platinum oxide in ethyl acetate. The product (mass spec. m.wt. 318), m.p. 184°C , absorbed in the infra-red at 1770 (γ -lactone) and 1710 cm^{-1} (cyclohexanone), and in the n.m.r., the singlets at $\tau 4.64$ and 5.1 found in the enone, were no longer present. A sharp singlet was observed at $\tau 7.62$ (2H) and an unresolvable multiplet at $\tau 7.1$ (2H) in addition to the three quaternary methyl groups ($\tau 8.76$, 8.9 and 9.08) and the secondary methyl group ($\tau 9.14$ doublet).

If the hydroxyl group were at C₁, C₃, C₁₁ or C₁₂, as was considered initially from the splitting pattern of the carbinol proton in the n.m.r. of lactone β , it is difficult to assign the two one proton singlets obtained on dehydration of dihydrolactone β , as the protons of a cis-double bond have a coupling constant of value $J = 8-12$ c/sec.

Consequently, it is difficult to attribute a hydroxyrosenonolactone structure to lactone β on the results of the reactions just described, although the O.R.D. curve and the n.m.r. spectrum are strong evidence in favour of this assignment.

Birch and Rickards⁽⁸⁷⁾ have assigned the tentative structure (XCIX) to lactone β on the basis of evidence which is at present unpublished. However, it is equally difficult to accommodate our results on this basis and we have no compelling reason to doubt that lactone β possesses the normal rosane skeleton.

In view of the small amounts of lactone β at our disposal it is proposed to prepare a suitable heavy atom derivative for X-ray structural analysis.

EXPERIMENTAL

Metabolites of T. Roseum Link

20 litres of Czapek-Dox and corn steep liquor⁽³²⁾ was seeded with spores of T. roseum. The mould was grown for three weeks as a surface culture, and the broth decanted from the mycelium. A little chloroform was added to the broth and mycelium to effect sterilisation.

The broth was extracted into ethyl acetate and the extracts were washed with sodium bicarbonate, water and thereafter dried. The solvent was removed to give a brown gum (4 gm) and the components of this were separated by p.t.l.c. on five (1000 x 200 x 1 mm.) plates using ethyl acetate: light petroleum as eluant. The following table summarises, in increasing polarity, the products obtained from chromatography of the broth extract.

BAND	WEIGHT	CONTENT
I	120 mg	Desoxyrosenonolactone ⁽⁴⁴⁾
II	160 mg	Rosenonolactone ⁽³⁷⁾⁽³⁸⁾
III	150 mg	Lactone α .
IV	50 mg	Lactone β ⁽³²⁾⁽³⁴⁾
V	1.3 gm	Trichothecin ⁽³³⁾

Pure samples of all these compounds were obtained as shown by t.l.c. with ethyl acetate : light petroleum (1 : 1) as eluant.

Lactone α (LXXX)

This metabolite was isolated as described and crystallised from ether as prisms, m.p. 180°C ; $[\alpha]_{\text{D}} -162^{\circ}$, ($c = 1.0$, chloroform); $\text{U}_{\text{max}}^{\text{CCl}_4}$ 3590, 1788, 1722, 1640 cm^{-1} ; $\lambda_{\text{max}}^{\text{EtOH}}$; No intense absorption above 220 m. On addition of sodium hydroxide solution, $\lambda_{\text{max}}^{\text{EtOH}}$ 260 m μ , $\sum 1990$, 222 m μ , $\sum 3300$. 1H quartet centred at T6.0 (CH-OH). (Found: C, 71.9; H, 9.5. $\text{C}_{20}\text{H}_{28}\text{O}_4$ requires C, 72.2; H 8.5%.)

Dihydrolactone α (LXXXII)

Lactone α (7 mg) was added to a suspension of 10% palladium charcoal (10 mg) in ethyl acetate (5 ml) and one mole of hydrogen was observed to be taken up. The solution was filtered through cellulose powder and the solvent removed to give dihydrolactone α (LXXXII), (5 mg) which crystallised from light petroleum, m.p. 174°C ; $\text{U}_{\text{max}}^{\text{CCl}_4}$ 3590, 1787, 1721 cm^{-1} . (Mass spec. m.wt. 334. $\text{C}_{20}\text{H}_{30}\text{O}_4$ requires 334).

Reduction of Lactone α (LXXX) with Sodium Borohydride

Lactone α (10 mg) was dissolved in methanol (2 ml), sodium borohydride (10 mg) added, and the solution left at room temperature for 1 hour. Work up by the addition of water, extraction into ethyl acetate, and removal of the solvent afforded two diols (LXXXIII)

and (LXXXIIIa), (7 mg), epimeric at C₇, as seen from t.l.c.

Separation of the mixture by p.t.l.c. was unsuccessful. Leaving the mixture of diols in acetone plus anhydrous copper sulphate for 2 days at 20°C yielded the cis acetone (LXXXVa), (3 mg), plus the unchanged trans-diol (LXXXIIIa), (3 mg). Hydrogenation of (LXXXVa) in ethanol over 10% palladium charcoal afforded the dihydro-acetone (LXXXV), as seen from t.l.c. and m.p. 167-168°C., m.m.p. 167-168°C.

Reduction of Lactone α (LXXX) with Adams' Catalyst in Acetic Acid

Lactone α (70 mg) was hydrogenated in "analar" acetic acid (10 ml) over platinum oxide (20 mg) for 24 hours at 20°C. Filtration of the solution through cellulose powder and removal of the acetic acid by azeotroping it with benzene, afforded the dihydro-diol (LXXXIV), (58 mg), shown to be homogeneous by t.l.c. (LXXXIV) crystallised from ether as fine needles, m.p. 215-216°C; $[\alpha]_D^{25} + 25^\circ$, (c = 0.4, chloroform); $\nu_{\text{max}}^{\text{CCl}_4}$ 3643, 3615, 3550 (bonded -OH), 1777 cm.⁻¹; 1H multiplets centred at τ 5.9 and 6.3 ($\text{CH}_2\text{-OH}$). (Found: C, 71.2; H, 9.5.

$\text{C}_{20}\text{H}_{32}\text{O}_4$ requires C, 71.4; H, 9.6%).

Acetone of the Dihydrodiol

The dihydrodiol (LXXXIV), (30 mg), was added to a suspension of copper sulphate (anhydrous) (1 gm) in "analar" acetone (10 ml). After 2 days at 20°C the solution was filtered through cellulose powder, and

the solvent removed to afford the acetonide (LXXXV), (22 mg), which crystallised from ether as fine needles, m.p. 167-168°C; $[\alpha]_D + 36.4$ (c = 0.45, chloroform); $U_{\max}^{\text{nujol}} 1770 \text{ cm.}^{-1}$; 1H quartets centred at T5.65 and 6.0 ($-\text{CH}-\text{O}-$)₂C(CH₃)₂. (Found: C, 73.6; H, 9.3. C₂₃H₃₆O₄ requires C, 73.4; H, 9.6%).

Action of ethanolic potassium hydroxide on lactone α (LXXX)

A solution of lactone α (20 mg) in benzene (sodium dried) (1 ml) was added to a 5% ethanolic potassium hydroxide solution (3 ml) and was kept at 20°C for 45 mins. Water was added, the solution extracted with ethyl acetate, the extracts combined, washed with water, dried and the solvent removed in vacuo. The product (XC) (14 mg), purified by p.t.l.c. using ethyl acetate: light petroleum (3 : 7) as eluant, crystallised from ether - light petroleum, m.p. 156-157°C; $[\alpha]_D + 4^\circ$, (c = 0.27, chloroform); $U_{\max}^{\text{CCl}_4} 3560, 1782 \text{ cm.}^{-1}$; 1H doublet centred at T5.33 (J = 6 c/sec) ($-\text{C}_6\text{H}-\text{O}-$) and at T7.48 (J = 6 c/sec) ($-\text{C}_5-\text{H}$). (Mass spec. m.wt. 332. C₂₀H₂₈O₄ requires 332).

Action of Methane Sulphonyl Chloride on Lactone

Lactone α (20 mg) in dry pyridine (1 ml) with methane sulphonyl chloride (0.05 ml) was heated on a steam bath for 1 hour. Water was added, the solution extracted into ethyl acetate, dried and the solvent removed, any excess pyridine being azeotroped off with benzene. The

product after purification by p.t.l.c., was found to be identical with (XC); same retention time on t.l.c.; i.r., ($U_{\max}^{CCl_4}$ 3560, 1782 cm^{-1}); n.m.r., (T5.33 (1H, d), T7.48(1H, d)), m.p. 156-157°C; m.m.p. 156-157°C; $[\alpha]_D + 4^\circ$, ($c = 0.5$; chloroform).

Acetylation of (XC)

(XC) (14 mg) was acetylated in "analar" acetic anhydride (0.3 ml) and dry pyridine (0.5 ml) at 20°C for 12 hours. The reagent was removed in vacuo and the product, a dark brown gum, purified by p.t.l.c. using ethyl acetate: light petroleum (3 : 7) as eluant. The acetate, (XC_a) was crystallised from light petroleum as large needles, m.p. 170°C, $U_{\max}^{CCl_4}$ 1783, 1750 cm^{-1} ; 1H doublets centred at T4.63 (>CH-O-) and at T7.46 ($\text{>C}_5\text{-H}$). (Mass spec. m.wt. 374. $\text{C}_{22}\text{H}_{30}\text{O}_5$ requires 374).

Reduction of Lactone α (LXXX) with Lithium Aluminium Hydride

Lactone α (30 mg) was dissolved in ether (sodium dried) (20 ml) and lithium aluminium hydride (50 mg) added. The solution was refluxed for 5 hours, and worked up as previously described to afford the oily tetrol (XCII), $U_{\max}^{CCl_4}$ 3619, 3530, 3352 cm^{-1} ; AB quartet at T5.45 and 6.70 ($J = 11$ c/sec) ($\text{-CH}_2\text{-OH}$), 1H doublet centred at T5.6 ($\text{>C}_6\text{H-OH}$); 1H quartet centred at T6.2 ($\text{>C}_7\text{H-OH}$). (Mass spec. m.wt. 338. $\text{C}_{20}\text{H}_{34}\text{O}_4$ requires 338).

Acetylation of the Tetrol (XCII)

The tetrol was left in pyridine (1 ml) and acetic anhydride (0.3 ml) at 20°C for 24 hours. Removal of the solvent in vacuo afforded a dark brown gum, found to be two products of very similar polarity after purification by p.t.l.c.

Reduction of (XC) with Lithium Aluminium Hydride

(XC) (30 mg) was refluxed in ether (sodium dried) with lithium aluminium hydride (50 mg) for 5 hours. On the normal work up, an oily tetrol (XCIII) was obtained, $\nu_{\text{max}}^{\text{CCl}_4}$ 3616, 3552, 3340 cm^{-1} ; AB quartet at τ 5.5 and 6.7 ($J = 11$ c/sec)($-\text{CH}_2 - \text{OH}$); 1H broad singlet at τ 5.84 ($J = 3$ c/sec) ($>\text{C}_6\text{H} - \text{OH}$); 1H quartet at τ 6.18 ($>\text{C}_7\text{H} - \text{OH}$).

Acetylation of the Tetrol (XCIII)

The tetrol (20 mg) was left in pyridine (1 ml) and "analar" acetic anhydride (0.5 ml) at 20°C for 24 hours. Work up, as previously described, yielded two diacetates which failed to separate by chromatographic techniques.

Oxidation of Dihydrolactone α (LXXXII) with Bismuth Oxide

To a briskly stirring solution of dihydrolactone α (LXXXII) (40 mg), bismuth oxide (35 mg) was added and the suspension heated on a steam bath for 1 hour. The solution was then refluxed for 10 mins, with

continuous stirring, filtered, taken up in ethyl acetate, washed with water, dried and the solvent removed, any remaining acetic acid being azeotroped with benzene. The resulting product, an acid, was esterified with diazomethane and was shown to be one spot on a chromatoplate. A purple colouration was observed on spraying a t.l.c. plate with alcoholic ferric chloride. The ester (LXXXVI) crystallised with difficulty from ice cold light petroleum, m.p. 121°C ; $\text{U}_{\text{max}}^{\text{CCl}_4}$ 3550, 1735, 1660 cm^{-1} ; 1H singlet at τ 3.5 ($\text{C}=\text{C}-\text{OH}$), 3H singlet at τ 6.33 ($-\text{COOCH}_3$); λ_{max} 257 $\text{m}\mu$, \sum 5590, 300 $\text{m}\mu$, \sum 3696, shifting to λ_{max} 356 $\text{m}\mu$, \sum 3096 on addition of sodium hydroxide.

Acetylation of (LXXXVI)

The diosphenol methyl ester (LXXXVI) (20 mg) was left overnight at 20°C in dry pyridine (1 ml) and "analar" acetic anhydride (0.8 ml). The reaction was worked up in the normal way to yield a gum, (LXXXVII) (10 mg) which crystallised with difficulty from ether - light petroleum as prisms, m.p. 117°C ; $\text{U}_{\text{max}}^{\text{CHCl}_3}$ 1765, 1730, 1650 cm^{-1} ; λ_{max} 250 $\text{m}\mu$, \sum 11,800; 3H singlets at τ 6.4 ($-\text{COOCH}_3$) and at τ 7.75 ($-\text{CH}_3-\text{CO}-\text{O}-$). (Mass spec. m.wt. 388. $\text{C}_{23}\text{H}_{32}\text{O}_5$ requires 388).

Attempted Oxidation of Lactone α

1. Lactone α (5 mg) was dissolved in analar acetone and cooled to 0°C. Jones oxidant (0.05 ml) was added and the solution kept at 0°C for 10 mins. The desired dione failed to form, the only product of the reaction being acidic, as seen from t.l.c. Variations of time, concentration and temperature failed to produce the desired product.
2. Lactone α (5 mg), chromium trioxide (3 mgs) and acetic acid (0.5 ml) were kept at 0°C for 1 hr. Work up by addition of water, extraction with ethyl acetate and removal of the solvent afforded unchanged lactone α (LXXX) and an acidic product as shown by t.l.c.
3. Ruthenium tetroxide solution⁽⁸³⁾ was prepared by suspending ruthenium dioxide (0.49 gm) in carbon tetrachloride (50 ml). Sodium metaperiodate solution (3.2 gm in water (50 ml)) was added and the solution stirred at 0°C for 1 hour. The clear carbon tetrachloride layer was separated off, filtered through glass wool and then shaken with a freshly prepared solution of sodium metaperiodate (1 gm in water (50 ml).) till the yellow colour of the carbon tetrachloride phase persisted.

To a solution of lactone α (LXXX) (5 mg) in carbon tetrachloride (1 ml) and water (0.05 ml), the freshly prepared ruthenium tetroxide solution was added, until the yellow colour persisted in the

carbon tetrachloride layer. The solution was stirred at 20°C for 2 hours, 2-propanol added to destroy any excess ruthenium tetroxide, and thereafter the solution was filtered, extracted with carbon tetrachloride, the extracts washed with water, dried, and the solvent removed to afford only acidic products as seen from t.l.c., instead of the desired dione.

Attempted opening of the Hemiketal (XC)

1. (XC) (3 mg) was dissolved in ethanol (0.5 ml) and 3N hydrochloric acid (0.1 ml) added and the solution left at 20°C for 1 hour. Work up by addition of water and extraction into ethyl acetate yielded only unchanged hemiketal (XC) as seen from t.l.c.
2. (XC) (3 mg) was dissolved in ethanol and 3N sodium hydroxide added and the solution left for 1 hour at 20°C. Work up by addition of water and extraction into ethyl acetate afforded unchanged hemiketal (XC) as shown by t.l.c. Increase in time, and concentration had no effect on (XC).
3. Hemiketal (XC) (5 mg) in dry pyridine (0.4 ml) and thionyl chloride (0.1 ml) was kept at 20°C for 12 hours and worked up by addition of water and extraction into ethyl acetate. No change was observed in (XC) as seen from t.l.c.

Attempted preparation of the Thioketal of Lactone α .

Lactone α (5 mg) was left in acetic acid (0.5 ml) ethane dithiol (0.1 ml) and boron trifluoride etherate (0.1 ml) at 20°C for 5 hours. Work up as previously described afforded a considerably less polar product which did not possess a γ -lactone ring, (no absorption in the infra-red at 1770 cm^{-1}).

Dihydrorosenonolactone

Rosenonolactone (200 mg) was hydrogenated in ethyl acetate over 10% palladium charcoal. Filtration of the solution through cellulose powder and removal of the solvent afforded dihydrorosenonolactone (195 mg), which crystallised from ether - light petroleum, m.p. 158-159°C, (lit. value⁽³⁸⁾ m.p. 138°C).

Furfurylidene Dihydrorosenonolactone (LXXXVIII)

Dihydrorosenonolactone (150 mg) was dissolved in ethanol (18 ml) and 15% aqueous sodium hydroxide (1 ml) and freshly distilled furfuraldehyde (120 mg) were added to the solution, which was left for 12 hours at 20°C. The solution was filtered to give a solid (LXXXVIII) (120 mg) which crystallised from methanol as yellow needles, m.p. 191°C; (lit. value⁽⁵⁴⁾ 192-193°C.); $U_{\text{max}}^{\text{nujol}}$ 1770, 1680, 1600 cm^{-1} ;

λ_{max} 322 $\text{m}\mu$, \sum 23,000.

Ozonolysis of (LXXXVIII)

Furfurylidene-dihydrosesenonolactone (LXXXVIII) (100 mg) was dissolved in acetic acid (10 ml) and ozonised at 20°C for 2 hours. Powdered zinc was added and the solution left overnight at 20°C, and thereafter the solution filtered through cellulose powder. Most of the acetic acid was removed in vacuo, the remainder being azeotroped with benzene. The crude product (LXXXIX) (60 mg), after purification by p.t.l.c. using ethyl acetate: light petroleum (1 : 4) as eluant, crystallised from methanol, m.p. 174°C, (lit. value⁽⁵⁴⁾ 173-176°C). The diosphenol (LXXXIX) showed a strong black-green colour with alcoholic ferric chloride solution. $U_{\max}^{\text{CHCl}_3}$ 3400, 1760, 1670, 1640 cm^{-1} ; λ_{\max} , 187 $\text{m}\mu$, \sum 11,150; shifting to λ_{\max} 350 $\text{m}\mu$ on addition of potassium hydroxide.

Action of Acetic Acid on the Diosphenol (LXXXIX)

The diosphenol (LXXXIX) (40 mg) was refluxed in acetic acid (3 ml) for 1 hour and thereafter the acetic acid removed in vacuo. The compound formed was an acid, which on esterification with diazomethane was shown to be one main product (LXXXVI) by t.l.c. Purification by p.t.l.c. using ethyl acetate: light petroleum (1 : 4) as eluting solvent afforded a compound identical with the diosphenol methyl ester (LXXXVI) prepared directly from the action of acetic acid -

bismuth oxide on dihydro-lactone α (LXXXII), as shown by t.l.c.

n.m.r. i.r. and u.v.

Bromination of Dihydrorosenonolactone

Dihydrorosenonolactone (200 mg) was dissolved in glacial acetic acid (5 ml) and 0.3 ml of a solution of bromine in acetic acid (3 gm in 10 ml) was added. One drop of hydrogen bromide was added and the solution left at 20°C for 45 minutes. Work up by the addition of water, extraction into ethyl acetate, washing of the extracts with aqueous sodium bicarbonate solution and with water and then drying and removal of the solvent, gave a gum (185 mg), seen to contain three components from t.l.c. The least polar of these was obtained pure by p.t.l.c. with ethyl acetate: light petroleum (1 : 4) as eluant, affording the 6-bromo-ketone (XCIV), m.p. 141°C; $[\alpha]_D^{20}$ -70, (c = 0.6, chloroform); $U_{max}^{CCl_4}$ 1770, 1725 cm^{-1} ; λ_{max} 308 m μ , \sum 62; 1H doublet centred at τ 5.9 (J = 12 c/sec) ($>CH-Br$). (Found: C, 60.3; H, 7.2. $C_{20}H_{29}O_3 Br$ requires C, 60.4; H, 7.3%).

Dehydrobromination of the 6-Bromo-Ketone (XCIV)

Anhydrous lithium bromide⁽⁵⁵⁾ (7 mg) was dissolved in dimethyl formamide (2 ml) and lithium carbonate (anhydrous) (12 mg) was added with continuous stirring. The solution was heated to 95°C under nitrogen, the α -bromo-ketone (XCIV) (20 mg) added and the reaction kept at this

temperature for 3 hours. Water containing a little acetic acid was then added, the aqueous solution extracted into ether, the extracts washed well with water, dried and the solvent removed to afford the enone (XCV), which crystallised from ice cold light petroleum, m.p.

167°C; $[\alpha]_D^{20} \pm 0^\circ$, (c = 0.2; chloroform); $U_{\max}^{CCl_4}$ 1779, 1679 cm^{-1} ;

λ_{\max} 235 m μ , \sum 10,884; 1H singlet at T4.2 ($\text{>C} = \text{CH}-$). (Found C, 75.7; H, 9.3 $\text{C}_{20}\text{H}_{28}\text{O}_3$ requires C, 75.9; H, 9.0%).

Epoxidation of Rosenonolactone

Rosenonolactone (30 mg) was dissolved in chloroform (2 ml) and m-chloroperbenzoic acid (22 mg) added. The solution was kept at 20°C for 12 hours and worked up by addition of calcium hydroxide⁽⁸²⁾ until neutral and then filtered. Removal of the solvent yielded the desired

epoxide (XCVII) (20 mg) which crystallised from chloroform-ether,

m.p. 176°C; $[\alpha]_D^{20} -102^\circ$, (c = 0.4, chloroform) $U_{\max}^{CHCl_3}$ 1770, 1720 cm^{-1}

1H multiplets at T7.4, 7.6 and 7.8. (Found C, 71.6; H, 8.5.

$\text{C}_{20}\text{H}_{28}\text{O}_4$ requires C, 72.2; H, 8.5%).

Attempted opening of the Epoxide Ring

The epoxide (XCVII) (5 mg) was dissolved in benzene (sodium dried) (1 ml), freshly distilled boron trifluoride etherate (0.1 ml) added, and the solution left at 20°C for 10 mins. Water was added and the aqueous solution extracted into ether, the extracts dried and the solvent removed.

T.l.c. showed that the desired aldehyde had not been formed, the proximate product being unstable to the conditions used.

Decrease of time and temperature failed to produce the aldehyde by straightforward opening of the epoxide ring.

Epoxide of Lactone α

Lactone α (4 mg) was dissolved in chloroform (1 ml) and m-chloroperbenzoic acid (4 mg) added. The solution was left at 20°C for 12 hours and worked up as previously described⁽⁸²⁾. T.l.c. showed that one product, the desired epoxide had been formed. $\nu_{\text{max}}^{\text{CHCl}_3}$ 3600, 1780, 1720 cm^{-1} .

The epoxide (2 mg) was dissolved in benzene (1 ml), boron trifluoride (0.1 ml) added and the reaction kept at 0°C for 1 min. Work up in the normal way, failed to give the desired product (XCVI), but resulted in complete decomposition, as seen from t.l.c.

Osmylation of Rosenonolactone

Rosenonolactone (35 mg) in dry pyridine (1.5 ml) with osmium tetroxide (25 mg) was left at 20°C for 12 hours. Aqueous sodium bisulphite was then added⁽⁸⁰⁾ and the solution stirred until a clear orange layer containing the cis-glycol separated out. The aqueous pyridine solution was then extracted into ethyl acetate, the extracts washed well with water, dried, and the solvent removed to afford two

keto diols as seen from t.l.c. one in major and the other in minor amount. Separation by p.t.l.c. with ethyl acetate: light petroleum (3: 2) as eluant gave the diol (XCVIII) obtained in higher yield, which crystallised from ether-chloroform, m.p. 188°C ; $[\alpha]_{\text{D}} -110^{\circ}$, ($c = 0.5$, chloroform); $\text{U}_{\text{max}}^{\text{CCl}_4}$ 3580, 3618 cm^{-1} (Found: C, 68.3; H, 8.5. $\text{C}_{20}\text{H}_{30}\text{O}_5$ requires C, 68.5; H, 8.6%).

Osmylation of Lactone α

Lactone α (15 mg) ..., dry pyridine (0.5 ml) and osmium tetroxide (10 mg) were kept at 20°C for 12 hours. Work up as previously described and then t.l.c. of the product showed that the desired diol had not been formed, the only products being acidic.

Lactone β

This metabolite, isolated as described, crystallised from ether as prisms m.p. 221°C ; $[\alpha]_{\text{D}} -124^{\circ}$, ($c = 0.56$, chloroform); $\text{U}_{\text{max}}^{\text{CCl}_4}$ 3610, 1782, 1722 cm^{-1} ; 1H multiplet at $\tau 5.9$ ($>\text{CH} - \text{OH}$). (Mass spec. m.wt. 332. $\text{C}_{20}\text{H}_{28}\text{O}_4$ requires 332).

Oxidation of Lactone β with Jones Oxidant

Lactone β (45 mg) was added to "analar" acetone (4 ml), cooled to 0°C , Jones oxidant⁽⁸⁵⁾ (0.5 ml) added and the solution kept at 0°C for 10 mins. Work up by addition of water, extraction into ethyl acetate, the extracts washed with water, dried and the solvent removed,

yielded an immediately crystalline compound (40 mg).

Recrystallisation from ether gave needles, m.p. 198°C ; $[\alpha]_{\text{D}} -100^{\circ}$,
 (c = 0.5, chloroform); $U_{\text{max}}^{\text{CCl}_4}$ 1767, 1710, 1640 cm^{-1} . (Mass spec.
 m.wt. 330. $\text{C}_{20}\text{H}_{26}\text{O}_4$ requires 330).

Action of methanolic potassium hydroxide on the Dione

The dione (35 mg) obtained above, was dissolved in benzene (2 ml) and 5% methanolic potassium hydroxide (2 ml) added. The solution was left at 20°C for 15 mins. and thereafter worked up by addition of water, extraction into ethyl acetate, and removal of the solvent to afford a gum (30 mg). The crude product was found to contain three compounds by t.l.c., the more polar component (9 mg) being separable from the other two by p.t.l.c. using ethyl acetate: light petroleum (1 : 3) as eluant. This compound crystallised from ether - light petroleum as needles, m.p. 119°C ; $U_{\text{max}}^{\text{CCl}_4}$ 1735, 1714 cm^{-1} ; 3H singlet in the n.m.r. at $\tau 6.4$ ($-\text{COOCH}_3$) (Mass spec. m.wt. 362).

Attempts of separate the other two components obtained from base treatment of the dione by p.t.l.c. were unsuccessful.

Dehydration of Dihydro Lactone β

Hydrogenation of lactone β (22 mg) in ethanol (5 ml) over 10% palladium charcoal afforded dihydrolactone β , $U_{\text{max}}^{\text{CHCl}_3}$ 1770, 1720 cm^{-1} . (Mass spec. m.wt. 334. $\text{C}_{20}\text{H}_{30}\text{O}_4$ requires 334).

Dihydrolactone (20 mg) in dry pyridine (1.5 ml) with phosphorous oxychloride (0.02 ml) was heated on a steam bath, under nitrogen, for 30 mins. Water was added and extraction into ethyl acetate afforded a dark brown gum (19 mg) found to be homogeneous by t.l.c. Purification by p.t.l.c. with ethyl acetate: light petroleum (1 : 3) as eluting solvent yielded a solid which crystallised from light petroleum m.p. 194°C ; U_{max} 1763, 1707 cm^{-1} ; λ_{max} , no intense absorption above $220\text{ m}\mu$; ^1H singlets in the n.m.r. at $\tau 4.64$ and 5.1 . (Mass spec. m.wt. 316. $\text{C}_{20}\text{H}_{28}\text{O}_3$ requires 316).

Hydrogenation of Dehydration Product

The dehydration product from dihydrolactone β (9 mgs) was hydrogenated in ethyl acetate (3 ml) over platinum oxide (20 mg), to afford an immediately crystalline saturated lactone (6 mg). Recrystallisation from ether gave needles, m.p. 184°C ; $U_{\text{max}}^{\text{CCl}_4}$ 1770, 1700 cm^{-1} . (Mass spec. m.wt. 318. $\text{C}_{20}\text{H}_{30}\text{O}_3$ requires 318).

Hydrogenation of the dehydrogenation product from lactone (2 mg) in methanol (2 ml) over 10% palladium charcoal afforded only acidic products and no starting material or saturated lactone, as shown by t.l.c. on silica gel and on silica gel-silver nitrate.

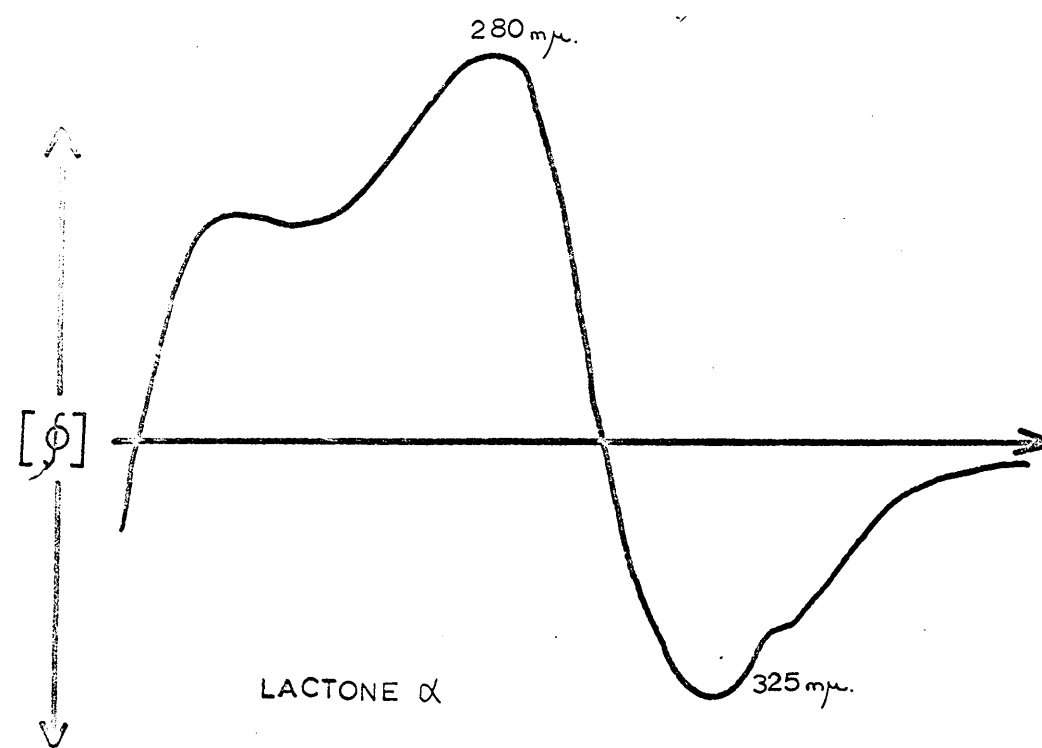
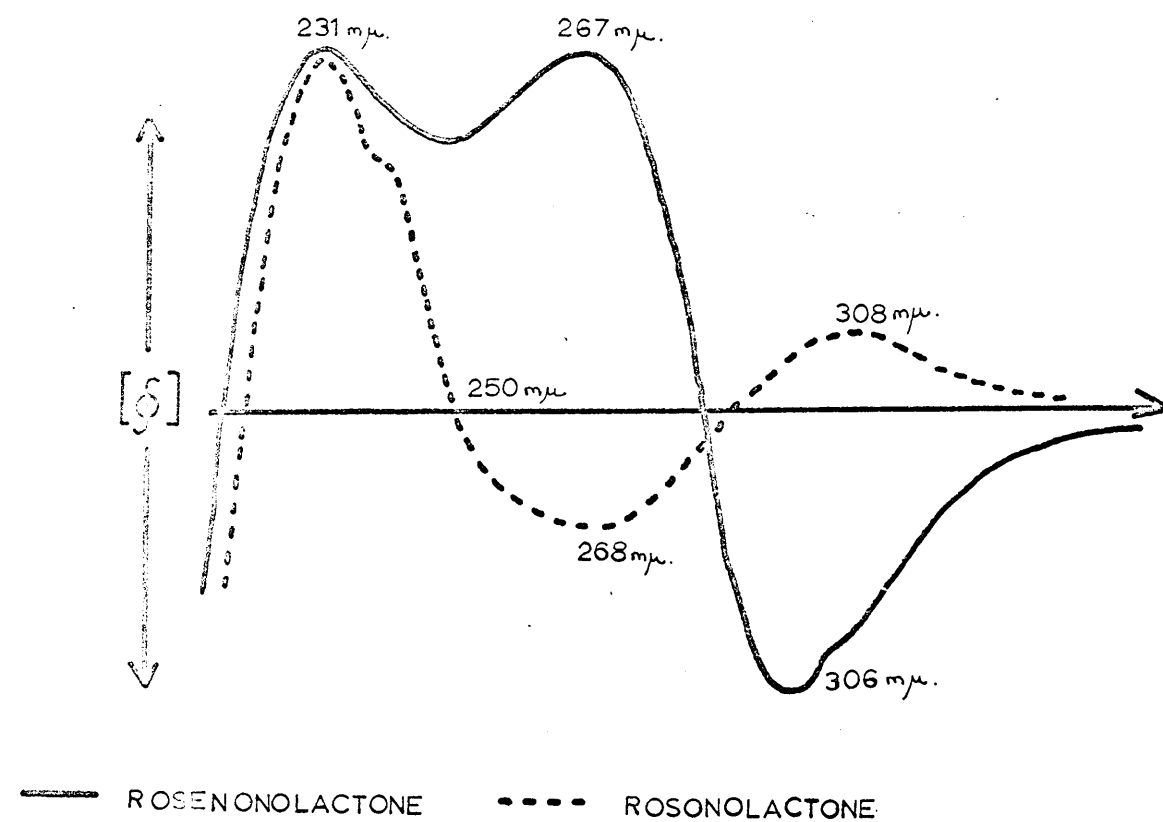
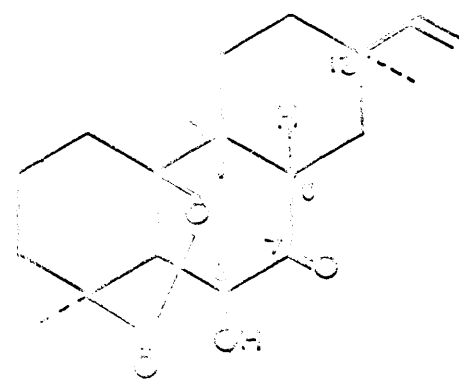
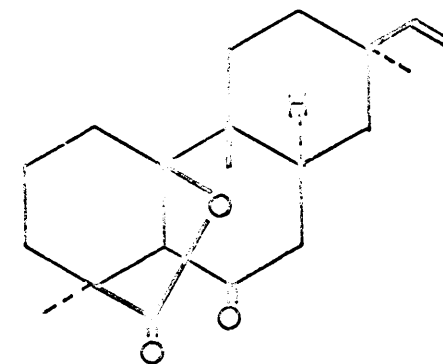


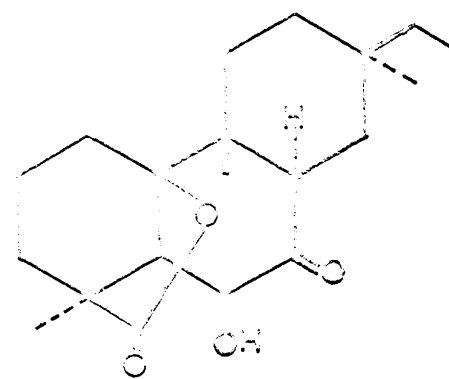
FIGURE 5



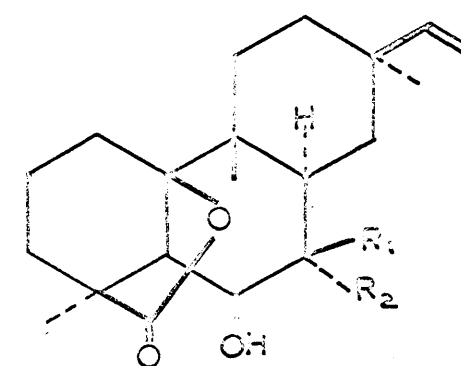
LXXX



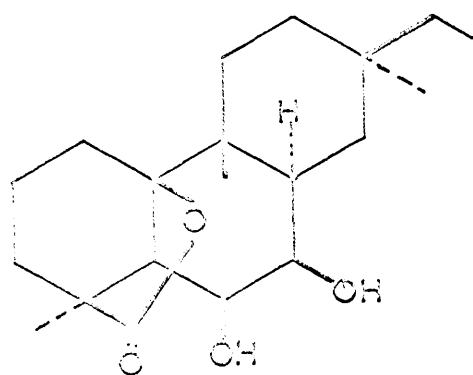
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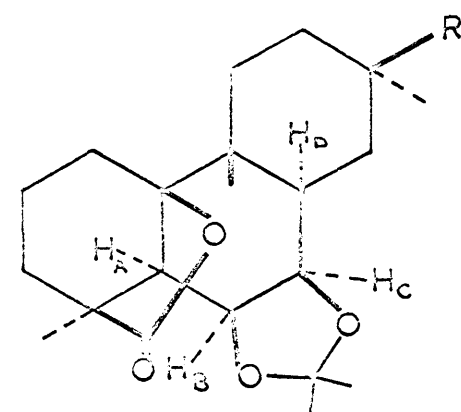
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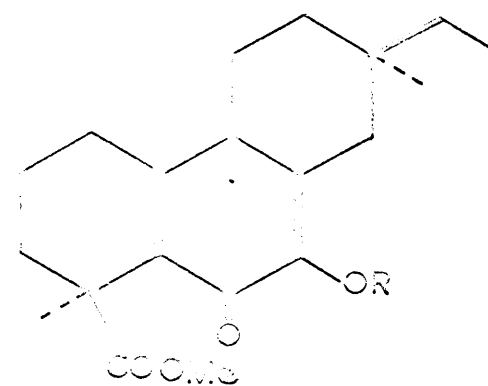
LXXXIII $R_1 = -OH$ $R_2 = -H$
LXXXIII $_{\alpha}$ $R_1 = -H$ $R_2 = -OH$



LXXXIV

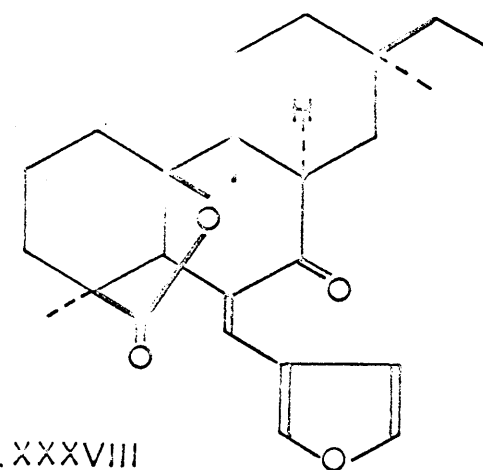


LXXXV $R_1 = -CH_2-CH_3$
LXXXV $_{\alpha}$ $R_1 = -CH=CH_2$

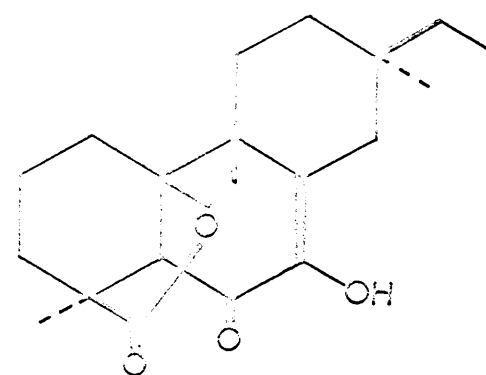


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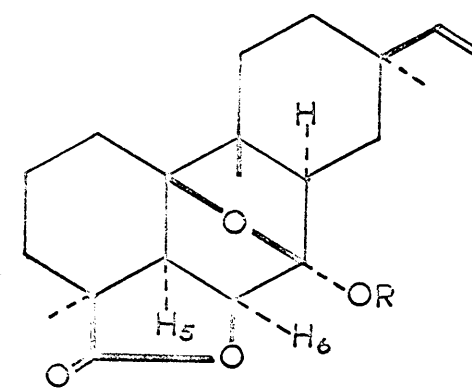
LXXXVII R = CH₃CO-



LXXXVIII

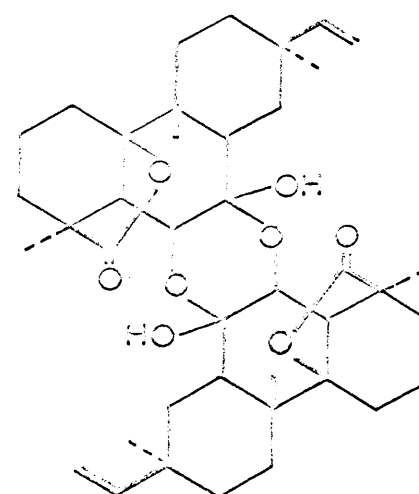


LXXXIX

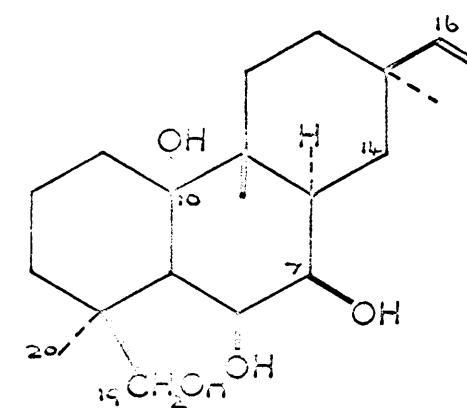


XC R = H

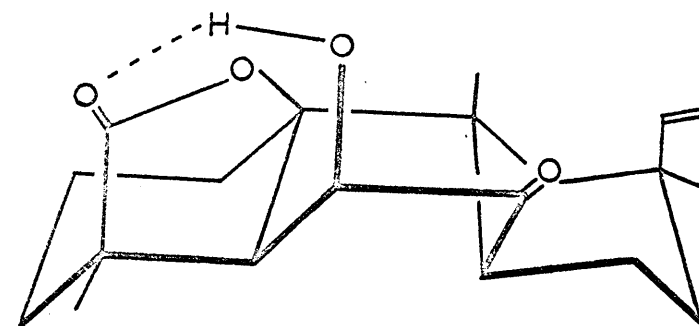
XC_α R = CH₃CO-



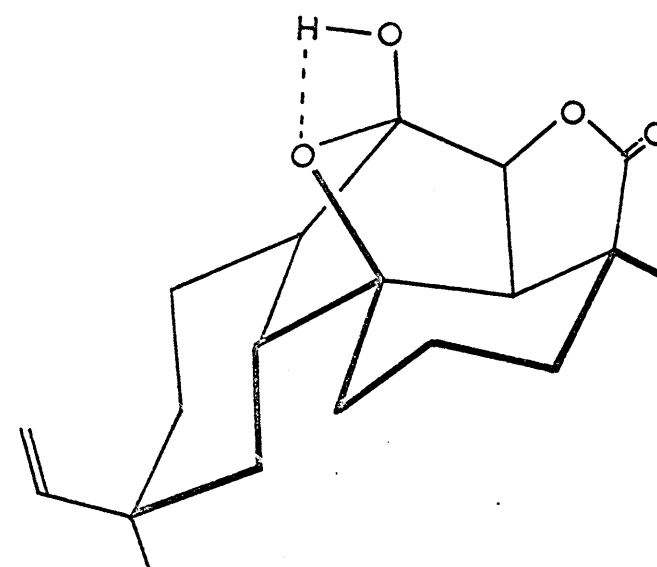
XCI



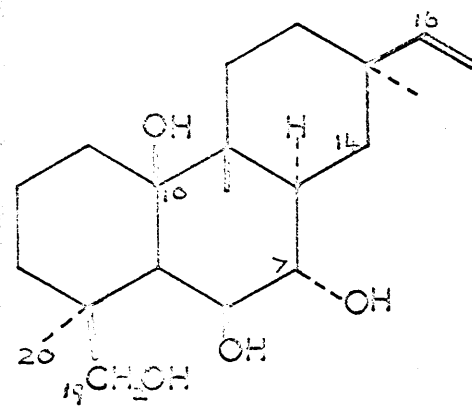
XCII



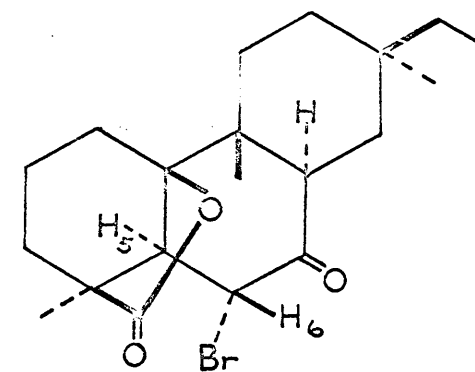
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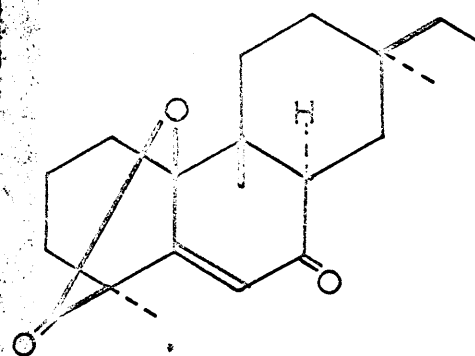
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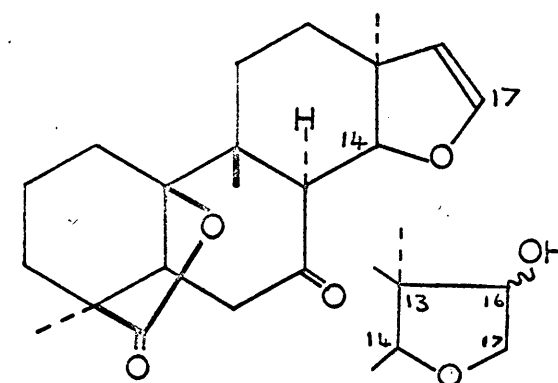
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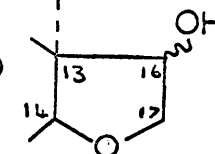
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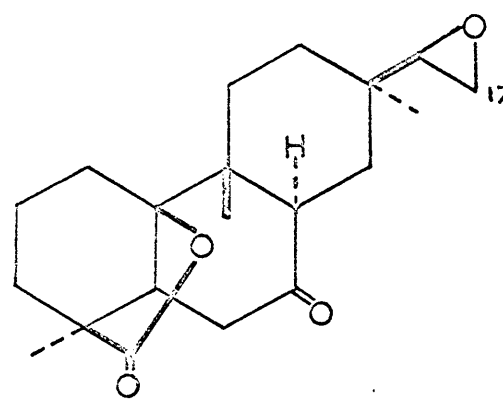
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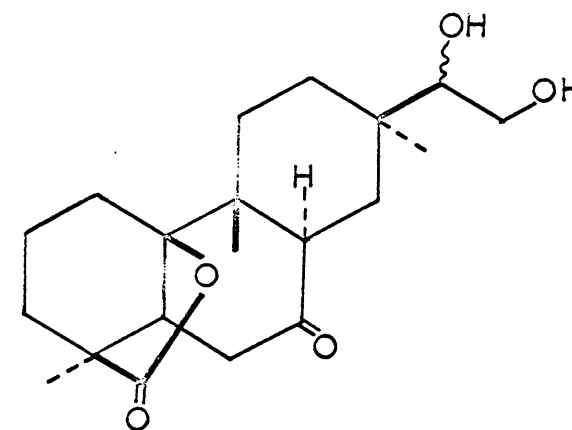
XCVI



XCVI α



XCVII



XCVIII

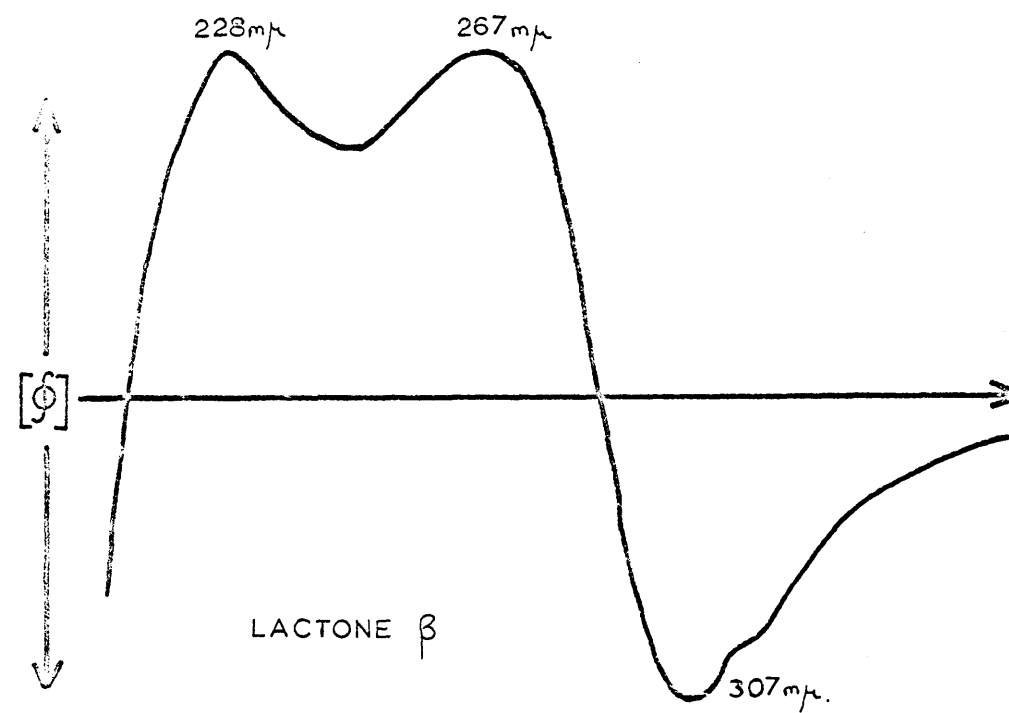
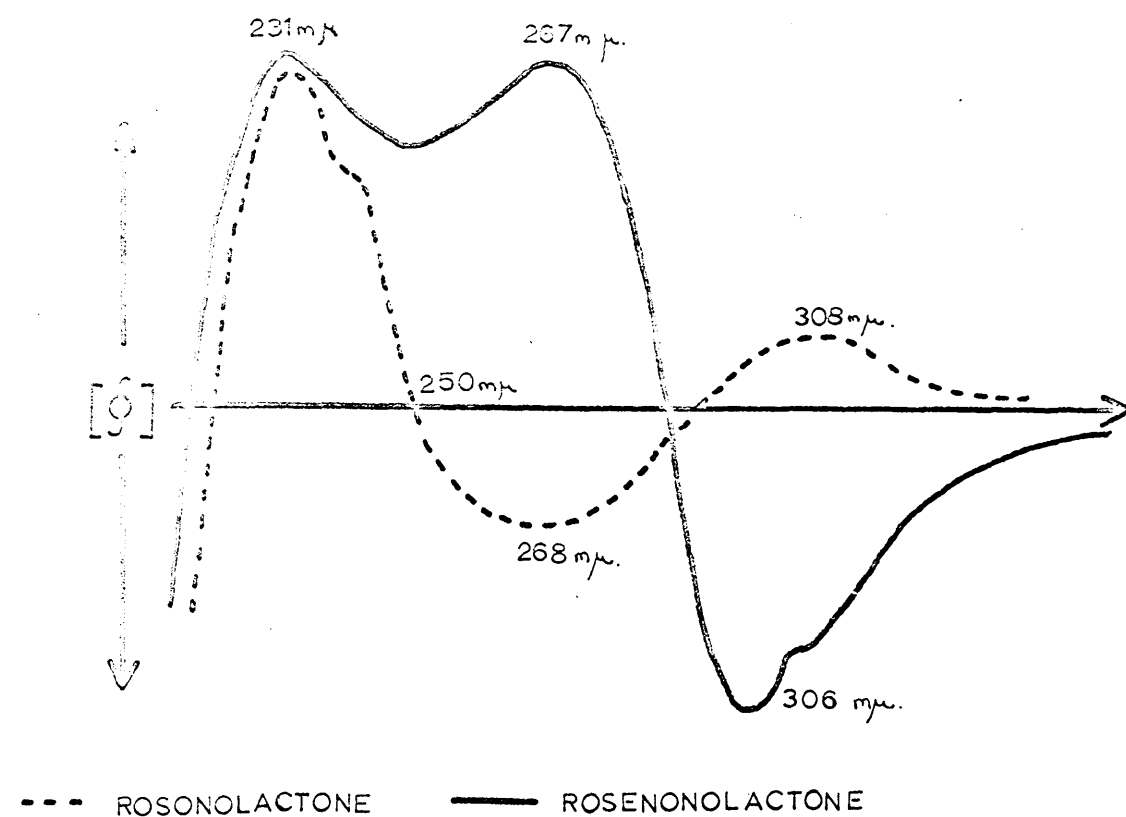


FIGURE 7

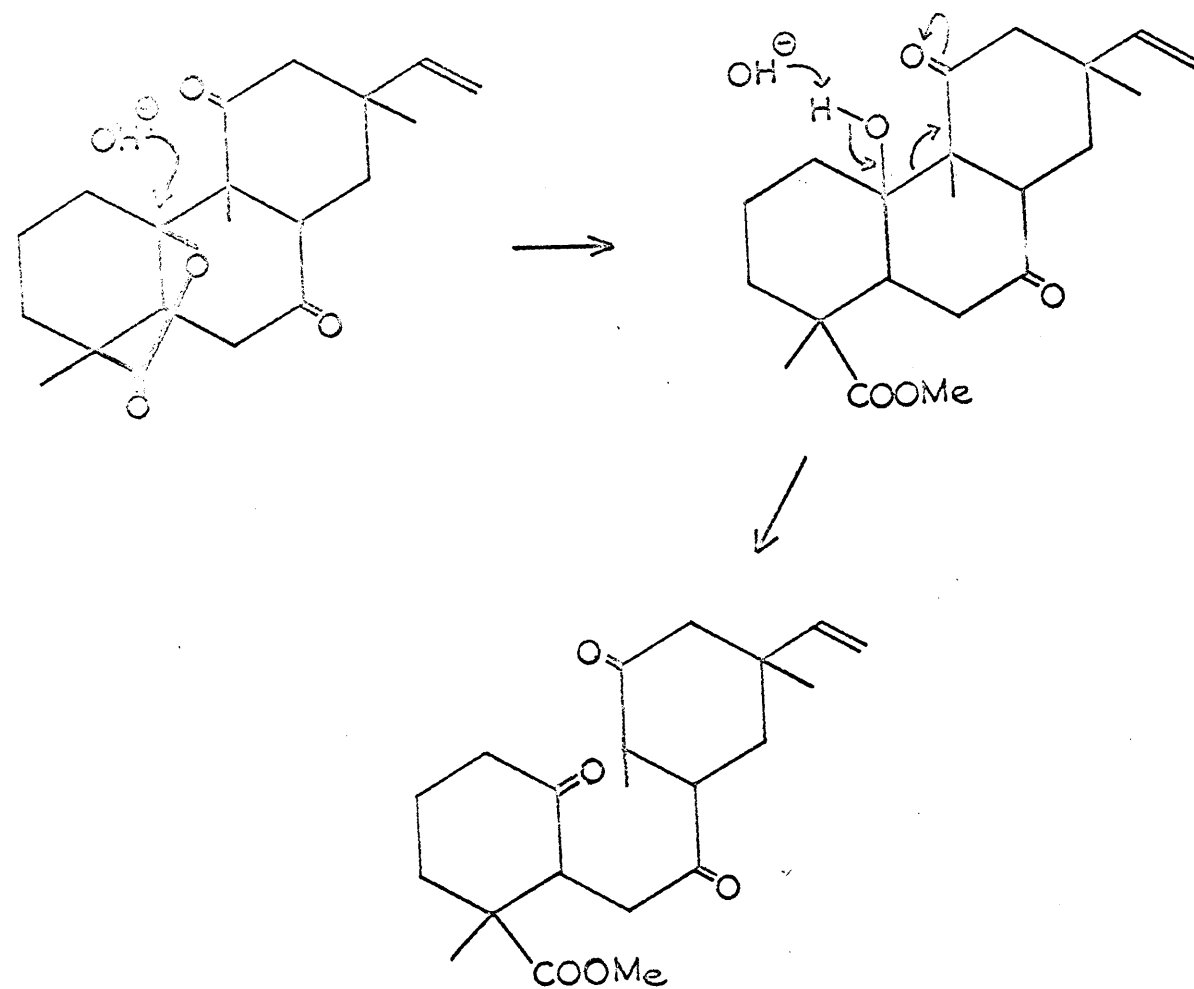
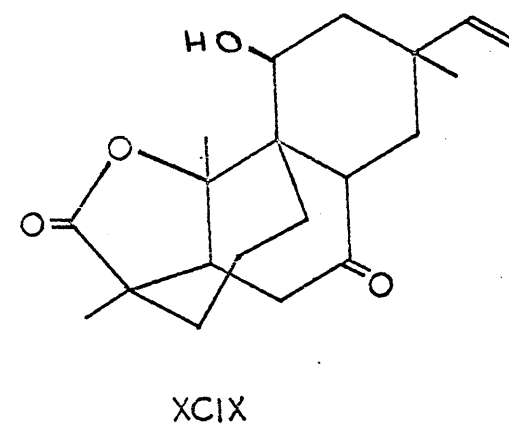


FIGURE 8



THE ERYTHROXY DIOLS

Recently, three interesting isomeric diols have been isolated from Erythrocydon monogynum, all based on the enantio-rosane skeleton (C). Diol X (CI), $C_{20}H_{34}O_2$, a completely saturated compound, was found to possess a tetra substituted cyclopropane ring by the presence in the n.m.r. spectrum of characteristic signals at high field (two 1H doublets at τ 9.46 and 9.88 ($J = 4.5$ c/sec). Initially, from the presence of this cyclopropane ring, it was considered that diol X may be related to trachylobane (CII), but this became less probable when the sequence $CMe.CHOH.CH_2OH$ was revealed. Therefore, a rearranged pimarane with a hydroxylated side chain, became a more plausible suggestion for the basic skeleton of diol X. Darutigenol (CIII) has been shown to possess this type of hydroxylated system. The other major diol, Y, (CIV), had an isolated vinylidene group as the sole element of unsaturation. This was evident spectroscopically from the infra-red, ultra-violet and n.m.r. spectra and also from the formation of a nor-ketone (CV) upon ozonolysis of Y-acetonide (CIVa).

The n.m.r. spectrum of the diacetate derived from diol Y exhibited, between τ 4.9 and 6.2, a twelve line system which was virtually superimposable on the corresponding region in the spectrum of diol X diacetate. The only difference was a slightly broadened singlet (τ 5.5, 2H),

attributable to the vinylidene group already mentioned, and superimposed on lines five and six of the twelve line spectrum. This suggested the similarity of the diol functions in the erythroxy diols X and Y, and location of the structurally dissimilar elements at points in the molecule remote from the diol functions. Like diol X, diol Y was considered to be a rearranged pimarane of structure (CIV), and two pieces of experimental evidence were found to support this theory. First ozonolysis of the diol Y acetonide (CIVa) yielded a nor-ketone acetonide (CV acetonide), in which the vinylidene group had been replaced by an oxygen with the loss of one carbon atom, and the appearance of new bands (CCl_4) at 1710 cm^{-1} (cyclohexanone) and 1420 cm^{-1} ($-\text{CH}_2-$ next to $-\text{CO}-$) in the infra-red spectrum. Secondly, isomerisation under acidic conditions of diol Y acetonide, led, after replacement of the acetonide function, to the acetonide of the naturally occurring diol Z, ((CIV), $\Delta^{3(4)}$ instead of $\Delta^{4(17)}$; 2H signal at T5.5 replaced by 1H signal at T4.85 (vinyl H).) From biogenetic considerations and the experimental results, diol Y either possessed the structure (CIV) or its antipode, although the structure (CVI), (or its antipode) had to be considered as a possible alternative.

The close structural relationship between diols X and Y was further emphasised by the following results. Reaction of either acetonide X or Y, with a saturated solution of hydrogen chloride in dry chloroform

at 20°C for 30 minutes, and replacement of the acetonide grouping led in each case, to a mixture of at least four products, containing two common constituents, as assessed by gas-liquid chromatography and confirmed by isolation. These major products were shown to be 1. diol Z acetonide and 11. the acetonide of a new diol (CVIIa), considered to have this structure because of the absence of olefinic protons in the n.m.r., but giving a positive tetranitromethane test. An alternative structure (CVIII) had to be considered for this rearrangement product, but integration of the appropriate region in the n.m.r. spectrum (T7.8 - T8.3) indicated that there were four and not six allylic protons and therefore structure (CVII) was more plausible. A direct comparison of the diene, derived from the diol (CVII) or (CVIII) by the Corey reaction⁽⁵⁶⁾, with the diene (CIX) obtained by acid isomerisation of pimaradiene⁽⁵⁷⁾, showed that the two dienes differed in their gas chromatographic properties and this was strong evidence in favour of structure (CVII). Final confirmation, that this was a correct structural assignment was obtained by synthesis of the diene (CX) from rosenonolactone (XLVII), whose absolute stereochemistry has been established. This diene (CX) was found to be identical in all respects, except for the sign of rotation, with the diene from the diol (CVII). This direct correlation, a part of which will be discussed more fully in this thesis, defines the

structure and absolute stereochemistry of diol Y as (CIV).

Two alternative structures were possible for diol X on the basis of the acid isomerisation products, namely (CI) or (CXI). Initially, definite evidence could not be found to distinguish between them, although structure (CI) was more strongly favoured for two reasons. First, if the cyclopropane ring were attached as in (CXI), on acid cleavage, the C₉ carbonium ion would be expected to participate to a certain extent, resulting in the formation of (CVIII). No evidence for this was observed. Secondly, the mass spectra of the acetonides X and Y were indistinguishable. Although this area of mass spectrometry is not well documented, a direct analogy was drawn with cycloartanyl and lanost-8(9)-enyl acetates, whose fragmentation patterns are almost identical.

The position of the cyclopropane ring in diol X has now been established by direct correlation with another component of E₁ monogynum, the triol Q (CXII). The position of the cyclopropane ring in triol Q (CXII) has been defined unambiguously by X-ray analysis,⁽⁵⁸⁾ and is located at positions 4-5.

Oxidation of triol Q acetone (CXIIa) and thereafter Wolff Kischner reduction, transformed it into diol X acetone⁽⁵⁹⁾ (CIa). Thus, the structure of diol X has now been confirmed as (CI).

Another compound isolated from the trunk wood of E.monogynum and closely related to triol Q, is triol P (CXIII), so assigned because of its behaviour on dehydration. Exposure of (CXIV) to thionyl chloride afforded two olefinic acetonides, (CXV), and the previously obtained (CVIIa). Assignment of the double bond in (CXV) to position 5-6 rather than 1-10 rested on its close resemblance to rimuene (XXIII), whose structure has now been established by the conversion of it and diol Y (CIV) respectively into the nor-hydrocarbons (CXVI) and its antipode.⁽²⁴⁾ The structure of rimuene has been confirmed by total synthesis.⁽⁶⁰⁾

Kitahara and his colleagues⁽⁶¹⁾ have recently isolated from Thuopsis dolabrata, the hydrocarbon dolabradiene (CXVII), possessing the same carbon skeleton as the tricyclic diterpenoids from E. monogynum, but of antipodal configuration, except at C₁₃. This has been confirmed by a direct correlation of dolabradiene (CXVII) with erythroxy diol Y (CIV).⁽⁶²⁾

DISCUSSION

Confirmation that the erythroxy diols X, Y and Z were antipodally related to the rosane skeleton was obtained by conversion of rosenonolactone (XLVII) and the ene-diol (CVII), respectively into the diene (CX) and its antipode.

Reduction of rosenonolactone (XLVII) with lithium aluminium hydride in tetrahydrofuran yielded an oily triol (CXVIII), characterised as its crystalline dihydro derivative, $C_{20}H_{36}O_3$, m.p. $140-141^{\circ}C$, $[\alpha]_D + 105^{\circ}$. Reaction of the unsaturated triol with 1.5 molar equivalents of toluene-p-sulphonyl chloride afforded a mixture of the ether toluene-p-sulphonate (CXIX) and the corresponding alcohol (CXX). A five molar excess of toluene-p-sulphonyl chloride yielded only one product (CXIX), as seen by t.l.c. and confirmed by the n.m.r. spectrum, which possessed two doublets centred at $\tau 2.35$ (2H) and 2.9 (2H) and a singlet $\tau 7.6$ (3H), all attributed to the toluene-p-sulphonate residue. Attempts to purify this or the mixture of ether toluene-p-sulphonate (CXIX) and the alcohol (CXX) by chromatography, resulted in the elimination of the toluene-p-sulphonate group to yield the $\Delta^{7(8)}$ -ether (CXXI). Support for this structure was provided by the infra-red spectrum ($CHCl_3$) which had bands at 1040 cm^{-1} (ether) and 1640 and 910 cm^{-1} (olefinic double bond) and a one proton multiplet at $\tau 4.75$ in

the n.m.r., spectrum, in addition to those absorptions seen in the ether (CXXII). The mixture of ether toluene-p-sulphonate (CXIX) and the alcohol (CXX) were therefore reduced with lithium aluminium hydride in tetrahydrofuran and the resulting 7-deoxy-ether (CXXII) separated from the 7-hydroxy-ether (CXX) by chromatography. The 7-deoxy-ether (CXXII), characterised as the dihydro derivative,

$C_{20}H_{34}O$, absorbed in the infra-red ($CHCl_3$) at 1640, 1040 and 910 cm^{-1} ,

and in the n.m.r. spectrum, the ether protons appeared as a singlet

at $\tau 6.25$ (2H). The 7-hydroxy-ether (CXX) had a band ($CHCl_3$) at

3600 cm^{-1} (hydroxyl) and the proton ($\sim CH-OH$) at C_7 was seen as a

multiplet ($\tau 6.05$, 1H) in the n.m.r., in addition to the ether protons,

($\tau 6.4$, s, 2H). This ether-alcohol (CXX) was characterised as the

p-nitro benzoate, $C_{27}H_{35}NO_5$ m.p. $157-158^\circ C$, $[\alpha]_D^{+20} 0^\circ$. Exposure

of the ether (CXXII) to ethanolic hydrogen chloride at $20^\circ C$, readily

transformed it into the previously reported crystalline dienol⁽⁵⁴⁾ (CXXIII),

$C_{20}H_{32}O$, m.p. $95^\circ C$, $[\alpha]_D^{+58} 0^\circ$, further characterised as the

crystalline p-nitro benzoate, $C_{27}H_{35}O_4N$, m.p. $158-160^\circ C$, $[\alpha]_D^{-18} 0^\circ$.

Infra-red absorption ($CHCl_3$) at 3600 cm^{-1} (hydroxyl) and 1640 cm^{-1}

(vinylidene group), an AB quartet at $\tau 6.48$ and 6.80 ($J = 12\text{ c/sec}$)

($-CH_2 - OH$) and no vinyl protons in addition to those of the ether (CXXII),

confirmed the structure (CXXIII) for the dienol.

The conversion of this primary alcohol into the corresponding hydrocarbon (CX) proved to be a matter of some difficulty. The route eventually followed, despite the low yield, was the oxidation of the alcohol (CXXIII) with chromic anhydride in acetic acid at 0°C, to a mixture of acidic and neutral products. From this mixture the unstable aldehyde (CXXIV) was separated as the least polar component, by rapid chromatography. A one proton singlet at T0.7 denoted the presence of the aldehyde group, as did absorption in the infra-red (CHCl_3) at 1730 cm^{-1} . The aldehyde (CXXIV) was immediately converted to the more stable thioketal by reaction with ethane dithiol and boron trifluoride etherate, and the n.m.r. spectrum of this compound (CXXV) showed a singlet at T6.84 (4H) assigned to the thiol group. Desulphurisation with Raney nickel in refluxing acetone gave the desired hydrocarbon (CX), $[\alpha]_{\text{D}}^{-117^\circ}$, as the sole product of the reaction.

Other methods were tried in an attempt to obtain the hydrocarbon (CX) in better yield than that afforded by the route just described. The use of Sarrett and Jones reagents in varying proportions of oxidant, concentration and temperature, failed to produce the desired aldehyde, only more polar products (t.l.c.) than the alcohol (CXXIII) being obtained. The attempted use of lead tetra-acetate⁽⁶³⁾ in pyridine also proved unsuccessful as an oxidising agent. Preparation of the chloroformate with phosgene and subsequent decomposition of this with triethyl amine

and dimethyl sulphoxide⁽⁶⁴⁾ produced only the unchanged alcohol (CXXIII), instead of the desired aldehyde (CXXIV). Formation of the toluene-p-sulphonate of the alcohol (CXXIII) and thereafter reduction with lithium aluminium hydride in ether yielded two hydrocarbons (possibly formed by rearrangement), which failed to separate by chromatography over silica gel-silver nitrate. All efforts to prepare the benzyl thioether⁽⁶⁵⁾ of the alcohol (CXXIII), prior to its reduction with Raney nickel, failed.

The diol (CVII) obtained from either diols X or Y upon acid isomerisation, was transformed into the hydrocarbon (CXXVI) by its (CVII) conversion into the thiocarbonate⁽⁵⁶⁾ and prolonged exposure of this to boiling triethyl phosphite. This diene (CXXVI), $[\alpha]_D + 112^\circ$, was identical in its i.r. u.v., n.m.r., and mass spectra and in its behaviour on thin-layer chromatography and g.l.c. (10% Apiezon L; 1% CHDMS; 10% PEGA.), but of opposite rotation when compared with the diene (CX), $[\alpha]_D - 117^\circ$, from rosenonolactone. Thus, the erythroxy diols X, Y and Z are related antipodally to rosenonolactone and this defines their stereochemistry at C₈ and C₁₃, since the absolute configuration of rosenonolactone has been established at these centres.

By defining the structure of the ene-diol (CVII), one of the acid isomerisation products from diol Y, the constitution and absolute

stereochemistry of diol Y has been placed beyond all doubt, except for C₁₆ which remains undefined.

EXPERIMENTAL

Reduction of Rosenonolactone with Lithium Aluminium Hydride

Rosenonolactone (XLVII) (700 mg) in dry tetrahydrofuran (50 ml) was refluxed with lithium aluminium hydride (400 mg) for 2 hours. The solution was cooled and a saturated solution of sodium sulphate added dropwise, until the excess lithium aluminium hydride was destroyed. The suspension was filtered and the solution, on evaporation, yielded the triol (CXVIII), an oil (650 mg). $\nu_{\text{max}}^{\text{CHCl}_3}$ 3500, 1640 cm^{-1} .


Hydrogenation of the Triol (CXVIII)

The triol (CXVIII) (30 mg) was hydrogenated in methanol (5 ml) with 10% palladium charcoal as catalyst. The derived dihydrotriol crystallised from pentane, m.p. 140-141°C; $[\alpha]_D + 105^\circ$, (c = 0.8, chloroform); $\nu_{\text{max}}^{\text{CHCl}_3}$ 3500 cm^{-1} . (Found: C, 74.3; H, 10.9. $\text{C}_{20}\text{H}_{36}\text{O}_3$ requires C, 74.0; H, 11.2%).

Tosylation of the Triol (CXVIII)

Toluene-p-sulphonyl chloride (1 gm) was added to the triol (CXVIII) (600 mg) in dry redistilled pyridine (3 ml) and left at 20°C for 12 hours. Water was added and the oily precipitate filtered, washed well with water, and then taken up in ether. The ethereal solution was washed with water, dried and the solvent removed in vacuo.

T.l.c. using ethyl acetate: light petroleum (3 : 7) as eluant showed that the desired ether toluene-p-sulphonate (CXIX) had been formed as the major product and the corresponding alcohol (CXX) in minor amount. Separation of these two components by column or preparative layer chromatography proved unsuccessful, the toluene-p-sulphonate being, under these conditions, transformed into one less polar (t.l.c.) product, the unsaturated ether (CXXI). $\nu_{\text{max}}^{\text{CHCl}_3}$ 1640, 1020, 910 cm^{-1} . ^1H multiplet at τ 4.7, in addition to those present in the ether (CXXII).

A large excess (5 molar) of toluene-p-sulphonyl chloride was added to the mixture (CXIX) and (CXX) (20 mgs), obtained as described above, in pyridine (1 ml). The solution was left at 20°C for 48 hours and worked up as described previously. One product, the toluene-p-sulphonate (CXIX) (15 mg) was obtained, (t.l.c.), which failed to crystallise. $\nu_{\text{max}}^{\text{CHCl}_3}$ 1600, 1180, 1120, 1040 910 cm^{-1} . ^2H doublets at τ 2.25 and 2.7 (aromatic protons); ^3H singlet at τ 7.58 (CH_3 -). .

Elimination of the Tosylate group

The mixture of ether toluene-p-sulphonate (CXIX) and the corresponding alcohol (CXX) (550 mg) were refluxed for 12 hours, in dry redistilled tetrahydrofuran (100 ml) with lithium aluminium hydride (600 mg) as a reducing agent. Work up as previously described, yielded an oil (490 mg), containing two components as seen from t.l.c. which

were separated by column chromatography (Alumina Grade III).

Elution with light petroleum gave first, the oily ether (CXXII)

(340 mg), which failed to crystallise. $U_{\max}^{\text{CHCl}_3}$ 1640, 1040, 910 cm^{-1} .

Signals in the n.m.r. at τ 6.25 (2H, s); 9.03 (3H, s); 9.08 (3H, s);

9.12 (3H, s). (Mass spec. m.wt. 288. $\text{C}_{20}\text{H}_{32}\text{O}$ requires 288).

Continued elution with light petroleum then gave the hydroxy-

ether (CXX), (60 mg). $U_{\max}^{\text{CHCl}_3}$ 3600, 1640, 1040, 910 cm^{-1} .

Dihydro ether (LXVIII)

Reduction of the ether (CXXII) (40 mg) in ethanol (5 ml) over

10% palladium charcoal yielded the dihydro-ether (LXVIII), (30 mg),

an oil. $U_{\max}^{\text{CHCl}_3}$ 1040, 910 cm^{-1} . 2H singlet in the n.m.r. at τ 6.25

(- CH_2 - O -). (Found: C, 82.7; H, 11.7. $\text{C}_{20}\text{H}_{34}\text{O}$ requires

C, 82.7; H, 11.8%).

p-Nitrobenzoate of the Hydroxy-ether (CXX)

To the hydroxy-ether (CXX) (20 mg) in dry pyridine (1 ml) was

added a solution of p-nitrobenzoyl chloride (15 mg) in benzene (1.5 ml),

and the solution left at 20°C for 12 hours. Work up by addition of

water and extraction into ether, afforded the p-nitrobenzoate of (CXX),

which crystallised from methanol - light petroleum m.p. 157-158°C;

$[\alpha]_D^{20} + 0^\circ$, (c = 0.33, chloroform); $U_{\max}^{\text{CHCl}_3}$ 1710, 1610, 1520 cm^{-1} .

(Found: C, 71.5; H, 7.8. $\text{C}_{27}\text{H}_{35}\text{O}_5\text{N}$ requires C, 71.5; H, 7.8%).

Conversion of the Ether (CXXII) to the unsaturated alcohol (CXXIII)

The ether (400 mg) was refluxed for 90 mins. in ethanol (15 ml) and hydrochloric acid (1.5 ml). Removal of the ethanol in vacuo, dilution with water, and extraction into ether, yielded a white solid (360 mg), shown to be mainly one product by t.l.c. Chromatography over alumina (II) furnished, on elution with light petroleum, the desired unsaturated alcohol (CXXIII) (350 mg). Sublimation at $95^{\circ}/0.1$ mm. afforded a white crystalline compound, m.p. 95°C . (lit. value⁽⁵⁴⁾ $106-108^{\circ}\text{C}$). $U_{\text{max}}^{\text{nujol}}$ 3600, 1640 cm^{-1} ; AB quartet at T6.48 and T6.80 ($J = 12$ c/sec) ($-\text{CH}_2 - \text{OH}$); no vinyl protons in addition to those in the ether (CXXII).

p-Nitrobenzoate of the Unsaturated Alcohol (CXXIII)

The alcohol (CXXIII) (20 mg) in dry pyridine (1 ml) was left at 20°C for 12 hours with p-nitrobenzoyl chloride (20 mg). Water was added and the aqueous layer extracted into ether to afford the desired p-nitrobenzoate, which crystallised from light petroleum m.p. $158-160^{\circ}\text{C}$, $[\alpha]_{\text{D}} -18^{\circ}$, ($c = 0.2$, chloroform). (Found C, 74.0; H, 8.2. $\text{C}_{27}\text{H}_{35}\text{O}_4\text{N}$ requires C, 74.1; H, 8.1%).

Oxidation of the Alcohol (CXXIII) to the Aldehyde (CXXIV)

The unsaturated alcohol (CXXIII) (240 mg) and chromium trioxide (80 mg) were kept in "analar" acetic acid (6 ml) at 0°C for 1.5 hours. The reaction product (220 mg), obtained by dilution with water and extraction into ether contained, in addition to the required aldehyde, at least four more polar components (t.l.c.). The aldehyde (CXXIV) (24 mg) which separated cleanly (t.l.c.) by elution from silica-gel with benzene: light petroleum (1 : 4), was stable in nitrogen at 0°C.

$\nu_{\text{max}}^{\text{CHCl}_3}$ 1730 cm^{-1} (no band at 3600 cm^{-1}); 1H singlet at T0.59, (-CHO).

Thioketal (CXXV) of the Aldehyde (CXXIV).

The aldehyde (21 mg) was kept in dry ether (2 ml) at 20°C for 5 hours, together with boron trifluoride etherate (0.2 ml) and ethane dithiol (0.2 ml). Dilution with 5% aqueous sodium hydroxide and extraction into ether afforded the thioketal (CXXV) (17 mg) which was purified by p.t.l.c., yielding a colourless oil (14 mg), $[\alpha]_D^{+20}$, ($n_D^{20} = 1.05$, chloroform); 4H singlet at T6.81 ($\text{CH}_2 - \text{S} -$)₂.

Reduction of the Thioketal (CXXV) with Raney Nickel

Raney nickel (200 mg) was deactivated by refluxing it in "analar" acetone for 2 hours. The thioketal (CXXV) in "analar" acetone (1 ml) was added and refluxing continued for a further 2 hours.

Filtration of the solution through cellulose powder, removal of the solvent and p.t.l.c. of the crude product with light petroleum as eluant, afforded the desired diene (CX) (7 mg), Found to be homogeneous by t.l.c. on silica-gel and silver nitrate - silica gel. $[\alpha]_D -117^\circ$, ($c = 1.98$ chloroform); $U_{\text{max}}^{\text{CHCl}_3}$ 1640, 1000, 915 cm^{-1} ; n.m.r. methyl signals at τ 9.15 (3H), 9.03 (3H), 8.96 (3H). (Found; mass spec. m.wt. 272.2539. $\text{C}_{20}\text{H}_{32}$ requires 272.2504). The i.r. and n.m.r. properties of this diene are indistinguishable from those of the diene (CXXVI) derived from diol Y (CIV).

Oxidation of the Alcohol (CXXIII)

1. Use of Sarrett or Jones reagents in varying proportions of oxidant, concentration and temperature proved unsuccessful, only more polar products than the alcohol (CXXIII) being obtained as seen by t.l.c.
2. The use of lead tetra-acetate⁽⁶³⁾ as an oxidising agent also failed to produce the required aldehyde.

Attempted preparation of the Aldehyde (CXXIV) via the Chloroformate⁽⁶⁴⁾

The alcohol (CXXIII) (30 mg) in dry ether, was treated with an ethereal solution of phosgene (0.15 ml) at room temperature for 2 hours⁽⁶⁴⁾. The reaction was studied by running i.r. spectra throughout, the

disappearance of the -OH absorption at 3600 cm^{-1} and the appearance of the chloroformate absorption at 1780 cm^{-1} indicating the completion of the reaction. On removal of the solvent, redistilled dimethyl sulphoxide (0.5 ml) was added and the reaction maintained at 20°C for 15 mins with stirring. Dry triethyl amine (0.1 ml) was added with cooling and the stirring continued for 20 mins. at 20°C . Water was added and the aqueous layer extracted into ether. The isolated product was found to be identical with that of the starting material (CXXIII). Variations in temperature, duration of each stage and alterations in concentration, failed to produce the desired aldehyde (CXXIV).

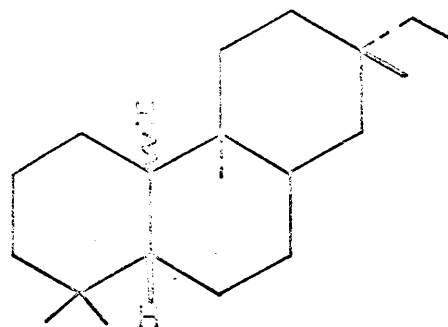
Attempted preparation of the Diene (CX) via the Toluene-p-Sulphonate

The tosylate of the alcohol (CXXIII) was prepared by dissolution of the alcohol (28 mg) in dry pyridine (1 ml) and addition of toluene-p-sulphonyl chloride (30 mg). The reaction was left at room temperature for 12 hours and work up as previously described, afforded the required tosylate (25 mg), $\nu_{\text{max}}^{\text{CHCl}_3}$ 1610, 1180, 1120 cm^{-1} . Sodium benzyl mercaptide⁽⁶⁵⁾ prepared from benzyl mercaptan (0.2 ml) and sodium (2 mg), was taken up in dimethyl formamide (0.2 ml) and a solution of the above tosylate in dimethyl formamide added. The reaction was kept at 100°C for 1 hour under nitrogen and worked up by the addition of 5% aqueous sodium hydroxide and extraction into ether. The ether

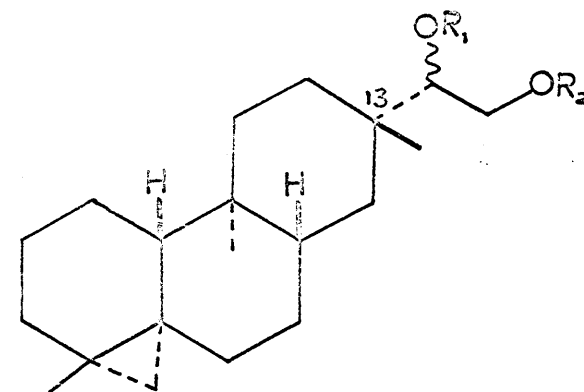
extract was washed well with water, dried and the solvent removed. T.l.c. showed that the desired thioether had not been formed and that the tosylate of the alcohol (CXXIII) was unstable to the conditions used.

Attempted conversion of the Alcohol (CXXIII) directly
to the Diene (CX)

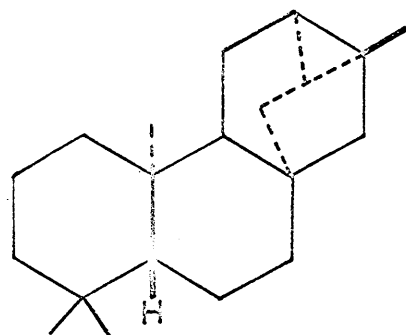
The tosylate of the alcohol (CXXIII) (60 mg) was prepared as described above and this was refluxed with lithium aluminium hydride in dry ether for 5 hours. The reaction product, obtained in the usual manner, yielded a clear oil (40 mg), which was shown to be two products by t.l.c. over silica gel - silver nitrate. Efforts to separate the hydrocarbons by p.t.l.c. over silica gel - silver nitrate failed.



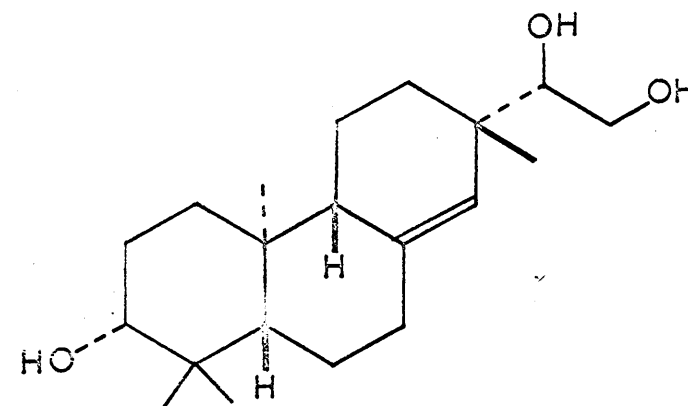
C



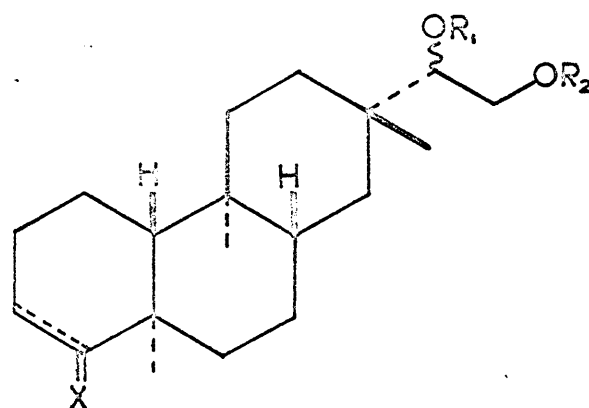
CI $R_1=R_2=H$
 CI α $R_1+R_2=>C(CH_3)_2$



CII



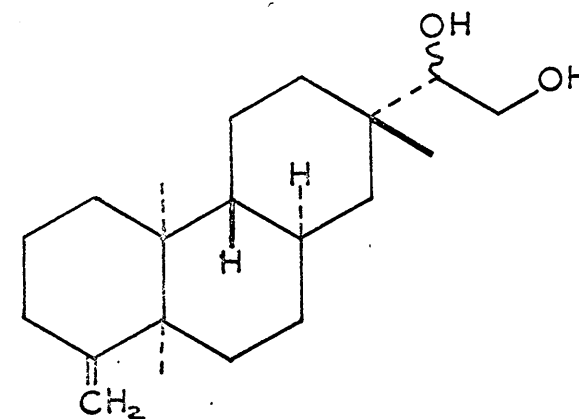
CIII



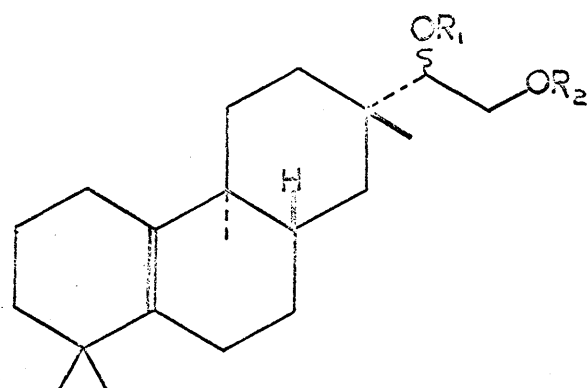
CIV. $X=CH_2, R_1=R_2=H$

CIV α $X=CH_2, R_1+R_2=>C(CH_3)_2$

CV $X=O, R_1=R_2=H$

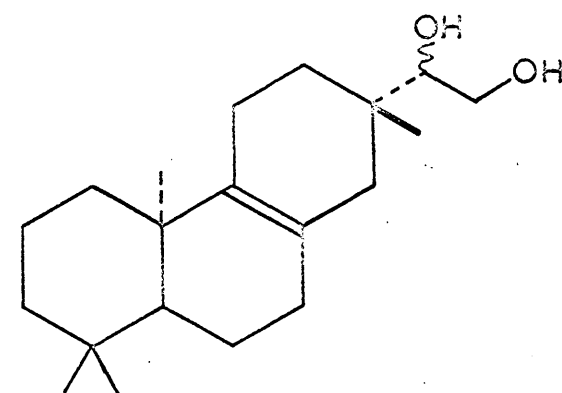


CVI

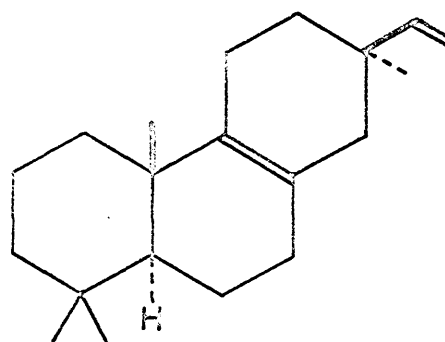


CVII $R_1 = R_2 = H$

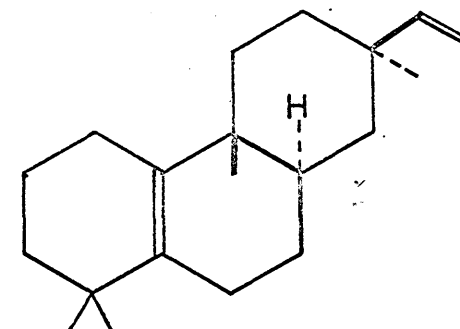
CVII α $R_1 + R_2 = C(CH_3)_2$



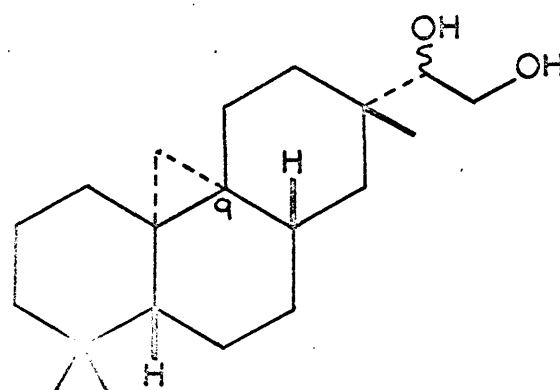
CVIII



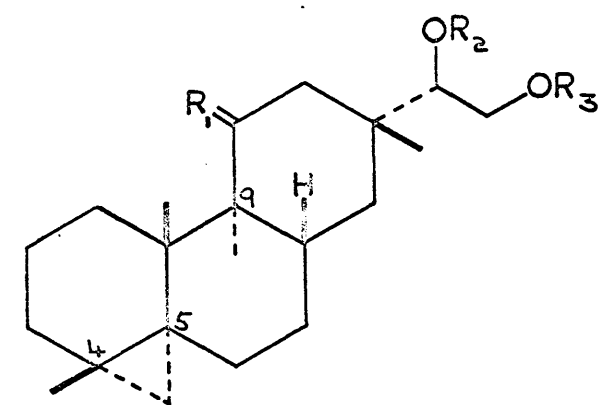
CIX



CX

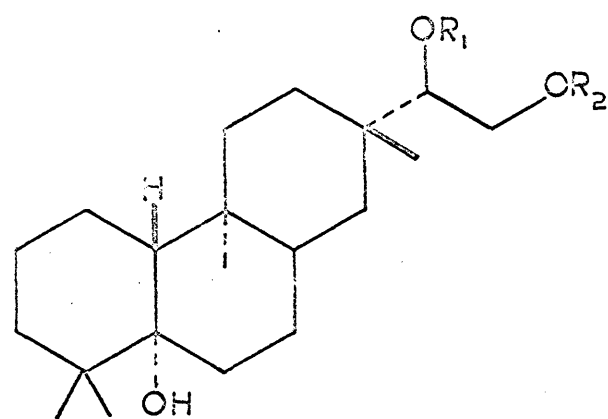


CXI



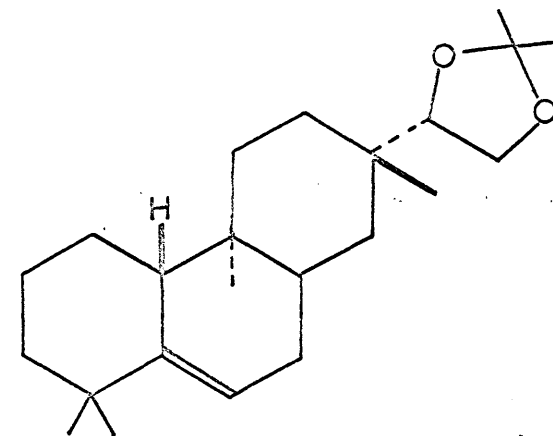
CXII $R_1 = \alpha OH, H. R_2 = R_3 = H$

CXII α $R_1 = \alpha OH, H. R_2 + R_3 = C(CH_3)_2$

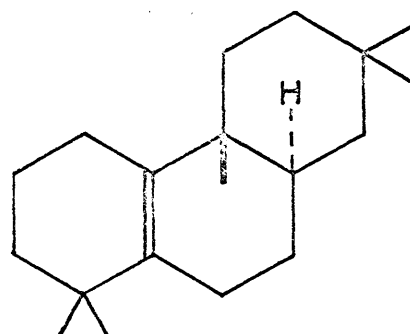


CXIII $R_1 = R_2 = H$

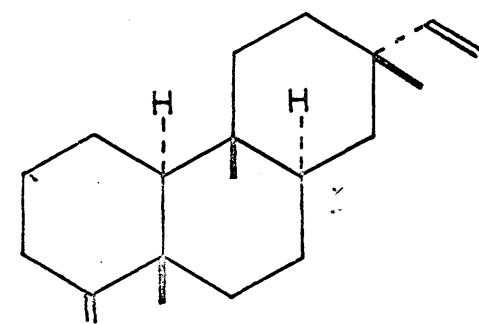
CXIV $R_1 + R_2 = C(CH_3)_2$



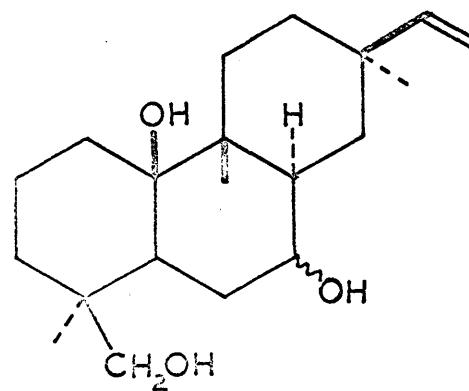
CXV



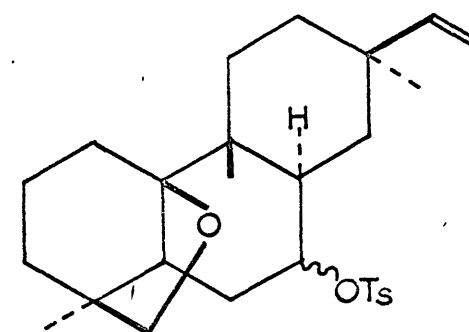
CXVI



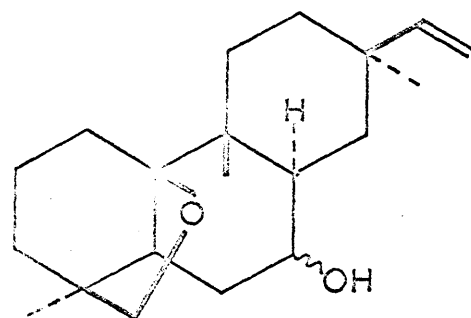
CXVII



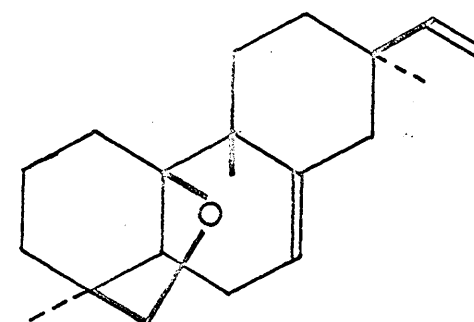
CXVIII



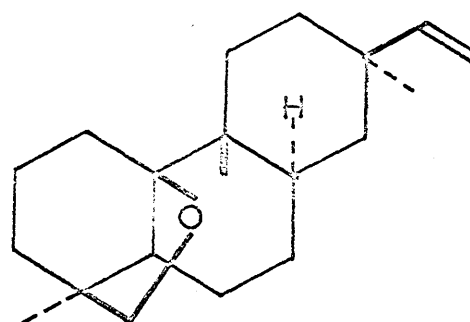
CXIX



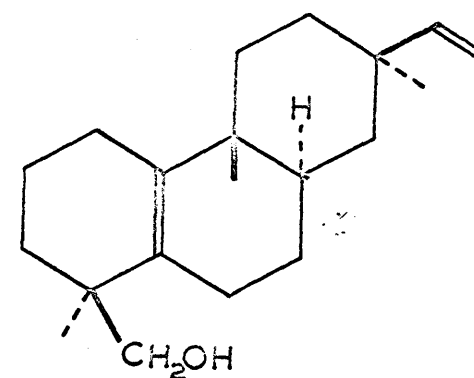
CXX



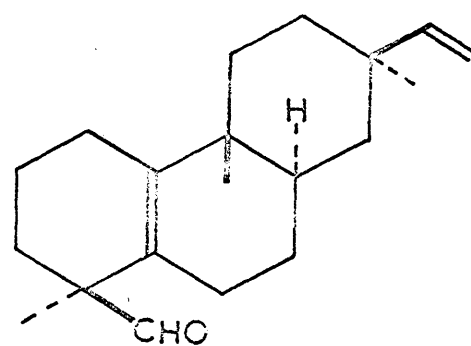
CXXI



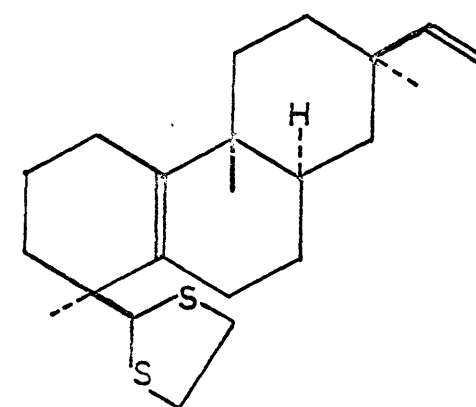
CXXII



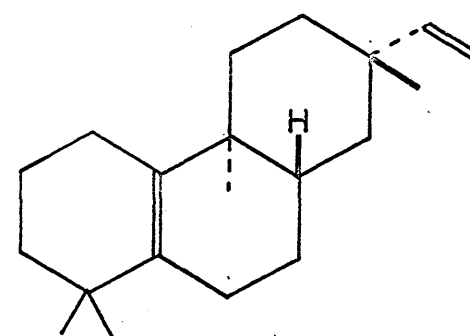
CXXIII



CXXIV



CXXV



CXXVI

EPERUIC AND LABDANOLIC ACIDS

Cocker and Halsall⁽⁶⁷⁾ established the structure of labdanolic acid as (CXXVII) and assigned the stereochemistry at positions C₅, C₉, and C₁₀ by degradation of the acid to the C-17 acid, identical with the acid (CXXIX)⁽⁶⁸⁾ of established stereochemistry from marrubiin and ambrein. The configuration at C₈ was assigned by comparison of the molecular rotation differences between sclareol (CXXXIIa) and manool (CXXXIIb) and between labdanolic acid and its dehydration product (CXXXIVa). Hence, all but the configuration at C₁₃ was established for labdanolic acid.

King and Jones⁽⁶⁹⁾ proposed the structure (CXXXa) for eperuic acid, relating it to the dehydration product from labdanolic acid. Rotation measurements on corresponding derivatives from the acids (CXXVII) and (CXXXa) were found to be of similar magnitude but of opposite sign. This led to the startling conclusion at the time, that eperuic acid had a carbon skeleton (XXII), which was the mirror image to that of rings A and B of all the then known di- and tri- terpenoids and steroids. This result had been confirmed⁽⁶⁹⁾ by relation of the C-17 acid from the eperuic series to (CXXIX) from ambrein. Despite the fact that their melting points were similar (m.p. 134-135°C, vs. 136-137°C) and their rotations almost the same ($[\alpha]_D$ -29.5°, vs. +33°),

though of opposite sign, these workers only claimed them as stereoisomers and not as antipodes.

Studies by Djerassi and Marshall⁽⁷⁰⁾ compared the rotatory dispersion curves of the nor-ketones of the eperuic and labdanolic series in an attempt to assign the absolute configuration of these compounds. The curve of the keto-ester (CXXXIII) from eperuic acid was virtually the mirror image of that from the corresponding labdanolic keto-ester (CXXXIV). As these curves were not exact mirror images, as required for antipodes, and as a fairly large discrepancy existed in the melting points (m.p. 223°C , vs. 190°C) of the oximes of the keto-esters, Djerassi attributed the stereochemical difference between eperuic and labdanolic acid to isomerism at C_9 , the other possible asymmetric centre C_{13} , being overlooked. This configuration gave eperuic acid the structure (CXXXV), possessing a trans-syn backbone, a situation not previously encountered in diterpenoid chemistry.

Labdanolic methyl ester had been assigned the 'R' configuration (CXXXVI) at C_{13} by Lederer and Bory⁽⁷²⁾ from molecular rotation data but this became insecure when it was demonstrated⁽⁷¹⁾ that hydrogen bonding may produce anomalous results. Barltrop and his associates⁽⁷¹⁾ carried out hydrogen bonding studies on both labdanolic and 13-*epi*-labdanolic methyl ester in carbon disulphide and obtained one sharp band for both esters at 3540 cm^{-1} . From this single sharp band, they assumed

that no intramolecular hydrogen bonding participated in either labdanolic methyl ester or its C₁₃ epimer and consequently, they supported Lederer's assignment of the 'R' configuration at C₁₃ for methyl labdanolate (CXXXVI). Barltrop did not carry out any dilution studies on methyl labdanolate or its 13-epimer to discover whether the hydroxyl frequency altered, though from its frequency of absorption (3540 cm.⁻¹) it would definitely appear to be bonded. (Normal unbonded hydroxyl absorption in carbon disulphide, 3600-3650 cm.⁻¹). However, infra-red studies on the labdane series by Raphael and his colleagues⁽⁷³⁾ showed that methyl labdanolate exhibited hydrogen bonding involving the formation of a ten membered ring (CXXXVII) and this conformation existed to a detectable extent in very dilute solutions of carbon tetrachloride. These results were at variance with those of Barltrop, Bigley and Rogers⁽⁷¹⁾ and thus the stereochemistry of labdanolic and eperuic acid was once more open to question.

Until recently, eperuic acid was the only diterpenoid to be assigned the trans-syn backbone. This anomaly was resolved by comparison⁽⁷⁴⁾ of the nor-keto-esters (CXXXVIII) and the related oximes (CXXXIX) derived from eperuic, labdanolic and 13-epi-labdanolic acids. The three keto-esters (CXXXVIII) showed no detectable difference (apart from sign) in their rotatory dispersion or circular dichroism, but comparison of the three acid oximes (CXXXIX) demonstrated conclusively that eperuic acid and

13-epi-labdanolic acid were antipodal (m.p. 224-226°C, $[\alpha]_D - 82^\circ$; m.p. 223-226°C, $[\alpha]_D + 87^\circ$, respectively). Inversion at C₉ might have occurred during the formation of the keto-esters and to eliminate this possibility, diol esters (CXL) of the three acids were prepared by osmylation of the olefinic esters (CXXXb). Once more the diol esters from eperuic and 13-epi-labdanolic acid were indistinguishable except for sign of rotation (m.p. 88-90°C, $[\alpha]_D + 4^\circ$; m.p. 88-90°C, $[\alpha]_D - 5^\circ$, respectively), but both differed from the diol ester from labdanolic acid (m.p. 77-79°C, $[\alpha]_D - 20^\circ$).

Thus, the normal trans-anti backbone was established for eperuic acid (XXII) in accordance with all other diterpenoids.

There now remained in doubt only the absolute configurations at C₁₃ in eperuic and labdanolic acids.

DISCUSSION

In an attempt to define the relative configuration of the C₁₃ methyl groups in eperuic acid (XXII) and labdanolic acid (CXXVII), Graham and Overton⁽⁸⁹⁾ converted the side chains of the respective acids into a third cyclohexane ring. This they did by cyclisation of the C₈ nor-ketones (CXXXVIII) from the two series to form the hydroxy-ester (CXLI). It was hoped that from these tricyclic derivatives, it might be possible to deduce the C₁₃ configuration from the coupling constants in the n.m.r. spectrum between H-13 and H-14, diaxial coupling for vicinal protons being greater than axial-equatorial coupling. However, under the conditions used (methoxide in refluxing toluene) epimerisation occurred⁽⁸⁹⁾ at C₁₃ affording the same hydroxy-ester from either of the nor-ketones of the two series.

Another approach to the problem of determining the absolute stereochemistry at the C₁₃ positions of the labdanolic and eperuic acids was to form the corresponding methyl-ketones (CXLII) and (CXLIII), obtained from the respective acids by reaction with methyl lithium⁽⁷⁵⁾. By conversion of these derivatives to the C₈ nor-ketones (CXLIV) and (CXLV) and thereafter cyclisation to the corresponding hydroxy-ketones (CXLVI) and (CXLVII) under conditions that should preserve intact the configurations at C₁₃, it was anticipated that the absolute stereochemistry

of eperuic and labdanolic acids at this centre could be determined. It was hoped that the C_{14} proton, being adjacent to the carbo-methoxy group would be easily identified as a low field doublet.

In the labdane series the methyl ketone (CXLII), $C_{21}H_{38}O_2$, prepared readily from the corresponding acid had U_{max}^{CC14} at 1720 cm.^{-1} (ketonic carbonyl) and exhibited a singlet τ 7.88; 3H, (\underline{CH}_3 CO); in the n.m.r. spectrum. An additional product, the tertiary alcohol (CXLVIII), $C_{22}H_{42}O$, was formed by the presence of excess methyl lithium.

Dehydration of the hydroxy-ketone (CXLII) with phosphorous oxychloride, afforded preferentially the enone (CXLIX), along with a very small percentage of the isomer (CL), as shown by t.l.c. on silica gel-silver nitrate, the product of reaction appearing primarily as one spot, only a faint shadow of a less polar product (CL) being visible. This result was confirmed by the absence of a vinyl methyl group in the n.m.r. spectrum and by the two broad singlets (τ 5.5, 1H; τ 5.15, 1H) assigned to the exomethylene double bond, also seen as bands ($\underline{CHC1}_3$) at 1650 and 890 cm.^{-1} in the infra-red spectrum.

The enone (CXLIX) was osmylated to yield two diols (CLI), epimeric at C_8 and these were cleaved, without prior separation, with sodium periodate in aqueous methanol. The resulting nor-ketone (CXLIV), $C_{20}H_{34}O_4$, m.p. $192 - 194^\circ\text{C}$, $\underline{CHC1}_3$ 1710 cm.^{-1} , was cyclised in

ethanolic potassium hydroxide to the hydroxy-ketone (CXLVI), $C_{20}H_{34}O_2$, m.p. $202^{\circ}C$, with extraordinary ease. (CXLVI) could also be obtained from the nor-ketone (CXLIV), by reflux in benzene with toluene-p-sulphonic acid, (m.p., m.m.p., g.l.c., 1%, SE30; 1% QF1; 2% 20M Peg.). From the infra-red spectrum (CCl_4) it was observed that the hydroxyl group at C_8 was completely bonded (3486 cm.^{-1}) to the ketonic carbonyl group (1692 cm.^{-1}), even at high dilution. On cyclisation of the dione (CXLIV), it was anticipated that the carbo-methoxyl group would assume the more stable equatorial (β) configuration, and ring C a chair conformation. Consequently, a 13β methyl group would result in an axial-equatorial coupling between H-13 and H-14 and the alternative 13α methyl assignment would enforce the larger diaxial coupling between H-13 and H-14. Unfortunately, it was impossible to define the stereochemistry at C_{13} from interpretation of the n.m.r. spectrum, as the C_{13} proton was deshielded by the β -hydroxyl group at C_8 and its signal was thus shifted downfield and appeared at the same position as the signal from the proton at C_{14} . Using benzene as a solvent failed to make the interpretation of the spectrum any easier.

It is of interest to note that in an endeavour to form the acetate of the hydroxy-ketone (CXLVI) by reflux with acetic anhydride in pyridine, the sole product furnished was the dione (CXLIV).

A similar series of reactions was carried out with eperuic acid, the acid being transformed⁽⁷⁵⁾ initially into the methyl ketone (CXLIII), $C_{21}H_{36}O$, by reaction with methyl lithium. The singlet (τ 7.9, 3H) confirmed the formation of the methyl ketone and this was further shown by $U_{max}^{CCl_4}$ 1710 cm^{-1} (ketonic carbonyl). Cleavage of the exocyclic double bond was effected by osmylation of (CXLIII) affording a mixture of C_8 epimeric diols (CLII). These, without prior separation, on reaction with sodium periodate in aqueous methanol yielded the dione (CXLV). Attempts to cyclise this dione (CXLV) in ethanolic potassium hydroxide failed to produce the hydroxy-ketone (CXLVII) corresponding to (CXLVI) formed so readily in the labdane series. More stringent conditions were used in the hope of cyclising the dione (CXLV); 1. Potassium tertiary butoxide in tertiary butanol⁽⁷⁶⁾; 2. Sodium hydride in benzene; 3. Dimethyl sulphoxide and sodium hydride⁽⁷⁷⁾; all proved unsuccessful. This may be attributed to the fact that, if in the eperuic series, the C_{13} methyl group is assigned the α configuration, 1 : 3 diaxial interaction between this group and the potential (α) hydroxyl at C_8 may prevent cyclisation.

On reflux in benzene with toluene-p-sulphonic acid for 1.5 hours, cyclisation of the dione (CXLV) was effected to afford an enone, assigned the structure (CLIII) from spectral information. From the mass spectrum, the molecular weight, 288, showed loss of 18, indicating the loss of water and formation of a double bond upon cyclisation. As no olefinic protons

were observed in the n.m.r., the double bond must be tetra-substituted

and therefore must be situated at $\Delta^{8(9)}$, $\Delta^8(14)$, or $\Delta^{13(14)}$
 and therefore must be situated at $\Delta^{8(9)}$, $\Delta^8(14)$, or $\Delta^{13(14)}$.
 Abso:

Absorption in the infra-red (CCl_4) at 1708 cm^{-1} indicates the presence of a carbonyl group and this was supported by the ultra-violet

spectrum $\lambda_{\text{max}} 207 \text{ m}\mu$, $\sum 3440$, $285 \text{ m}\mu$, $\sum 160$; no change

on addition of sodium hydroxide. Consequently, the double bond was

assigned to the $\Delta^{8(9)}$ position. The proton at C_{14} , being allylic and

adjacent to a carbonyl group, was seen as a complex multiplet at $\tau 7.5$

due to its vicinal coupling with H_{13} , and 1 : 4 coupling with $\text{H}_{12} (\alpha)$

and $\text{H}_8 (\alpha)$. In view of the uncertainty attending the preferred conformation

of the rather mobile cyclohexene ring C, no conclusion could be derived from

the n.m.r. spectrum concerning the configuration at C_{13} .

When either the dione (CXLIV) or the hydroxy-ketone (CXLVI) from

labdanolic acid was refluxed in benzene for 5 hours along with toluene-p-

sulphonic acid, an enone (CLIV) was obtained, which was indistinguishable

from the enone (CLIII) by t.l.c. and mass spectrometric analysis, but

differed in its behaviour on g.l.c. (5% QF1). From infra-red (U_{max}

1710 cm^{-1}), ultra-violet ($\lambda_{\text{max}} 209 \text{ m}\mu$, $\sum 2765$; $285 \text{ m}\mu$, $\sum 130$; no

change on addition of potassium hydroxide) and n.m.r. spectra (no olefinic

protons), and identical fragmentation pattern with that of (CLIII), the double

bond in the enone (CLIV) was assigned to the $\Delta^{8(9)}$ position. The diffuse

multiplet at T7.55 (1H) was ascribed to the proton at C₁₄, but again this gave no indication of the configuration at C₁₃.

The C₈-nor-ketone (CXLIV) was considered to cyclise to the hydroxy-ketone (CXLVI) as shown in Figure 9. The failure of the eperuic dione (CXLV) to do so under the same conditions can be attributed to the 1 : 3 diaxial interaction experienced by the -OH group at C₈ with the methyl groups at C₁₀ and C₁₃, the double diaxial interaction being absent in the case of labdanolic acid. Thus, the action of base on the nor-ketones (CXLIV) and (CXLV) is persuasive evidence for the assignment of an α configuration to the methyl groups at C₁₃ in the labdanolic and eperuic series.

On acid catalysis, cyclisation of the nor-ketone (CXLV) occurred because the 1 : 3 diaxial interaction between C₈ and C₁₃ was relieved by removal of the C₈ hydroxyl group to afford the $\Delta^{8(9)}$ compound (CLIII); Figure 10.

The 13 α methyl group configuration was supported by the circular dichroism curves of the nor-ketones (CXLIV) and (CXLV), which showed that the compounds were almost antipodal, the $\Delta\epsilon$ max. values being -2.76 (CXLIV); + 2.12 (CXLV). Assuming that the contributions of the decalone systems were -2.34 and +2.34 in these two compounds, the contribution of the side chain carbonyl would be -0.32 for both. From the

work of Levene⁽⁹⁰⁾ and Djerassi⁽⁹¹⁾ a negative contribution would correspond to an α configuration for the methyl group at C₁₃. This result is in good agreement with the value for citronellal⁽⁸⁸⁾ of +0.22, in which the methyl group adjacent to the aldehyde residue is in a β configuration.

Confirmation of the α assignment to the methyl group at C₁₃ in the labdane series has been obtained by an X-ray analysis⁽⁹²⁾ of the p-bromophenacyl-ester derivative from labdanolic acid.

Efforts were made to obtain the isomeric $\alpha\beta$ unsaturated ketones from (CLIII) and (CLIV) as the conjugated form should be energetically more favourable. Prolonged reflux in alcoholic potassium hydroxide or with toluene-p-sulphonic acid failed to produce the desired $\alpha\beta$ unsaturated ketones, leaving the compounds unchanged (g.l.c. 1% QF1; 1% SE 30; 2% 20M Peg.). However, reflux in acetic acid, acetic anhydride and perchloric acid⁽⁷⁸⁾ afforded one product (CLV) from the labdanolic enone (CLIV) and three products from the eperuic enone (CLIII).

The compound (CLV) was found to be isomeric with the enone (CLIV) (mass spec. m.wt. 288), but differed in its retention time on g.l.c. (1% SE 30; 1% QF1.) and fragmentation pattern in the mass spectrum. No vinyl methyl group or vinyl protons were observed in the n.m.r., so that the double bond was assigned to the 8-14 position, giving the $\alpha\beta$ unsaturated ketone (CLV). This assignment conflicts with the infra-red ($\nu_{\text{max}}^{\text{CCl}_4}$ 1708 cm⁻¹)

and the ultra-violet spectra (λ_{max} 204 m μ , \sum 4000; 242 m μ , \sum 1200 and 285 m μ , \sum 170; no change on addition of aqueous sodium hydroxide.). This anomolous behaviour in the i.r. and u.v. may occur because the π orbitals of the double bond and the carbonyl group are prevented from overlapping as a result of steric interaction between the C₁₃ α methyl group and the 14 β carbo-methoxy group. Inhibition of conjugation in this type of system, resulting in greatly reduced values for the extinction coefficient, has been reported by Braude and Waight⁽⁷⁹⁾.

The three products from the enone (CLIII), failed to separate on chromatography, but gas-liquid/mass spectrometric analysis showed the components of the mixture to be of molecular weight : Peak 1, m.wt. 288; Peak 2, m.wt. 288; Peak 3, Parent at m/e 251. The fragmentation pattern of peak 1 was identical with that of (CLV), though their retention times differed on g.l.c. (1% QF1), and was therefore considered to be the $\alpha\beta$ unsaturated ketone (CLVI). The components of peaks 2 and 3 need to be investigated further before structures can be confidently assigned to them.

EXPERIMENTAL

Labdanolic acid (CXXVII)

Methyl labdanolate (CXXVIII) was hydrolysed⁽⁶⁷⁾ under reflux for one hour with a solution of sodium (400 mg) in ethanol (12 ml) and water (0.2 ml). The resulting acid was a gum (680 mg) and from t.l.c. no methyl ester remained.

Methyl Ketone (CXLII)

Methyl lithium was prepared⁽⁷⁵⁾ by addition over a period of five mins., of the redistilled methyl iodide (1.25 ml) in anhydrous ether (20 ml), to a stirred suspension of lithium (0.27 gm) in anhydrous ether (25 ml). The solution was refluxed for one hour and then filtered into a constant pressure dropping funnel. The resulting methyl lithium solution was added dropwise to a stirred solution of labdanolic acid (680 mg) in anhydrous ether (25 ml) under an atmosphere of nitrogen. The solution was refluxed for 90 mins., cooled and water added carefully to destroy the excess methyl lithium. The aqueous layer was extracted into ether, the extracts combined, washed well with water, dried and the solvent removed to yield a yellow oil (600 mg). T.l.c. revealed one major product and two minor products. P.t.l.c., using ethyl acetate: light petroleum (3 : 7) as eluant, yielded the desired methyl ketone (CXLII) (510 mg). Although this was homogeneous on t.l.c. it failed to crystallise, as did the oxime prepared in

the usual way. $U_{\max}^{\text{CHCl}_3}$, 3600, 1720 cm^{-1} ; 3H singlets at τ 7.88, 8.8, 9.15; 6H singlet at τ 9.22; 3H doublet centred at τ 9.08.

(Found: C, 77.8; H, 11.65. $\text{C}_{21}\text{H}_{38}\text{O}_2$ requires C, 78.20; H, 11.88%).

Extraction of the band more polar than the methyl ketone (CXLII) afforded the carbinol (CXLVIII) as a gum (40 mg). $U_{\max}^{\text{CHCl}_3}$ 3610 cm^{-1} ; 3H singlets, τ 8.85, 9.12; 6H singlets at τ 8.78, 9.2; 3H doublet at τ 9.0. (Found: C, 77.70; H, 12.24. $\text{C}_{22}\text{H}_{42}\text{O}_2$ requires C, 78.04; H, 12.04%).

The third component of the reaction product (20 mg) was not characterised.

Dehydration of the Methyl Ketone (CXLII)

The methyl ketone (CXLII) (450 mg) was dehydrated with phosphorous oxychloride (0.5 ml) in pyridine (2 ml) at 20°C overnight. The reaction was cooled in ice, water added carefully and the aqueous solution extracted well into ethyl acetate. The extracts were dried and the solvent removed, azeotroping with benzene to remove any remaining pyridine. The product (CXLIX), a gum (380 mg), containing little of the isomer (CL) as seen by t.l.c. on silica gel-silver nitrate and from the n.m.r. spectrum. $U_{\max}^{\text{CHCl}_3}$ 1720, 1650 cm^{-1} ; 1H broad singlet at τ 5.15 and 5.5 ($>\text{C}=\text{CH}_2$); absence of 3H singlet at τ 8.3 (vinyl methyl). (Found: C, 82.61; H, 11.74. $\text{C}_{21}\text{H}_{36}\text{O}$ requires C, 82.83; H, 11.92%).

Formation of the Diol (CLI) from (CXLIII)

The dehydration product (CXLIII) (350 mg) was dissolved in pyridine (2 ml) and osmium tetroxide (280 mg) added. The solution was left at 20°C for 12 hours and worked up as previously described⁽⁸⁰⁾. The product (CLI) (300 mg) was shown by t.l.c. to contain two diols of very similar polarity. No attempt was made to separate these, though the crude product was chromatographed to remove all impurities.

Cleavage of the Diol mixture (CLI)

The diol mixture (CLI) (200 mg) obtained above, was dissolved in methanol (5 ml) and an aqueous solution of sodium metaperiodate (180 mg) added. The reaction was maintained at 0°C for one hour, water added and the aqueous solution extracted into ethyl acetate. On drying and removal of the solvent a gum (80 mg) was formed, which crystallised with difficulty from icecold light petroleum to afford the diene, (CXLIV), m.p. 192-194°C; $U_{\max}(\text{CHCl}_3)$ 1710 cm^{-1} ; 3H singlets at τ 7.88, 9.02, 9.15, 9.3; 3H doublet at τ 9.05. (Found: C, 78.0; H, 11.54. $\text{C}_{20}\text{H}_{34}\text{O}_2$ requires C, 78.38; H, 11.18%).

Hydroxy-Ketone (CXLVI)

To a solution of the diene (CXLIV) (60 mg) in benzene was added 1% ethanolic potassium hydroxide (0.5 ml) and the reaction left at 20°C for 20 mins. Addition of water and extraction into ether afforded the hydroxy-

ketone (CXLVI) (60 mg), which crystallised from light petroleum, m.p.

202°C; $[\alpha]_D^{20} -65^\circ$, ($c = 0.5$, chloroform); $U_{\max}^{CCl_4} 3486, 1692 \text{ cm.}^{-1}$;

3H singlets at T7.8 and 9.0; 6H singlet at T9.15; 3H doublet at T9.18.

(Found C, 78.5; H, 11.1. $C_{20}H_{34}O_2$ requires C, 78.4; H, 11.2%).

Attempted acetylation of the Hydroxy-ketone (CXLVI)

The hydroxy-ketone (CXLVI) (10 mg) was dissolved in dry pyridine (0.5 ml), "analar" acetic anhydride (0.5 ml) added and the solution refluxed for 12 hours. Removal of the solvent in vacuo, and purification by p.t.l.c. afforded, as the sole product, the diene (CXLIV) (7 mg), as shown by t.l.c.; i.r., and n.m.r.

Dehydration of the Hydroxy-Ketone (CXLVI)

The hydroxy-ketone (CXLVI) (20 mg) in dry benzene (3 ml) and toluene-p-sulphonic acid (10 mg) were refluxed for 5 hours. Addition of water and extraction into ethyl acetate, afforded on removal of the solvent, the oily enone (CLIV) (13 mg) as the sole product, as seen from g.l.c.

(1% SE 30; 5% QF1; 1% QF1). $U_{\max}^{CCl_4} 1710 \text{ cm.}^{-1}$; $\lambda_{\max} 209 \text{ m}\mu$, $\int 2765; 285 \text{ m}\mu, \int 130$. Signals in the n.m.r. at T7.5 (1H, m); 7.98 (3H, s); 8.98 (3H, s); 9.14 (3H, s); 9.16 (3H, s); 9.1 (3H, d). (Mass spec. m.wt. 288. $C_{20}H_{32}O$ requires 288). Fragmentation pattern identical with that of (CLIII).

Isomerisation of the Enone (CLIV)

The enone (CLIV) (9 mg) was heated at 95°C with acetic acid (1 ml), acetic anhydride (0.1 ml) and 60% perchloric acid (0.05 ml)⁽⁷⁸⁾ for 20 mins. Water was added, the aqueous solution extracted into ethyl acetate, the extracts washed with sodium bicarbonate and then with water, dried and the solvent removed to yield a gum (CLV) (4 mg) as the only component, as shown by t.l.c. over silica gel-silver nitrate and g.l.c. (1% SE 30; 1% QF1; 1% CHDMS). $U_{\max}^{CCl_4}$ 1705 cm.⁻¹; λ_{\max} 204 m μ , \int 400, 285 m μ , \int 170. Signals in the n.m.r. at T7.92 (3H, s), 8.99 (3H, s), 9.02 (6H, s). (Mass spec. m.wt. 288. C₂₀H₃₂O requires 288).

Eperuic Acid (XXII)

Methyl eperuate (CXXXI) (700 mg) was hydrolysed as described for methyl labdanolate (CXXVIII). The resulting gum (XXII) (650 mg) which failed to crystallise, contained no methyl ester (CXXXI) as seen from t.l.c.

$U_{\max}^{CHCl_3}$ 3600, 1710 cm.⁻¹.

Methyl Ketone (CXLIII)

The methyl ketone (CXLIII) from eperuic acid (650 mg) was prepared (after the method prescribed by Meinwald⁽⁷⁵⁾), as described previously for labdanolic acid, furnishing a gum (600 mg). $U_{\max}^{CHCl_3}$ 1710, 1640 cm.⁻¹; 1H broad singlets in the n.m.r. at T5.2 and 5.5 ($\text{>C} = \text{CH}_2$); other signals at T7.9 (3H, s), 8.8(3H, s), 9.12 (3H, s), 9.18 (3H, d), 9.3 (3H, s).

(Found: C, 82.6; H, 11.7. $C_{21}H_{36}O$ requires C, 82.8; H, 11.9%).

Osmylation of the Methyl Ketone (CXLIII)

The methyl ketone (CXLIII) (550 mg) and osmium tetroxide (450 mg) were kept in pyridine at 20°C for 16 hours. Work up as previously described, afforded two diols (CLI) of very similar polarity (t.l.c.). These diols (250 mg) were separated by p.t.l.c. from the less polar impurities but were not separated from each other. $U_{max}^{CHCl_3}$ 3600, 1710 cm^{-1} .

Cleavage of the Diol Mixture (CLI)

To the epimeric diols (CLI) (250 mg) in methanol (5 ml) was added an aqueous solution of sodium periodate (160 mg) and the solution kept at 0°C for 1 hour. Working up in the usual way gave the dione (CXLV) (100 mg), which failed to crystallise after purification by p.t.l.c. $U_{max}^{CHCl_3}$ 1720 cm^{-1} ; signals in the n.m.r. at τ 7.94 (3H, s), 9.06 (3H, s), 9.18 (3H, s), 9.3 (3H, s), 9.1 (3H, d). (Found: C, 78.0; H, 11.1. $C_{20}H_{34}O_2$ requires C, 78.4; H, 11.2%).

Cyclisation of the Dione (CXLV) to the Enone (CLIII)

The dione (CXLV) (20 mg) and toluene-p-sulphonic acid (10 mg) in dry benzene (2 ml) were refluxed for 90 mins, and thereafter water added and the aqueous solution extracted into ether. The extracts on removal of

the solvent afforded the enone (CLIII) (10 mg) as a gum, found to be homogeneous from t.l.c. on silica gel-silver nitrate and from g.l.c. (1% SE 30; 1% QF1; 5% QF1). The enone (CLIII) differed in retention time from the enone (CLIV) of the labdane series only on one column (5% QF1). $\nu_{\text{max}}^{\text{CCl}_4}$ 1708 cm^{-1} ; λ_{max} 207 $\text{m}\mu$, \sum 3400, 285 $\text{m}\mu$, \sum 160. Signals in the n.m.r. at τ 7.5 (1H, m), 8.02 (3H, s), 9.07 (3H, s), 9.15 (6H, s), 9.17 (3H, d). (Mass spec. m.wt. 288. $\text{C}_{20}\text{H}_{32}\text{O}$ requires 288).

Attempted Cyclisations of the Dione (CXLV)

1. The diene (CXLV) (2 mg) was dissolved in dry benzene (0.5 ml) 1% ethanolic potassium hydroxide solution (0.1 ml) added and the reaction left at 20°C for 20 mins. Working up as previously described, afforded the unchanged dione (CXLV) as judged by t.l.c., g.l.c., i.r., and u.v.

Increasing the reaction time under the same conditions to 5 hours, afforded only unchanged starting material (CXLV).

2. To a solution of the dione (CXLV) (2 mg) in dry benzene (1 ml), 10% ethanolic potassium hydroxide solution (0.2 ml) was added and the reaction left at 20°C for periods of 3, 5 and 16 hours. In all cases the unchanged dione (CXLV) was recovered exclusively.

When the above solution was refluxed for 5 hours, there was again, no change.

3. To dry refluxing t-butanol⁽⁷⁶⁾ (10 ml), was added potassium (200 mg) and the refluxing continued until all the potassium had dissolved. This solution of potassium tertiary butoxide solution (1 ml) was added to the dione (CXLV) and the reaction left at 20°C for 15 mins. Addition of water, extraction into ethyl acetate and removal of the solvent afforded only unchanged dione (CXLV) (t.l.c., i.r., and u.v.).

Reflux of the dione (CXLV) (5 mg) in potassium tertiary butoxide (1 ml) under nitrogen for 30 mins. afforded an intractable mixture of products (numerous spots on t.l.c.).

4. The dione (CXLV) (5 mg) was dissolved in dry benzene (1 ml) and, sodium hydride (5 mg) added and the solution refluxed, a portion of the solution being removed after 30 mins., 1 hour and 16 hours. Extraction of the aqueous layer into ether afforded in each case unchanged dione (CXLV).

5. The methyl sulphonyl carbanion was prepared⁽⁷⁷⁾ by refluxing sodium hydride (50 mg) with excess dimethyl sulphoxide (3 ml) for 45 mins. under nitrogen. The solution was cooled to 20°C and the dione (CXLV) (10 mg) in dimethyl sulphoxide (1 ml) was added to this with stirring and under nitrogen. After 20 mins. dilute acetic acid (2 ml. in 10 ml water) was added with cooling and the resulting solution extracted into ether, the extracts dried and the solvent removed, to afford unchanged dione (CXLV). (t.l.c., i.r., and u.v.).

Lengthening the reaction time to 3 hours at 20°C and to 1 hour and 4 hours at reflux temperature gave, in all cases, unchanged diene (CXLV) as the sole product of reaction.

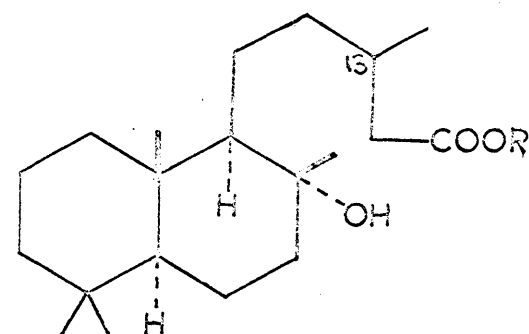
Isomerisation of the Enone (CLIII)

The enone (CLIII) (10 mg), "analar" acetic anhydride (0.1 ml), "analar" acetic acid (1 ml) and 60% perchloric acid (0.05 ml)⁽⁷⁸⁾ were refluxed for 30 mins. Working up as previously described afforded three products (6 mg) as judged from g.l.c. (1% QF1; 1% SE 30). These products failed to separate on t.l.c. but were analysed by g.c.m.s.

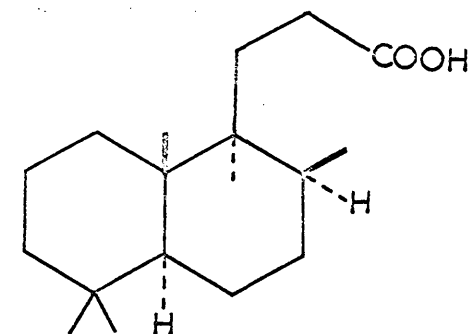
Peak 1. Mass spec. m.wt. 288. $C_{20}H_{32}O$ requires 288. Fragmentation pattern identical with that of (CLV) from the labdane series.

Peak 2. Mass spec. m.wt. 288. $C_{20}H_{32}O$ requires 288. Fragmentation pattern different from that of (CLVI).

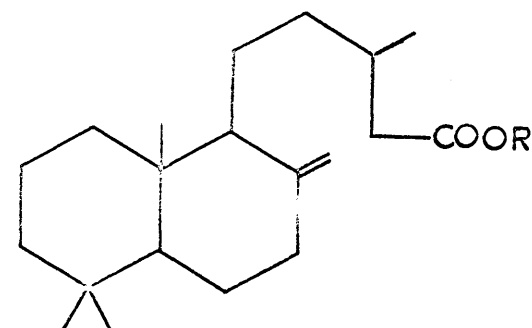
Peak 3. Present in the mass spectrum at 251.



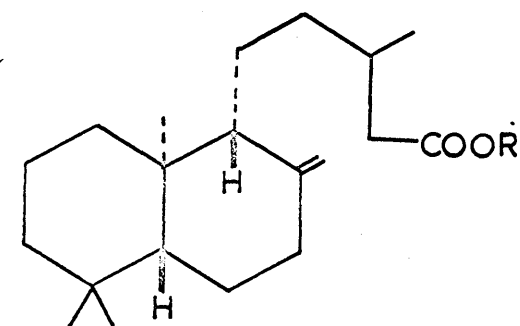
CXXVII $R = H$
CXXVIII $R = CH_3$



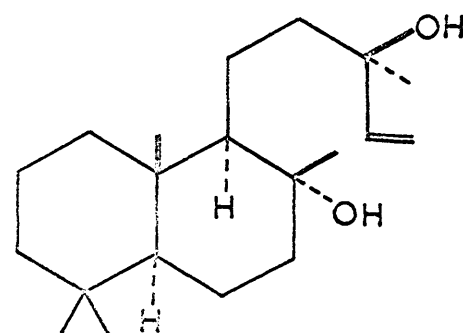
CXXIX



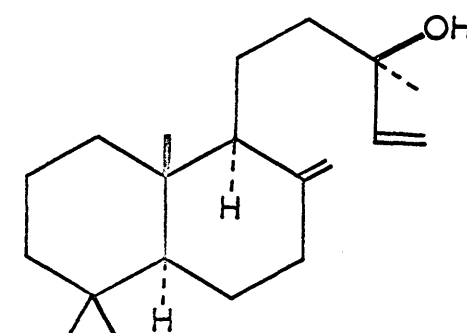
CXXXa $R = H$
CXXXb $R = CH_3$



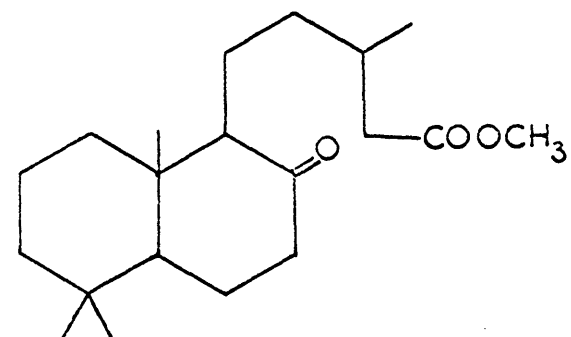
XXII $R = H$
CXXXI $R = CH_3$



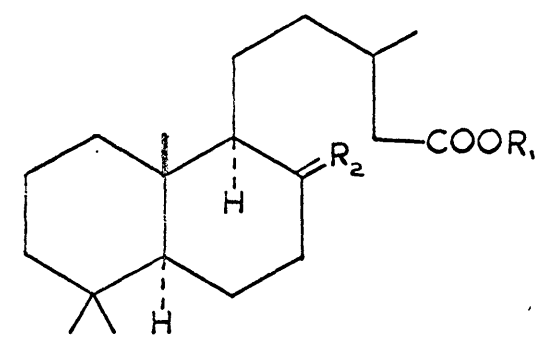
CXXXIIa



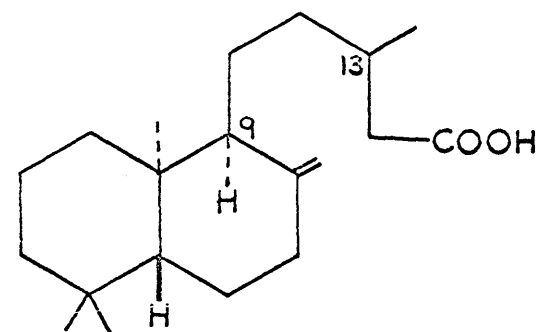
CXXXIIb



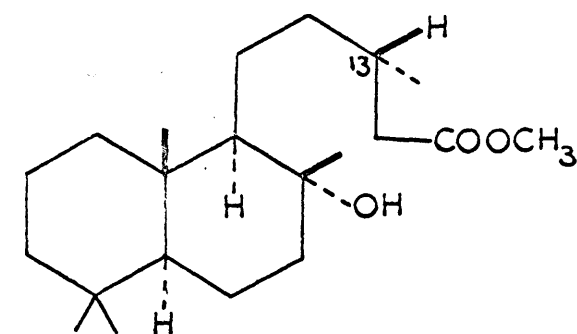
CXXXIII



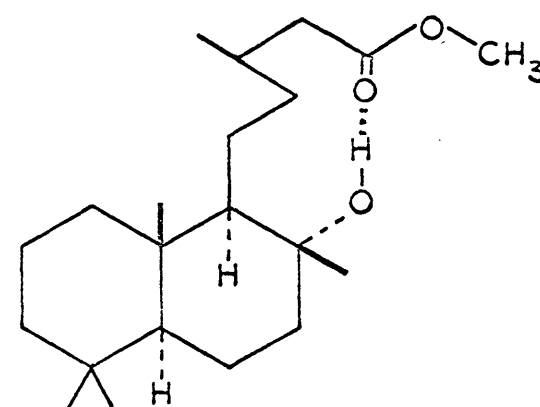
CXXXIV $R_1 = \text{CH}_3$ $R_2 = \text{O}$
CXXXIV α $R_1 = \text{H}$ $R_2 = \text{CH}_2$



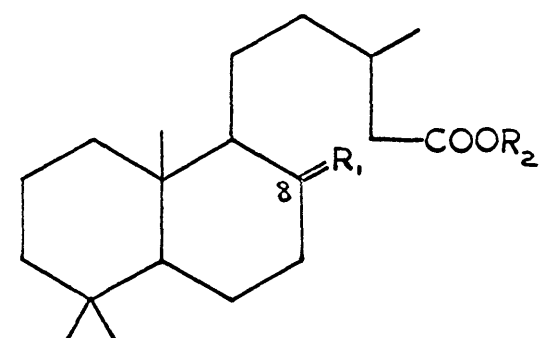
CXXXV



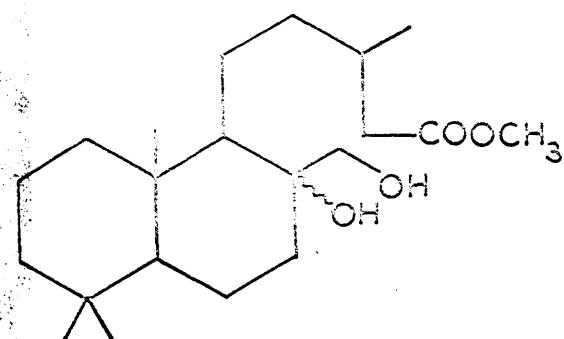
CXXXVI



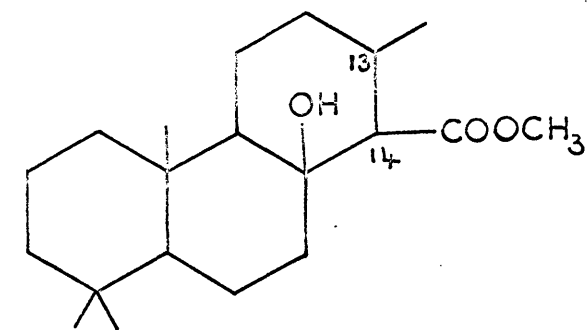
CXXXVII



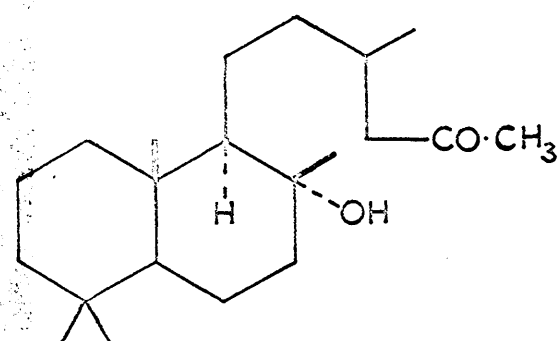
CXXXVIII $R_1 = \text{O}$ $R_2 = \text{CH}_3$
CXXXIX $R_1 = \text{NOH}$ $R_2 = \text{H}$



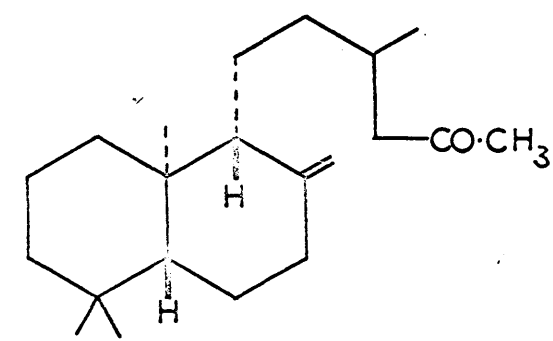
CXL



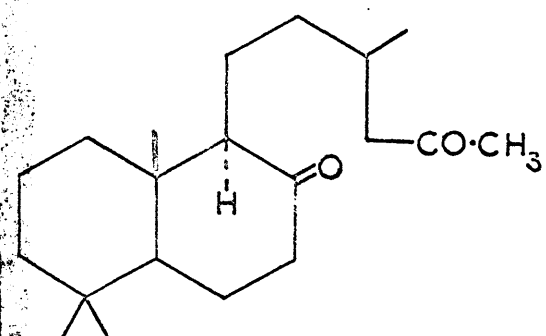
CXLI



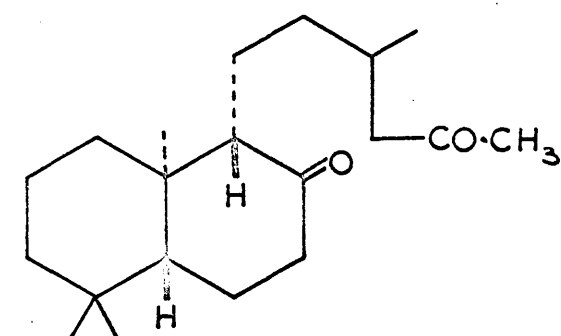
CXLII



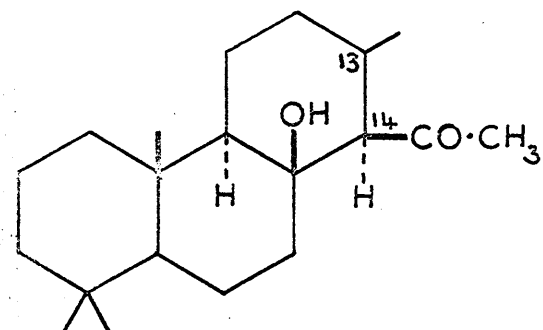
CXLIII



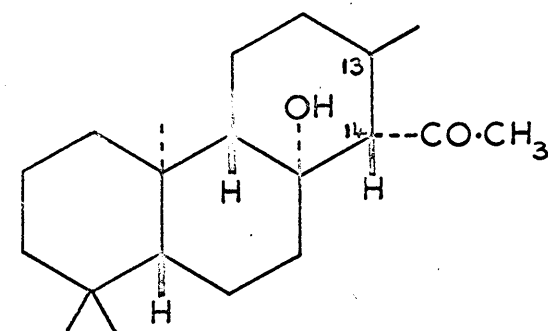
CXLIV



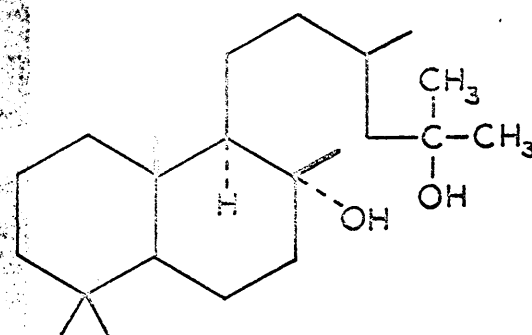
CXLV



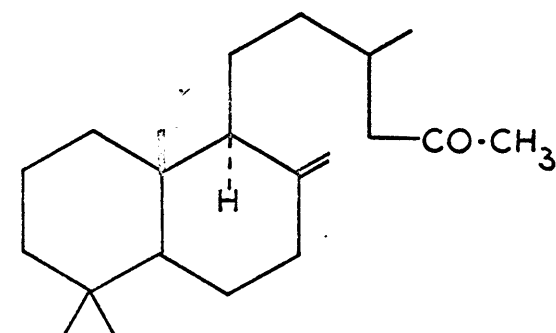
CXLVI



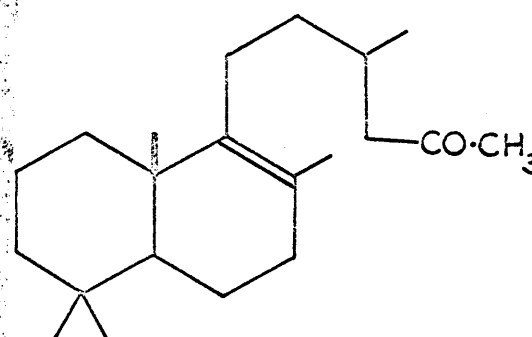
CXLVII



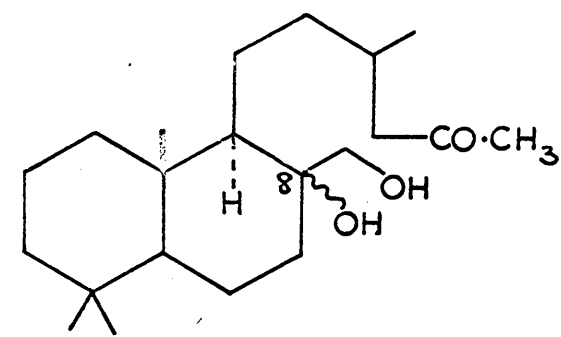
CXLVIII



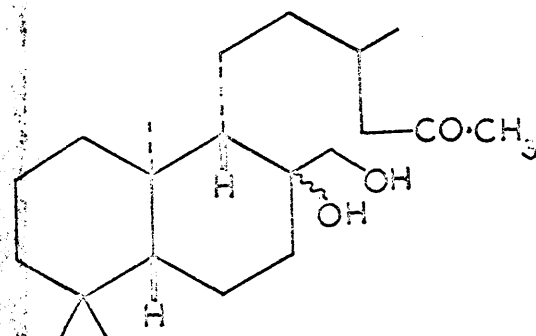
CXLIX



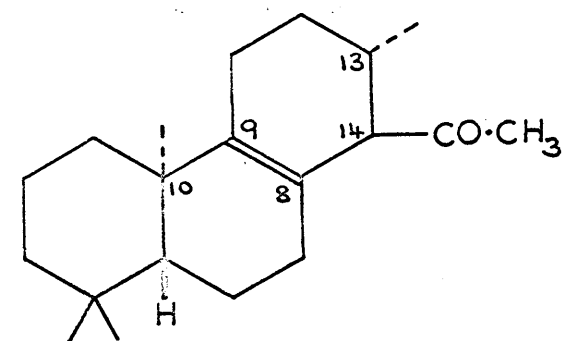
CL



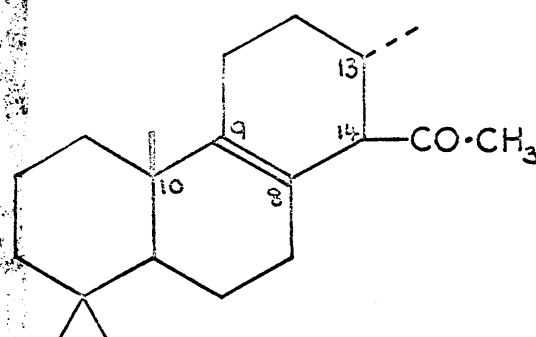
CLI



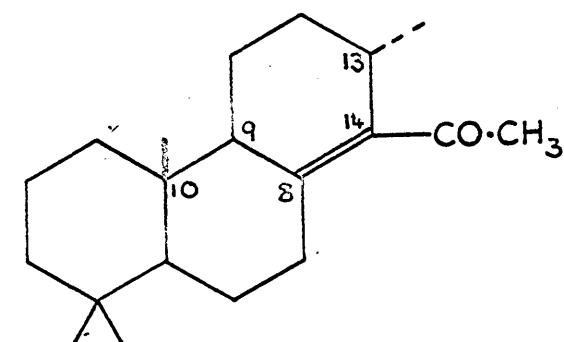
CLII



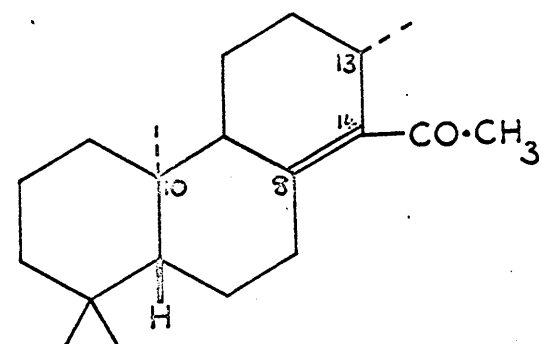
CLIII



CLIV



CLV



CLVI

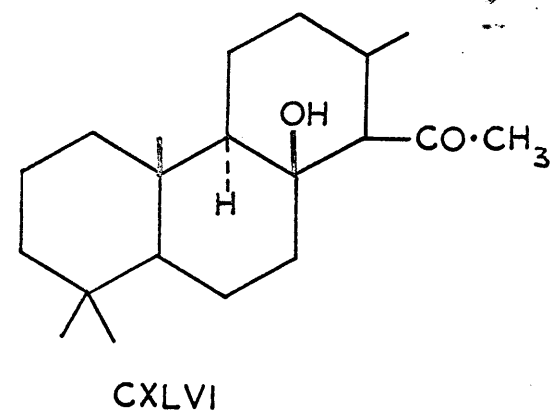
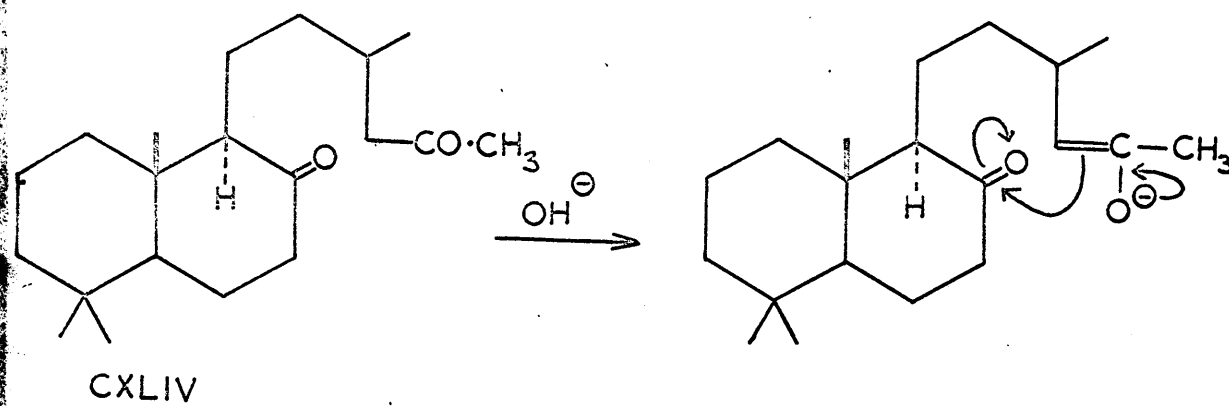


FIGURE 9

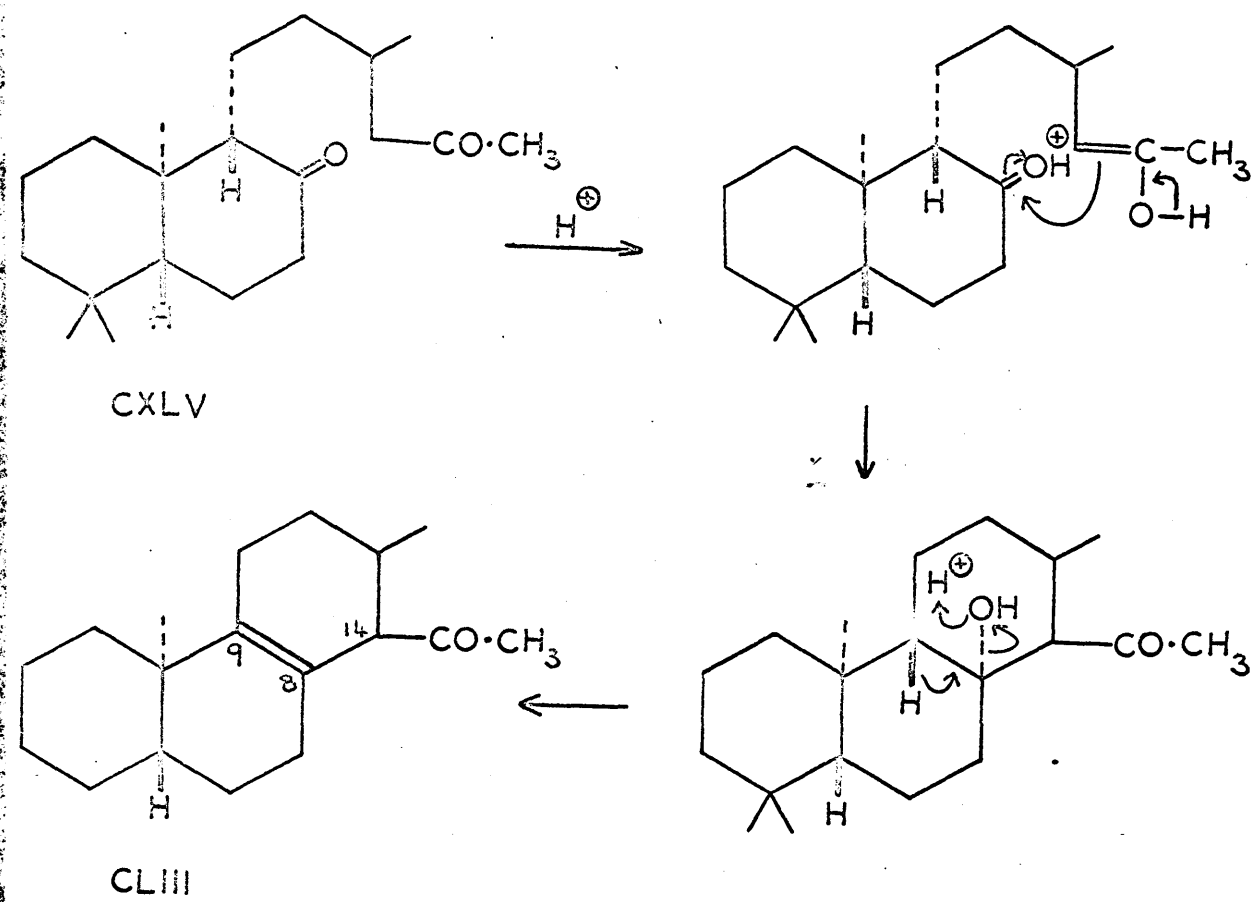


FIGURE 10

BIBLIOGRAPHY

1. G. WILLIAMS, Chem. News, 1860, 2, 206.
2. L. RUZICKA, Experientia, 1953, 9, 357.
3. R.B. WOODWARD and K. BLOCH,
J.Amer.Chem.Soc., 1953, 75, 2023.
4. W.G.DAUBEN, S.ABRAHAM, S.HOTTA, I.L.CHAIKOFF,
I.V.BRADLOW, H.L. and A.H. SOLOWAY,
J.Amer.Chem.Soc., 1953, 75, 3038.
5. K. BLOCH, Vitamins and Hormones, 1957, 15,
119.
6. A.J.BIRCH, R.W.RICKARDS, H.SMITH, A.HARRIS, and
W.B.WHALLEY, Proc. Chem. Soc., 1958, 223.
7. J.J.BRITT, and D.ARIGONI, Proc. Chem. Soc., 1958, 224.
8. R.G.LANGDON, and K.BLOCH, J. Biol. Chem., 1953, 200, 129.
9. A.J.BIRCH, R.W.RICKARDS and H.SMITH,
Proc. Chem. Soc., 1958, 192.
10. J.W.CORNFORTH, I.Y.GORE, and G.POPJAK,
Biochem. J., 1957, 65, 94.
11. J.W.CORNFORTH, G.D.HUNTER and G.POPJAK,
Biochem. J., 1953, 54, 597.
12. K.FOLKERS, D.E.WOLF, C.H.HOFMANN, P.E.ALDRIKH,
H.R.SKEGGS, and L.D.WRIGHT,
J.Amer. Chem. Soc. 1956, 78, 4499.
13. P.A.TAVORMINA, M.H.GIBBS, and J.W.HUFF,
J. Amer. Chem. Soc., 1956, 78, 4498;
6210.
14. D.ARIGONI, Experientia, 1958, 41, 153.

15. A.J.BIRCH, D.W.CAMERON, C.W.HOLZAPFEL, and
R.W.RICKARDS, Chem. and Ind., 1963, 374.
16. L.GUGLIELMETTI, Ph.D. Thesis, Zurich, 1962.
17. A.H.PHILIPS, T.T.TCHEN, and K.BLOCH,
Fed.Proc., 1958, 17, 289.
18. F.LYNEN, H.EGGERER, U.HENNING, and L.KESSEL,
Angew. Chem., 1958, 70, 738.
19. A. de WAARD, A.H.PHILIPS, and K.BLOCH,
J. Amer. Chem. Soc., 1959, 81, 2913.
20. F.LYNEN, B.W.AGRANOFF, H.EGGERER, U.HENNING, and
E.M.MOSLEIN, Angew. Chem., 1959, 71, 657.
21. S.CHAYKIN, J.LAW, A.H.PHILIPS, T.T.TCHEN, and K.BLOCH,
Proc. Nat. Acad. Sci. Wash., 1958, 44,
998.
22. H.RILLING, and K.BLOCH, J. Biol. Chem., 1959, 234, 1424.
23. A.I.SCOTT, G.A.SIM, G.FERGUSON, D.W.YOUNG, and
F.McCAPRA, J. Amer. Chem. Soc., 1962, 84, 3197.
24. J.D.CONNOLLY, R.McCRINDLE, R.D.H.MURRAY, and K.OVERTON,
Tetrahedron Letters, 1964, 30, 1983.
25. A.J.BIRCH, R.W.RICKARDS, H.SMITH, A.HARRIS, and
W.B.WHALLEY, Tetrahedron, 1959, 7, 241.
26. D.ARIGONI, Biosynthesis of Terpenes and Steroids,
Churchill, London, 1959, P.231.
27. D.ARIGONI, Gazz. Chim. Ital., 1962, 92, 884.
28. M.C. WOODS, K. NAKANISHI, and N.S. BHACCA,
Tetrahedron, 1966, 243.
29. W.G. DAUBEN, W.F. THIESSEN, and P.R. RESNICK,
J. Amer. Chem. Soc., 1962, 84, 2015.

30. H. GROTMAN, T. NORIS, M. SUMIMOTO, and A. MORRISON,
Tetrahedron Letters, 1964, 3879.
31. M. SHIRO, T. SATO, H.KOYAMA, Y.MOKI, K.NAKANISHI
and S.UYEO, Chem. Comms., 1966, 97.
32. G.G. FREEMAN, and R.I. MORRISON,
Biochem. J., 1948, 43, XXIII.
33. G.G. FREEMAN and R.I. MORRISON,
Biochem. J., 1949, 44, 1.
34. G.G. FREEMAN, R.I.MORRISON, and S.E. MICHEAL,
Biochem. J., 1949, 45, 191.
35. S.E. MICHEAL, Biochem. J., 1948, 43, XXIV.
36. W.O. GODFREDSSEN and S. VANGEDAL,
Proc. Chem. Soc., 1964, 188.
37. A. ROBERTSON, W.R. SMITHIES, and E. TITTENSOR,
J. Chem. Soc., 1949, 879.
38. A. HARRIS, A. ROBERTSON and W.B. WHALLEY,
J. Chem. Soc., 1958, 1799.
39. A. HARRIS, A.ROBERTSON and W.B.WHALLEY,
J. Chem. Soc., 1958, 1807.
40. G.A. ELLESTAD, B. GREEN, A. HARRIS and W.B. WHALLEY,
J. Chem. Soc., 1965, 7246.
41. M.R. COX, G. ELLESTAD, A.J.HANNAFORD, I.R. WALLWORK
and W.B. WHALLEY, J. Chem. Soc., 1965, 7257.
42. J.J. BRITT, and D. ARIGONI, Proc. Chem. Soc., 1958, 224.
43. T. CAIRNS, G. EGLINTON, A.I. SCOTT and D.W. YOUNG,
J. Chem. Soc., 1966 (A), 255.
44. W.B. WHALLEY, B. GREEN, D. ARIGONI, J.J. BRITT and
C. DJERASSI, J. Amer. Chem. Soc., 1959, 81, 5520.

45. H.H. INHOFFEN, S. SCHUTZ, P. ROSSBERG, O. BERGES,
K. NORDSIK, H. PLENIO, and E. HOROLDT,
Ber., 1958, 91, 2626.
46. N.K. KOCHETKOV, and A.I. USOV,
Tetrahedron, 1963, 973.
47. A.I. SCOTT, S.A. SUTHERLAND, D.W. YOUNG, L. GUGLIELMETTI,
D. ARIGONI and G.A. SIM, Proc. Chem. Soc., 1964, 19.
48. L.F. FIESER, J. Amer. Chem. Soc., 1954, 76, 1945.
49. R. STEVENSON and L.F. FIESER,
J. Amer. Chem. Soc., 1956, 78, 1409.
50. S.R. LANDAUER, AND H.N. RYDON,
J. Chem. Soc., 1953, 2224.
51. L. CAGLIOTTI and P. GRASSELLI,
Chem and Ind., 1964, 153.
52. G.L. O'CONNER, and H.R. NACE,
J. Amer. Chem. Soc., 1955, 77, 1578.
53. N.S. BHACCA and D.H. WILLIAMS,
Applications of N.M.R. Spectroscopy
to Organic Chemistry, Holden - Day,
P.100.
54. D.W. YOUNG, Ph. D. Thesis, Glasgow, 1963.
55. R. JOLY, J. WARNANT, G. NOMINE and D. BERTIN,
Bull. Soc. Chim. France, 1958, 366.
56. E.J. COREY and R.A.E. WINTER,
J. Amer. Chem. Soc., 1963, 85, 2677.
57. R.A. IRELAND and P.W. SCHIESS,
J. Org. Chem., 1963, 28, 6.
58. G. FERGUSON, J.W.B. FULKE and R. McCRINDLE,
Unpublished Results.

59. J.D. CONNOLLY, D.M. GUNN, R. McCRINDLE, R.D.H. MURRAY,
and K.H. OVERTON, *Tetrahedron Letters*, 1966, 19, 2109.
60. R.A. IRELAND and L.N. MANDER,
Tetrahedron Letters, 1964, 3453.
61. Y. KITAHARA and A. YOSHIKOSHI,
Tetrahedron Letters, 1964, 1755.
- 61a. Y. KITAHARA, A. YOSHIKOSHI and S. OIDA,
Tetrahedron Letters, 1964, 1763.
62. J.D. CONNOLLY, Y. KITAHARA, K.H. OVERTON, and A. YOSHIKOSHI,
Chem. Pharm. Bull. (Japan), 1965,
13, 603.
63. R.E. PARTCH, *Tetrahedron Letters*, 1964, 3071.
64. D.H.R. BARTON, B.J. GARNER and R.H. WIGHTMAN,
J. Chem. Soc., 1964, 1855.
65. C.A. HENRICK and P.R. JEFFERIES,
Chem and Ind., 1963, 1801.
66. J.D. CONNOLLY, R. McCRINDLE, R.D.H. MURRAY, A.J. RENFREW,
K.H. OVERTON, and A. MELERA,
J. Chem. Soc., 1966, (C), 268.
67. J.D. COCKER and T.G. HALSALL,
J. Chem. Soc., 1956, 4262.
68. D. BURN and W. RIGBY, *Chem. and Ind.*, 1955, 386.
69. F.G. KING and G. JONES, *J. Chem. Soc.*, 1955, 658.
70. C. DJERASSI and D. MARSHALL,
Tetrahedron, 1957, 1, 238.
71. D.B. BIGLEY, N.A.J. ROGERS and J.A. BARLTROP,
J. Chem. Soc., 1960, 4613.
72. LEDERER and BORY, *Croat. Chem. Acta.*, 1957, 29, 157.

73. A.J. BAKER, G.EGLINTON, A.G. GONZALEZ, R.J. HAMILTON
and R.A. RAPHAEL, J. Chem. Soc., 1962, 4705.
74. E.M. GRAHAM and K.H. OVERTON,
J. Chem. Soc., 1965, 126.
75. J. MEINWALD, J.J. TUFARIELLO and J.J. HURST,
J. Org. Chem., 1964, 2914.
76. G. STORK and F.H. CLARKE, J. Amer. Chem. Soc., 1961, 83, 3114.
77. E.J. COREY, R.B. MITRA and H.UDA,
J. Amer. Chem. Soc., 1964, 86, 485.
78. D.H.R. BARTON, O.C. BOCKMAN and P. de MAYO,
J. Chem. Soc., 1960, 2263.
79. E.A. BRAUDE and E.S. WAIGHT,
Prog. in Org. Chem., Vol. I, Chpt.4.
80. J.S. BARAN,
J. Org. Chem., 1960, 25, 257.
81. A.I. VOGEL,
A Text Book of Practical Organic
Chemistry, P.891, (Ed. 1956).
82. H.B. HENBEST and B. NICHOLLS,
J. Chem. Soc., 1957, 4608.
83. G. STORK, A. MEISELS and J.E. DAVIES,
J. Amer. Chem. Soc., 1963, 85, 3419.
84. H.B. HENBEST, and T.I. WRIGLEY,
J. Chem. Soc., 1957, 4596.
85. K. BAUDEN, I.M. HEILBRON, E.R.H. JONES, and B.C.L. WEEDON,
J. Chem. Soc., 1946, 39.
86. H. BROCKMAN and H. SCHODDER,
Ber., 1941, 74, 73.
87. A.J. BIRCH and R.W. RICKARDS,
Private Communication.

88. G. SNATZKE, Private Communication.
89. E.M. GRAHAM, Ph.D. Thesis, Glasgow, 1964.
90. P.A. LEVENE and A. ROTHAN, Rotatory Dispersion in "Organic Chemistry", Vol. II, Chapt. 21, H. Gilman, (Ed.), John Wiley & Sons, Inc., New York, 1938.
91. C. DJERASSI, Bull. Soc. Chim. France, 1957, 741.
92. G. FERGUSON and R.D. MELVILLE? Unpublished Results.