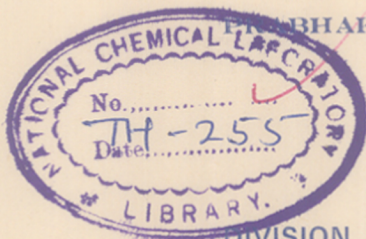


INVESTIGATION OF
NATURAL TERPENOIDS AND
THEIR TRANSFORMATION PRODUCTS

A **COMPUTERISED**
THESIS
SUBMITTED TO
THE UNIVERSITY OF POONA
FOR
THE DEGREE OF
DOCTOR OF PHILOSOPHY
IN
CHEMISTRY

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BY



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(P.P. Pai)

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CONTENTS

		Pages
General Introduction	••	1
General Remarks	••	11
<u>CHAPTER I</u>		
Isolation of α -anyrinstearata, β -anyrin and lupeol pamtates from the leaves and stalks of costus plant ana oxidation of β -anyrin and its acetate by Jonas' chromic acid reagent	••	13
<u>CHAPTER II</u>		
Oxidation studies on taraxasterol and its derivatives	••	53
<u>CHAPTER III</u>		
Oxidation stualas on (+)car-3-ene and its derivatives	••	111
<u>CHAPTER IV</u>		
Revised structure of nardostachone, a aaaquitarpanic ketoae, isolatad from	••	144

••••••••••

GENERAL INTRODUCTION

GENERAL INTRODUCTION

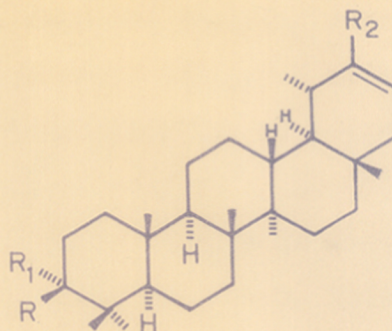
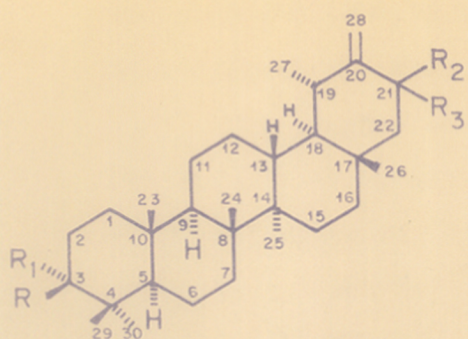
The study of natural products derived from the plant kingdom form an important aspect of organic chemistry. Studies of natural products not only increase and deepen our scientific knowledge but also provide a basis for highly developed industry.

The importance of the study of natural products in India can hardly be over emphasised, especially in view of the fact that India possesses a wealth of most abundant and diversified flora. This is but natural as this subcontinent is fortunate in having a variety of climatic and soil conditions, suitable for healthy growth of plants, herbs, grasses etc.

A great progress has been achieved in the chemistry of "Natural Products" as a result of the extensive work carried out by organic chemists in this field. Highly effective separation techniques such as precise fractionation, elaborate column chromatography, gas-liquid and thin layer chromatography and the modern physical methods of structure determination may be attributed to this phenomenal progress achieved during the last few years. The advent of gas-liquid and thin layer chromatography on preparative scale has opened up possibilities for the isolation of new compounds and the reaction products

in the purest form. The physical methods viz., ultraviolet, infrared, nuclear magnetic resonance spectroscopy, optical rotatory dispersion, circular dichroism, X-ray crystallography and mass spectrometry have revolutionized the successful elucidation of the structure and absolute configuration of organic compounds. Further, during this period, development of many new reagents and reactions have also helped in this work. The better understanding of the biogenetic pathways and organic chemical mechanistic theories also aided to arrive at the correct conclusions during the structure determinations. A brief summary of the present work is described below:

Though, extensive work has been carried out on the isolation and characterisation of the constituents, of both steam distilled^{1,2} as well as solvent extracted³ costus root oils (Sausurea lappa, Clarke), not much work has been reported on the constituents present in other parts of the plant. However, the hexane extract obtained by extraction of the leaves and stalks of the costus plant has been reported to contain triterpenoids from which only the isolation of taraxasterol (I) and its acetate (II) has been described⁴. On critical examination of the total hexane extract of the leaves and stalks, in addition to (I) and (II), three low melting triterpenoid esters viz. α -amyrinstearate, β -amyrinpalmitate and lupeol palmitate have been isolated⁵ by employing chromatography over alumina and silicic acid impregnated with silver nitrate,



I $R=OH, R_1=R_2=R_3=H.$

IV $R=OH, R_1=H, R_2=-CH_2OH.$

II $R=-O-\overset{\overset{O}{\parallel}}{C}-CH_3, R_1=R_2=R_3=H.$

V $R=-O-\overset{\overset{O}{\parallel}}{C}-CH_3, R_1=H, R_2=-CH_2OH.$

III $R, R_1=O, R_2=R_3=H.$

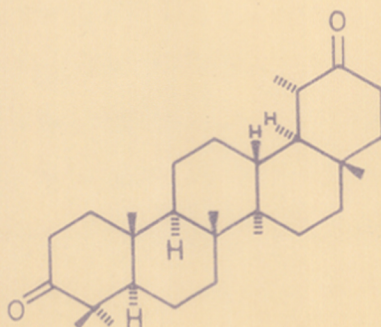
VI $R, R_1=O, R_2=-CH_2OH.$

X $R=-O-\overset{\overset{O}{\parallel}}{C}-CH_3, R_1=H,$

VII $R=-O-\overset{\overset{O}{\parallel}}{C}-CH_3, R_1=H, R_2=-CHO.$

$R_2, R_3=O.$

IX $R, R_1=O, R_2=-CHO.$



VIII

and characterised by spectral data and chemical reactions. This part of the work is described in Chapter I of the thesis.

The chapter also describes oxidation of β -amyrin and its acetate by Jones' chromic acid reagent⁶. In addition to the normal and allylic oxidation products, some more interesting oxidation products are also formed, which have been characterised by spectral data.

Chapter II deals with the oxidation studies of taraxasterol (I), its acetate (II) and taraxasterone (III) by m-chloroperbenzoic acid⁷ and also the oxidation studies of (I) and (II) by Jones' chromic acid reagent⁷ and selenium dioxide⁸.

Oxidation studies of taraxasteryl acetate (II) by perbenzoic acid has been described⁹ earlier. However, when taraxasterol (I), its acetate (II) and taraxasterone (III) are oxidised with m-chloroperbenzoic acid at 15°, the corresponding epoxides could not be isolated but their rearrangement products could be isolated and characterised⁷. These include the allylic alcohols (IV), (V) and (VI) and the corresponding C₂₀ nor-ketones.

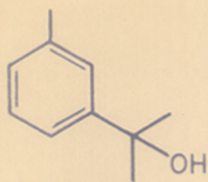
Jones' chromic acid oxidation⁷ of taraxasterylacetate (II) gives, in addition to the C₂₀ nor-ketoacetate, a conjugated aldehyde acetate (VII). When taraxasterol (I) is oxidised with the same reagent, in addition to the taraxasterone (III) and the diketone (VIII), the conjugated ketoaldehyde (IX) is also obtained.

Seleniumdioxide oxidation⁸ of taraxasterylacetate (II) gives, in addition to the normal conjugated ketoacetate (X), a selenoid which is formed by the reaction of two molecules of (II). Taraxasterol (I) with seleniumdioxide gives similar products.

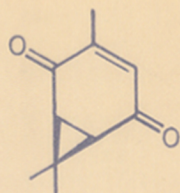
The work presented in Chapter III describes (1) oxidation studies on (+) car-3-ene by sodiumdichromate and acetic acid¹⁰ and (2) epoxidation studies on 4- α -acetyl-car-2-ene (XV) by m-chloroperbenzoic acid¹¹.

(+) Car-3-ene on oxidation with sodium dichromate and acetic acid¹⁰ gives as the main products (1) 8-hydroxy m-cymene (XI), (2) car-3-ene 2,5-dione (XII), (3) 1,1,4-trimethylcyclohepta, 2,4-diene-6-one (XIII) and (4) car-2-ene (XIV), which have been isolated by a single chromatography over alumina and characterised through spectral data and physical constants.

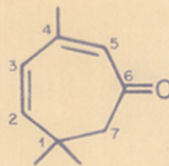
On treatment with acetic anhydride in the presence of zinc chloride (+) car-3-ene gives as the major product, 4- α -acetyl-car-2-ene¹² (XV). The latter on treatment with perbenzoic acid gives the corresponding α -epoxide. The epoxide on elution over alumina almost exclusively rearranges to a conjugated ketoalcohol (XVI). However, when treated with mineral acid in methanol, it affords paramenthane derivatives, identified as (XVII) and (XVIII).



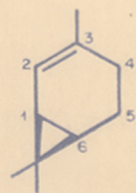
XI



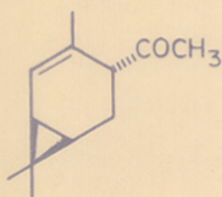
XII



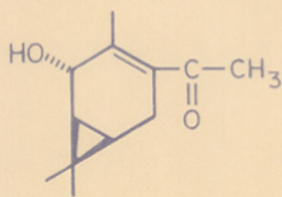
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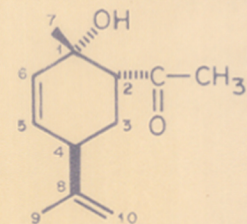
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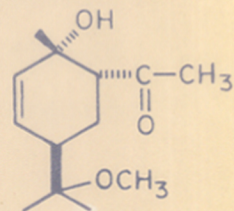
XV



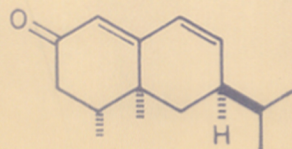
XVI



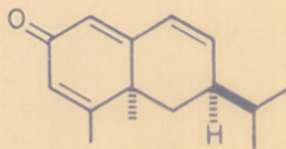
XVII



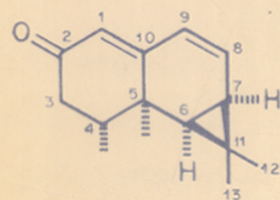
XVIII



XIX



XX



XXI

The work presented in Chapter IV describes the revision of the structure of nardostachone¹³, a sesquiterpenic ketone isolated from Nardostachys latamansi D.C.¹⁴. Sastry et al. assigned the structure¹⁵ (XIX) to the ketone. Later, Pinder¹⁶ synthesised a compound possessing the same structure (XIX) and found that the physical constants and spectral data of the synthetic specimen are not in agreement with those reported for the natural ketone. Pinder suggested an alternative structure (XX) to the ketone, on the basis of the spectral evidence already given by Sastry et al. However, the structure (XX), suggested by Pinder also does not fully explain the spectral characteristics of the compound. By careful and critical examination of the spectral data of the ketone, the revised tricyclic structure (XXI) possessing the aristolane skeleton is assigned to it.

During the course of these investigations liberal use of modern techniques such as infrared, ultraviolet, nuclear magnetic resonance and mass spectroscopy and thin layer chromatography has been made. For the isolation of the compounds, column chromatographic techniques using adsorbants like alumina, silica gel and silica gel impregnated with silver nitrate is employed.

For the sake of brevity, a general introduction to terpenoids has been avoided. The subject has already been covered adequately by a number of well-known publications of

which special mention may be made of the following:

- (1) Terpenes by J.L. Simonsen and W.C.J. Ross,
Vol.1-5 1957 edition (University Press, Cambridge).
- (2) Chemistry of Carbon Compounds, edited by
E.H. Rodds, Vol. IIB, IIC, 1953 edition (Elsevier
Publishing Co.).
- (3) The Essential Oils, Vol. 1-6, by E. Guenther
(D. Van Nostrand Co. Inc., N.Y.).

I gratefully acknowledge the help I received from
these publications, during the course of the investigations.

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16. A.R. Pinder, *Tetrahedron Lett.*, 413 (1970).

GENERAL REMARKS

1. All melting points and boiling points are uncorrected.
2. All temperatures are recorded on the Centigrade scale.
3. Unless otherwise stated, all rotations were taken in chloroform solutions. Concentrations are expressed in g/100 ml of the solution.
4. The ultraviolet spectra were recorded in ethanol solution on a Perkin-Elmer 350 and Carl-Zeiss 445069 ratio recording spectrophotometers.
5. The infrared spectra were recorded on a Perkin-Elmer infracord spectrophotometer, Model 137B and Model 221, with sodiumchloride optics.
6. The NMR spectra were taken in carbon-tetrachloride solution, unless otherwise mentioned, using tetramethylsilane as the internal reference on a A-60 and T-60 MC Varian instruments and the chemical shifts are measured in τ units.
7. The mass spectra were recorded on a CEC-21-110B mass spectrometer.
8. Acid washed activated alumina standardised as per Brockmann's procedure and silicic acid for chromatographic purposes, after activation, were employed for column chromatography.
9. TLC analysis were carried out on glass plates coated with a mixture of silicic acid and plaster of Paris (85:15:200 mesh)

and activated at 120° for 3 hr. Solvent systems used were pet.ether, benzene, ethylacetate and acetone or a suitable mixture of two or more of these solvents, depending upon the nature of the compounds. The plates were developed by keeping in an iodine chamber or by spraying with H_2SO_4 .

10. Numbers given to charts, figures and structures in each chapter of the thesis refer to that particular chapter only.

11. The list of references pertaining to each chapter has been given at the end of that chapter.

12. Unless otherwise stated, all solutions were dried over anhydrous sodiumsulphate.

13. Unless otherwise stated, all b.ps. refer to the bath temperatures.

14. In the list of IR bands given in the experimental section, the significant bands described in the theory are underlined.

15. A brief summary of each chapter is given at the beginning of that chapter.

16. In the description of NMR signals, the abbreviations brs, brd and brm means broad singlet, broad doublet and broad multiplet respectively.

CHAPTER - I

ISOLATION OF α -AMYRINSTEARATE, β -AMYRIN AND LUPEOL
PALMITATES FROM THE LEAVES AND STALKS OF COSTUS PLANT
AND OXIDATION OF β -AMYRIN AND ITS ACETATE BY JONES'
CHROMIC ACID REAGENT

S U M M A R Y

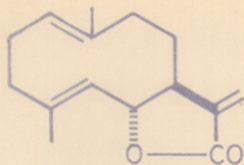
From the leaves and stalks of the costus plant, Saussurea Lappa, clarke, in addition to taraxasterol (VI) and taraxasteryl acetate (VII), three low melting triterpenoid-esters, Ester A, Ester B and Ester C have been isolated in the pure state. These have been identified as α -myrin-stearate (Ester A), β -myrin palmitate (Ester B) and lupeol palmitate (Ester C), by saponifying them, and identifying and characterising the alcohol and the acid components obtained after saponification.

Oxidation of β -myrin (XI) by Jones' chromic acid reagent furnishes in addition to the β -myrone (XVIII) and β -myrene 3,11-dione (XIX), an oxidodiketone to which a tentative structure (XXI) has been assigned on the basis of spectral data. The acetate (XII), on oxidation with the same reagent gives in addition to the known 11-keto β -myrin acetate (XIII), two other isomeric keto acetates which have been assigned structures (XXII) and (XXIV) on the basis of spectral data and chemical reactions.

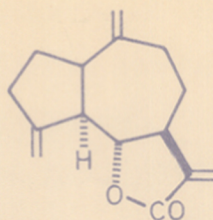
The costus plant, Saussurea Lappa, Clarke, belongs to the compositae family and is grown in India in Kashmir and the hill districts of Punjab at high altitudes¹. The roots of the plant are known to possess medicinal properties and have been prescribed in the indigenous system of medicine^{2,3,4}. The roots contain a fragrant principle which can be isolated either by steam distillation or solvent extraction. The essential oil obtained from the roots is used in high grade perfumary preparations. In our laboratory, a low temperature solvent extraction procedure for extracting costus root oil, using 40-60° pet.ether, has been developed. The oil obtained by this method is free from polymerisation and denaturing.

The systematic examination of the solvent extracted costus root oil resulted in the isolation of several compounds some of which are mentioned below. Costunolide (I), dehydrocostuslactone (II), costol (III), costic acid (IV) and aplotoxene (V) etc.⁵⁻¹⁴. However, the leaves and stalks of the costus plant have not been systematically examined so far, though the isolation of an alkaloid "saussurin" has been reported¹⁵ from them.

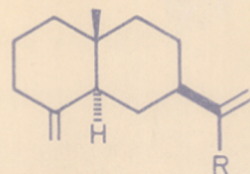
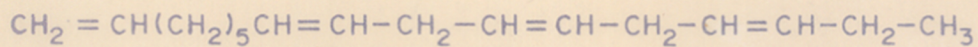
The isolation of taraxasterol (VI) and its acetate (VII) from the hexane extract of the leaves and stalks of the costus plant has been reported¹⁶ by C.P. Nambodripad et al. They extracted the leaves and stalks with hexane to give a dark



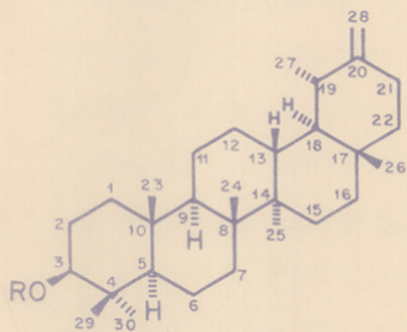
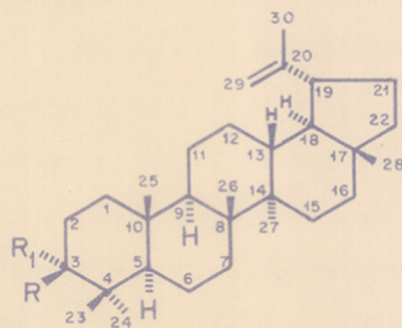
I



II

III $R = -CH_2OH$ IV $R = -COOH$ 

V

VI $R = H.$ VII $R = -COCH_3.$ XV $R = OH, R_1 = H.$ XVI $R = OCOCH_3, R_1 = H.$ XVII $R, R_1 = O.$ Ester C $R = OCO(CH_2)_{14}CH_3, R_1 = H.$

green coloured concentrate in 3.4% yield which on chromatography over alumina has been reported to yield taraxasteryl acetate (VII) and also taraxasterol (VI). These compounds have been identified by physical constants (m.p., m.m.p. and rotation) and spectral data (IR and NMR). Except these two compounds, no other compound has been reported from the extract of leaves and stalks. We therefore felt it desirable to isolate the other major compounds of the hexane extract and characterise them.

PRESENT WORK

When a pet.ether solution of the hexane extract is cooled to -18° for 48 hr., some solid separated which is filtered. TLC examination of the solid indicates it to consist of mainly two compounds. The solid on chromatography over alumina (Gr. III, 1:25 ratio) gives a crystalline solid m.p. $252-54^{\circ}$ (hexane + ethyl acetate), $(\alpha)_D + 99.1^{\circ}$, in the earlier fractions eluted with pet.ether. It has been identified by physical constants (m.p., m.m.p. and rotation) and spectral data (IR) as (VII). The fraction eluted with benzene gives a solid which on crystallisation from (hexane + ethylacetate) followed by acetone melts at 222° , $(\alpha)_D + 90^{\circ}$. This has been identified as taraxasterol (VI) by spectral data (IR) and physical constants (m.p. and m.m.p.).

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The mother liquor left after the removal of solids is taken for further examination. Initial examination of this extract by chromatography over alumina (Gr. I, 1:20 ratio) indicates that a major portion of the material is eluted with pet.ether in the earlier fractions. Examination of IR spectra of these fractions suggests it to consist of carbonyl compounds (1739 cm^{-1}); Since it fails to give any solid derivatives with 2,4 DNP, it was thought that the fraction contains mainly esters.

Based on these preliminary observations the mother liquor is chromatographed on alumina (Gr. I, 1:20 ratio) and eluted with pet.ether, pet.ether + benzene (1:1), pet.ether + benzene (1: 2), benzene and chloroform. The fraction eluted with pet.ether gives an oily liquid which on TLC analysis ($\text{AgNO}_3/\text{SiO}_2$, 20% benzene in hexane) indicates it to be a mixture of five compounds. In order to effect a rough separation between esters containing trisubstituted double bonds and those containing exocyclic methylenic double bonds, the above fraction is chromatographed initially on silicic acid impregnated with 10% silver nitrate, followed by rechromatography of some of the fractions obtained that are enriched in one component. By crystallisation of appropriate fractions, it has been possible to isolate three low melting esters, designated as A,B,C in pure state (TLC). From the pet.ether + benzene (1:1) fraction of the main chromatography,

teraxasteryl acetate (VII) has been isolated by rechromatography followed by crystallisation. Similarly taraxasterol (VI) has been isolated from the pet.ether + benzene (1:2) fraction of the main chromatography.

Ester A, m.p. $46-48^{\circ}$ (ethanol + acetone, 1:4) (α)_D + 61° (benzene, c, 1.84), $C_{48}H_{84}O_2$ shows in its IR spectrum (Fig. 1) a band at 1739 cm^{-1} ($>C=O$) and a strong band at 720 cm^{-1} characteristic of compounds containing more than three adjacent $-CH_2-$ groups in a chain. The NMR (CCl_4) spectrum indicates the presence of $-CH=C<$ grouping (signal at 4.8 τ). Since it fails to give any derivative, characteristic of ketones, it is suspected to be an ester. For identification, it is saponified to isolate the alcohol and the acid components.

The alcohol (VIII), $C_{30}H_{50}O$, M^+ 426, m.p. $183-84^{\circ}$ (hexane + 10% benzene), (α)_D + 83° (c, 1) shows in its IR spectrum a band at 3280 cm^{-1} (OH). It gives an acetate (IX), $C_{32}H_{52}O_2$, m.p. $220-222^{\circ}$ (EtAc), (α)_D + 81° (c, 2.1); IR bands at 1740 and 1252 (acetate); NMR (CCl_4) signals at; 9.2, 9.14, 8.98, 8.94 (24H, methyls at C_4 , C_8 , C_{10} , C_{14} , C_{17} , C_{19} and C_{20}); 8.04 (3H, s, acetate methyl at C_3); a triplet centred at 5.64 (1H, O, $J = 8\text{ Hz}$, C_3-H) and a broad singlet at 4.9 (1H, olefinic proton at C_{12}). Oxidation of (IX) with sodiumdichromate and acetic acid¹⁷ furnishes a conjugated ketoacetate (X), $C_{32}H_{50}O_3$, m.p. $274-76^{\circ}$ (EtAc),

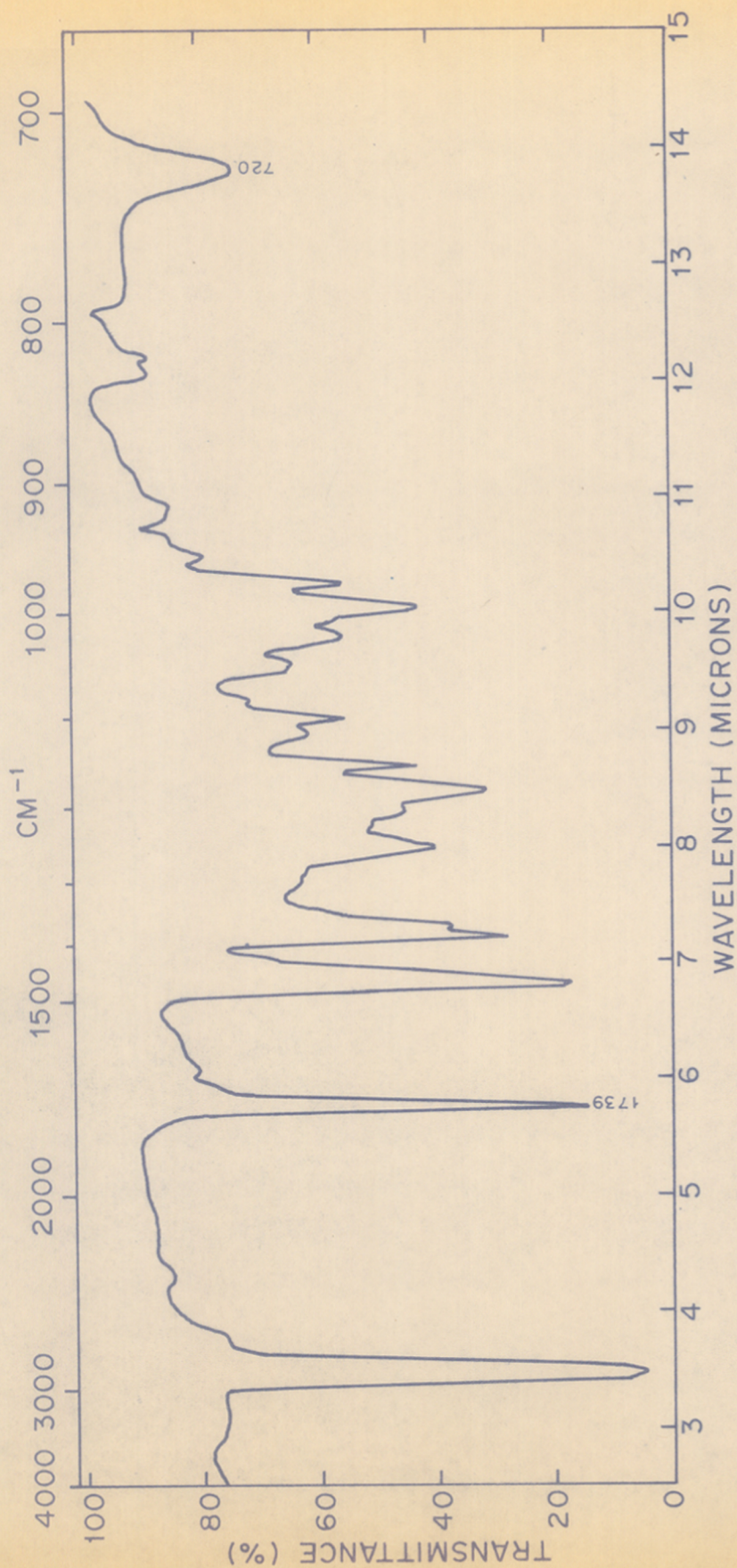


FIG. 1. IR SPECTRUM OF α -AMYRINSTEARATE (ESTER, A) (NUJOL)

(α)_D + 97° (c, 0.6). The ketoacetate (X) shows IR bands at 1730 ($>C=O$ of ester), 1250 (acetate), 1670 (conj. $>C=O$) and 1626 cm^{-1} (conj. $>C=CH-$); UV λ_{max} 248 m μ , ϵ_{max} 12000, NMR (CCl_4) signals at 9.2, 9.17, 9.04, 8.83, 8.78 (24H, methyls at C₄, C₈, C₁₀, C₁₄, C₁₇, C₁₉ and C₂₀); 8.0 (3H, s, acetate methyl at C-3); 7.75 (1H, s, C₉-H, adjacent to $>C=O$); a triplet centred at 5.6 (1H, J = 7.8 Hz, C₃-H) and at 4.6 (1H, s, olefinic proton at C₁₂). From these data the alcohol is identified as α -myrin (VIII) and the identity is confirmed by comparison of the physical constants and IR spectra of compounds (VIII), (IX) and (X) with those of an authentic samples reported in lit.^{18,19}

The acid component is crystallised from ethanol to give a solid m.p. 69°, C₁₈H₃₆O₂, M⁺ 284. It is identified as stearic acid from physical constants²⁰ and IR spectrum. The ester A therefore has been identified as α -myrin-stearate (Lit.^{21a} records for α -myrinsteearate m.p. 45°).

Ester B, C₄₆H₈₀O₂, M⁺ 664, m.p. 76-78° (acetone + 10% hexane), (α)_D + 57° (benzene, c, 2.8), shows IR (Fig. 2) absorption bands at 1736 ($>C=O$ of ester) and 720 cm^{-1} characteristic of compounds containing more than three adjacent -CH₂-groups in a chain. Its NMR (CCl_4) shows signals at 9.08, 9.02, 8.97, 8.67 (27H, methyls at C₄, C₈, C₁₀, C₁₄, C₁₇, C₂₀ and the end methyl of the aliphatic side chain of the ester moiety), 5.55 (1H, m, C₃-H) and at 4.84 (1H, brs,

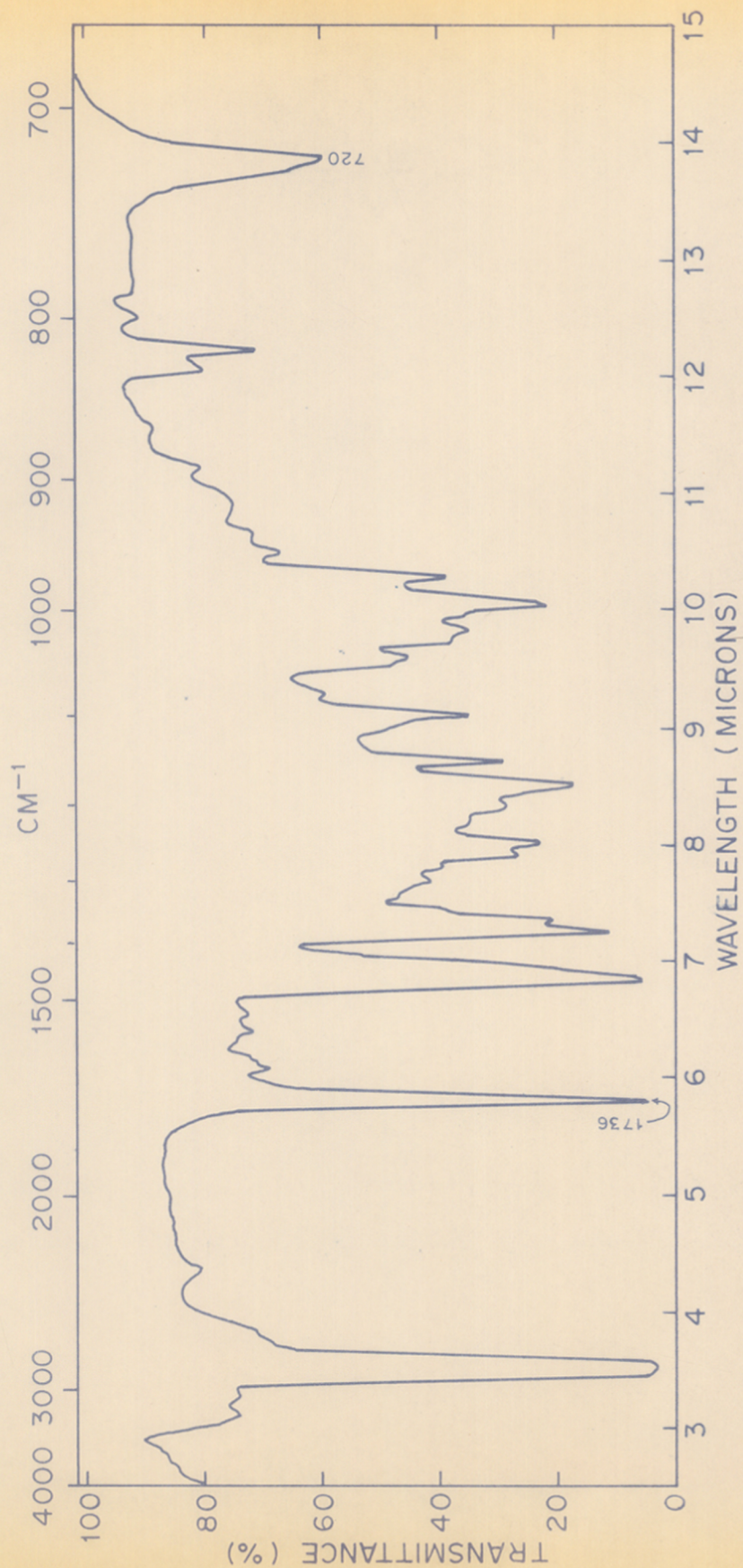
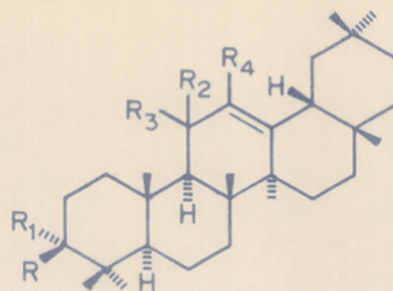
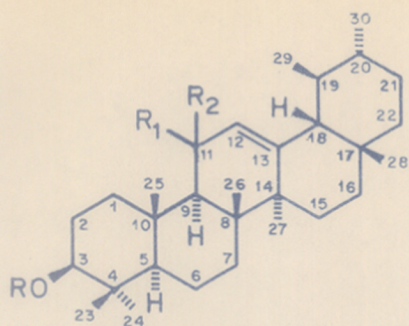


FIG. 2. IR SPECTRUM OF β -AMYRYNPALMITATE (ESTER, B) (NUJOL)



VIII $R=R_1=R_2=H$.

IX $R=-COCH_3$, $R_1=R_2=H$.

X $R=-COCH_3$, $R_1, R_2=O$.

Ester A $R=-CO(CH_2)_{16}CH_3$,
 $R_1=R_2=H$.

XI $R=OH$, $R_1=R_2=R_3=R_4=H$.

XII $R=-OCOCH_3$, $R_1=R_2=R_3=R_4=H$.

XIII $R=-OCOCH_3$, $R_1=R_4=H$, $R_2, R_3=O$.

XIV $R=-OCO(CH_2)_{14}CH_3$, $R_1=R_4=H$,
 $R_2, R_3=O$.

Ester B $R=-OCO(CH_2)_{14}CH_3$,
 $R_1=R_2=R_3=R_4=H$.

XVIII $R, R_1=O$, $R_2=R_3=R_4=H$.

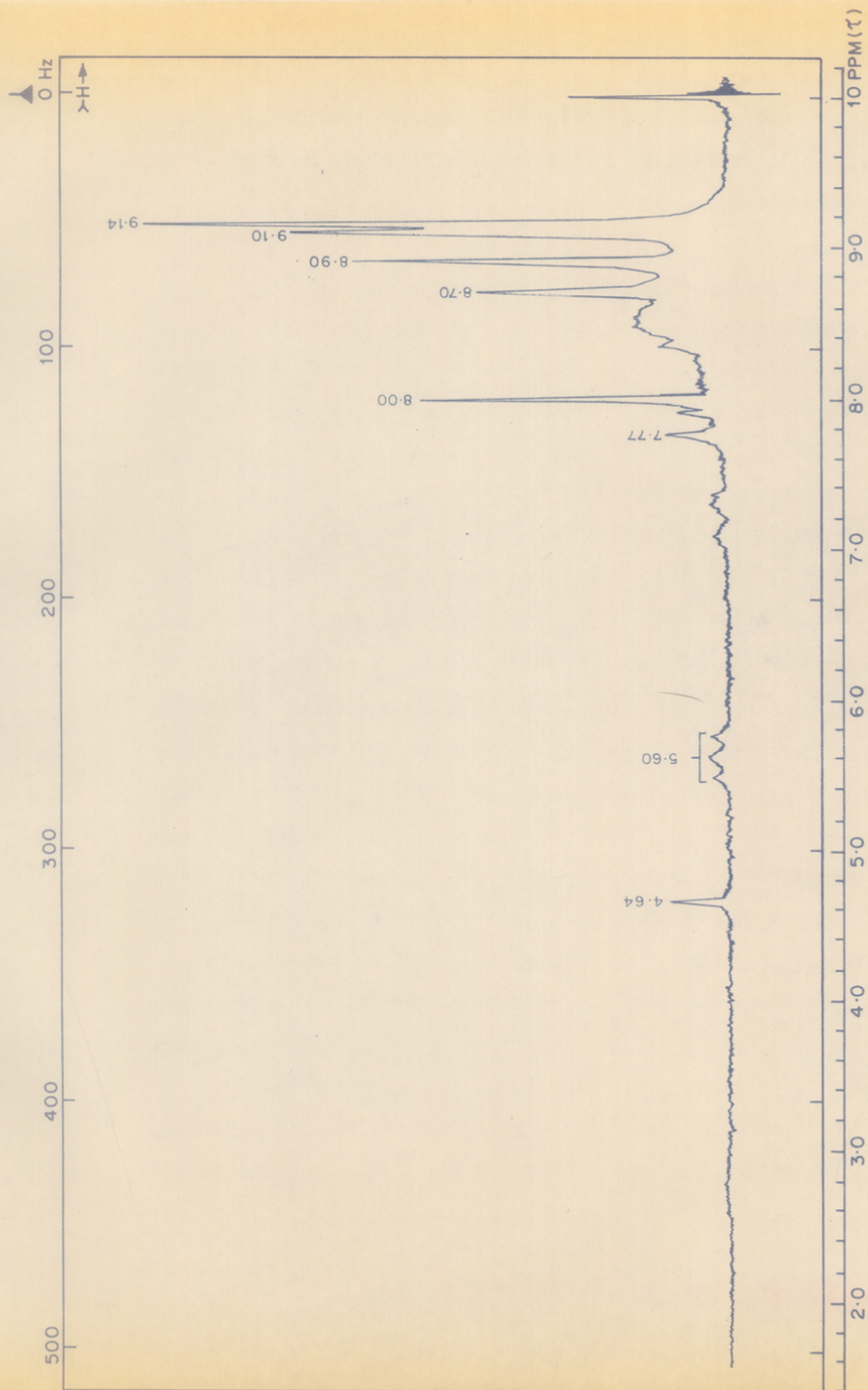
XIX $R, R_1=O$, $R_2, R_3=O$, $R_4=H$.

XXV $R_1=R_2=R_3=H$, $R=R_4=-O-\overset{\overset{O}{\parallel}}{C}-CH_3$.

olefinic proton at C₁₂). It is saponified to isolate the alcohol and the acid components.

The alcohol (XI), C₃₀H₅₀O, M⁺ 426, m.p. 195-96° (hexane + 10% benzene), $\alpha_D + 91^\circ$ (benzene, c, 2), shows IR bands at 3400 cm⁻¹ (OH); NMR (CCl₄) signals at 9.25, 9.23, 9.2, 9.15, 9.03, 8.95 (24H, methyls at C₄, C₈, C₁₀, C₁₄, C₁₇ and C₂₀), 6.87 (1H, t, J = 8 Hz, C₃-H) and at 4.87 (1H, brs, olefinic proton at C-12). It gives an acetate (XII), C₃₂H₅₂O₂, m.p. 236-38° (ethyl acetate), $(\alpha)_D + 82^\circ$ (c, 1.5). The compound (XII) shows IR bands at 1739 and 1253 cm⁻¹ (acetate); NMR (CCl₄) signals at 9.15, 9.1, 9.04, 8.92 (24H, methyls at C₄, C₈, C₁₀, C₁₄, C₁₇ and C₂₀); a triplet centred at 5.6 (1H, J = 10 Hz, C₃-H) and 4.89 (1H, brs, olefinic proton at C-12). On oxidation with sodium-dichromate-acetic acid¹⁷, compound (XII) furnishes a conjugated ketoacetate (XIII), C₃₂H₅₀O₃, m.p. 264° (ethyl-acetate), $(\alpha)_D + 110^\circ$ (c, 0.9); UV $\lambda_{\max}$, 247 mμ; $\epsilon_{\max}$ 15,360; IR bands at 1733 (>C = O of ester), 1250 (acetate), 1666 (conj. >C = O) and 1613 cm⁻¹ (conj. >C = CH). Its NMR (CCl₄) (Fig. 3) spectrum shows signals at 9.14, 9.1, 8.9, 8.78 (24H, methyls at C₄, C₈, C₁₀, C₁₄, C₁₇ and C₂₀); 8.0 (3H, s, acetate methyl at C-3), 7.77 (1H, s, C₃-H adjacent to >C = O); a triplet centred at 5.6 (1H, J = 8.4 Hz, C₃-H) and at 4.64 (1H, C-12 olefinic proton). From the above spectral data the alcohol (XI) has been identified as

* 8.05 (3H, s, acetate methyl at C₃);

FIG. 3. NMR SPECTRUM OF 11-KETO β -AMYRIN ACETATE (XIII) (CCl_4)

β -amyrin and the identity is further confirmed by comparison of physical constants and IR spectra of (XI), (XII) and (XIII) with those of authentic samples reported in lit.²²

The acid component $C_{16}H_{32}O_2$, m.p. 62° (ethanol), M^+ 256 is identified as palmitic acid from m.p.²³, analysis and spectral data. Ester B therefore, has been identified as β -amyrin palmitate (lit.^{21b} records for β -amyrin palmitate m.p. $77-78^\circ$, $(\alpha)_D + 54^\circ$).

Allylic oxidation of ester B by sodium dichromate-acetic acid¹⁷ furnishes the 11-keto- β -amyrinpalmitate (XIV), which has been identified by spectral data. Compound (XIV), m.p. 148° (ethyl acetate), $C_{46}H_{78}O_3$, M^+ 678 shows IR bands at 1736 ($>C=O$ of ester), 1660 (conj. $>C=O$) and 1613 cm^{-1} (conj. $>C=C<$); UV λ_{max} , 248 $m\mu$, ϵ_{max} 12500. Its NMR (CCl_4) spectrum shows characteristic signals at 9.13, 9.01, 8.87 (27H, methyls at $C_4, C_8, C_{10}, C_{14}, C_{17}, C_{20}$ and the end methyl of the aliphatic side chain of the ester moiety), 7.75 (3H, C_9 -H adjacent to $>C=O$ group and CH_2 -adjacent to ester $>C=O$), a triplet centred at 5.53 (1H, $J = 8.6$ Hz, C_3 -proton) and at 4.5 (1H, olefinic proton at C_{12}). From the above spectral data the compound has been assigned the structure (XIV).

Ester C, $C_{46}H_{80}O_2$, m.p. $77-78^\circ$ (acetone), $(\alpha)_D + 26^\circ$ (benzene, c, 2.8) shows IR (Fig. 4) bands at 1730 ($>C=O$ ester), 1639 , 887 ($>C=CH_2$) and 718 cm^{-1} (more than three $-CH_2-$

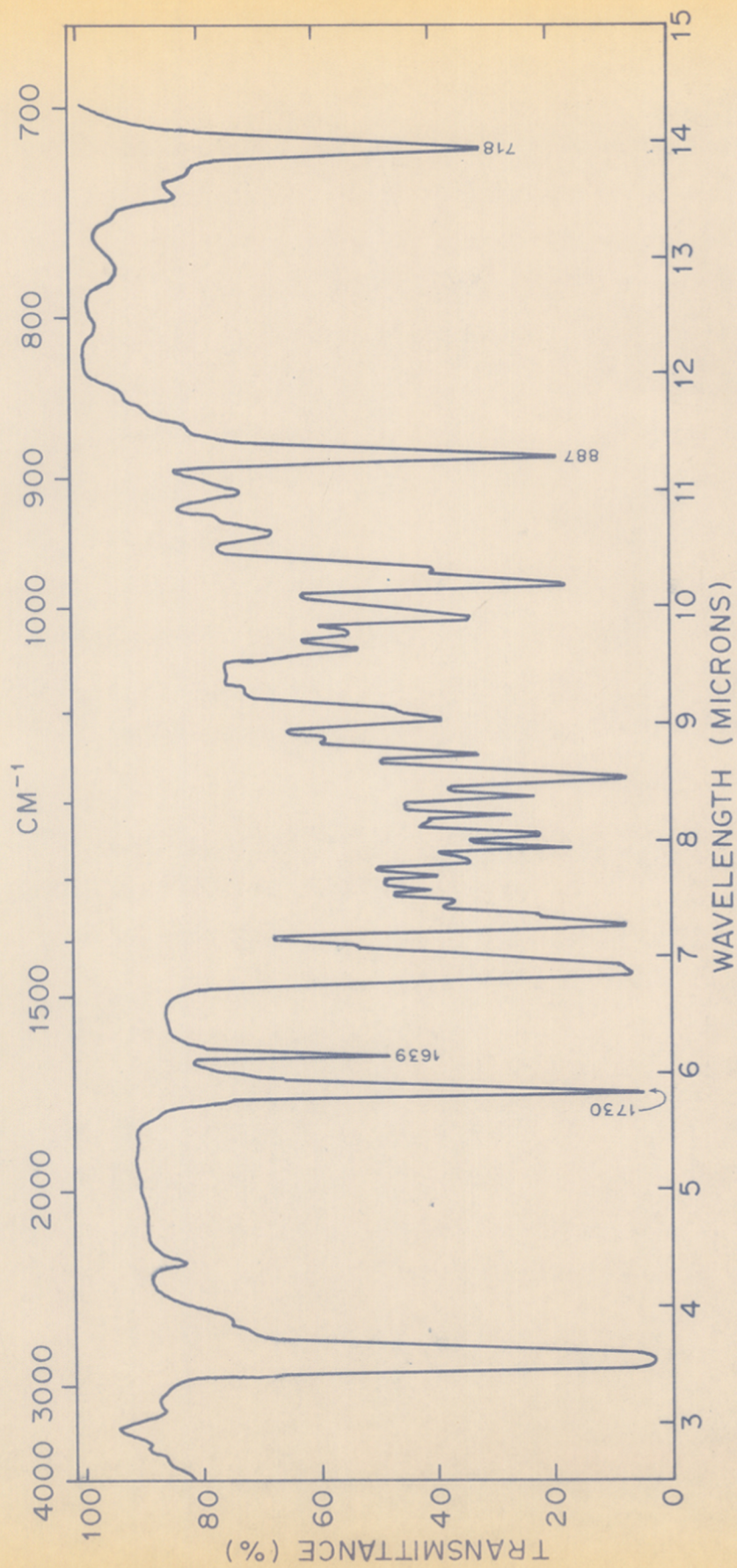


FIG. 4. IR SPECTRUM OF LUPEOLPALMITATE (ESTER, C) (NUJOL)

groups in a chain) and NMR (CCl_4) signals at (5.4, 2H, broad multiplet, $>\text{C} = \text{CH}_2$) in addition to the methyl signals and the signal due to $\text{C}_3\text{-H}$. It is saponified to isolate the alcohol and the acid components.

The alcohol (XV), $\text{C}_{30}\text{H}_{50}\text{O}$, $M^+ 426$, m.p. $214-16^\circ$ (methanol + acetone, 3:1), $(\alpha)_D + 28^\circ$ (c, 2.5); shows IR bands at 3400 (OH), 1639, 887 cm^{-1} ($>\text{C} = \text{CH}_2$); NMR (CCl_4) signals at 9.28, 9.22, 9.18, 9.04, 8.97 (18H, methyls at $\text{C}_4, \text{C}_8, \text{C}_{10}, \text{C}_{14}$ and C_{17}), 8.34 (3H, s, C_{20} methyl on double bond), 6.94 (1H, m, $\text{C}_3\text{-H}$) and at 5.4 (2H, brn, $>\text{C} = \text{CH}_2$). It gives an acetate (XVI) $\text{C}_{32}\text{H}_{52}\text{O}_2$, m.p. $217-18^\circ$ (ethyl acetate), $(\alpha)_D + 43^\circ$ (c, 1.2). The compound (XVI) shows IR bands at 1740, 1253 (acetate), 1642 and 885 cm^{-1} ($>\text{C} = \text{CH}_2$); NMR (CCl_4) signals at 9.24, 9.22, 9.17, 9.07, 8.97 (18H, methyls at $\text{C}_4, \text{C}_8, \text{C}_{10}, \text{C}_{14}$ and C_{17}), 8.35 (3H, s, C_{20} methyl on double bond), 8.02 (3H, s, acetate methyl), a triplet centred at 5.7 (1H, $J = 9$ Hz, $\text{C}_3\text{-H}$) and a multiplet centred at 5.4 (2H, $>\text{C} = \text{CH}_2$). Jones' chromic acid oxidation of (XV) gives a ketone (XVII) $\text{C}_{30}\text{H}_{48}\text{O}$, $M^+ 424$, m.p. $169-71^\circ$ (acetone), $(\alpha)_D + 57^\circ$ (c, 1.5). It shows IR bands at 1704 ($>\text{C}=\text{O}$), 1429 ($-\text{C}-\text{CH}_2$), 1645, 896 and 878 cm^{-1} ($>\text{C} = \text{CH}_2$); NMR (CCl_4) signals at 9.2, 9.04, 9.0, 8.97, 8.9 (18H, methyls at $\text{C}_4, \text{C}_8, \text{C}_{10}, \text{C}_{14}$ and C_{17}), 8.3 (3H, s, C_{20} methyl on double bond), 7.7 (2H, m, methylene protons at C_2 adjacent to $>\text{C} = \text{O}$) and a broad multiplet centred at 5.44 (2H, $>\text{C} = \text{CH}_2$). It gives

2:4 DNP derivative $C_{36}H_{52}O_4N_4$, m.p. 212-14°. From the above data alcohol (XV) has been identified as lupeol and the identity is confirmed by m.m.p. determination with an authentic sample and superimposable IR spectra of both (XV) and (XVI).

The acid component, m.p. 62° (ethanol), $C_{16}H_{32}O_2$, M^+ 256 has been identified as palmitic acid by m.p.²³, analysis and IR spectrum.

Ester C therefore, has been identified as lupeol palmitate (lit.^{21c} records for lupeol palmitate m.p. 79°).

Although β -amyrin palmitate has been isolated from natural sources²⁴⁻²⁶, the other two esters viz. α -amyrin stearate and lupeol palmitate appear to have been not isolated from the natural sources. However, they have been prepared synthetically^{21a,c}.

β -Amyrin (XI) on oxidation with chromic acid in acetic acid has been reported to give β -amyrene (XVIII)^{27,28} and β -amyrene, 3, 11-dione²⁹⁻³¹ (XIX). When β -amyrin-acetate (XII) is oxidised with potassiumpermanganate or hydrogen peroxide in acetic acid solution^{32,33} or chromic acid in acetic acid^{34,35} β -amyranonyl acetate (12,13 dihydro, 12-keto β -amyrin acetate) (XX) is obtained. Chromic acid oxidation³⁶ of β -amyrin acetate (XII) also gives β -amyrenonyl acetate (XIII) (11-keto β -amyrin acetate). In the oxidation of β -amyrin (XI) and β -amyrinacetate (XII) by chromic acid

in acetic acid, in addition to the oxidation of the functional group, allylic oxidation also takes place at C₁₁. We were interested to see whether the Jones' chromic acid oxidation of (XI) and (XII) give only the products with the oxidation of functional group or allylic oxidation products in addition to the normal products of oxidation of functional group.

PRESENT WORK

When oxidised with Jones' chromic acid reagent (1 hr. acetone, at room temperature), β -amyrin (XI) gives in addition to β -amyrone (XVIII) and β -amyrene, 3, 11-dione (XIX), two more compounds in minor quantity. These have been separated by chromatography (silicic acid) and purified by crystallisation. The least polar compound, C₃₀H₄₈O, M⁺ 424, m.p. 180-82° (acetone), (α)_D + 114° (c, 1.2), semicarbazone C₃₁H₅₁ON₃, m.p. 248-50° has been identified as β -amyrone (XVIII). It shows IR bands at 1710 (>C = O) and 1424 cm⁻¹ (-CO-CH₂); NMR (CCl₄) signals at 9.17, 9.12, 9.0, 8.94, 8.87 (24H, methyls at C₄, C₈, C₁₀, C₁₄, C₁₇ and C₂₀), 7.67 (2H, m, methylene protons at C₂ adjacent to >C = O) and at 4.87 (1H, brs, C₁₂ olefinic proton). The compound eluted next to (XVIII), C₃₀H₄₆O₂, M⁺ 438, m.p. 235-37° (hexane), (α)_D + 140° (c, 0.8); monosemicarbazone C₃₁H₄₉O₂N₃, m.p. 244-46°, UV $\lambda_{\max}$ 252 m μ , $\epsilon_{\max}$ 12500; IR bands at 1715 (>C = O), 1666 (conj. >C = O),

1613 (conj $>C = CH$) and 1426 ($-CO-CH_2$); NMR (CCl_4) signals at 9.1, 8.97, 8.84, 8.77, 8.67 (24H, methyls at C_4 , C_8 , C_{10} , C_{14} , C_{17} and C_{20}), 7.7 (2H, s, methylene protons at C_2 adjacent to $>C=O$) and at 4.5 (1H, s, olefinic proton at C_{12}), has been identified as β -anyrene, 3, 11-dione (XIX). (Lit.³¹ records for (XIX) m.p. 237°, monosemicarbazone m.p. 232°).

The compound eluted next to (XIX), a minor product, $C_{30}H_{46}O_3$, M^+ 454, m.p. 232-35° (hexane) shows IR bands at 1700 ($>C=O$) and 1405 cm^{-1} ($-C-CH_2$). Its NMR (CCl_4) spectra shows signals at 9.08, 9.0, 8.95, 8.8 (24H, methyls at C_4 , C_8 , C_{10} , C_{14} , C_{17} and C_{20}), a multiplet centred at 7.62 (2H, C_2 methylenic protons adjacent to $>C=O$) and two doublets centred at 7.37, 7.42 (2H, $J = 6$ Hz each, the α and β -protons of the C_{12} methylene group which is adjacent to the 11-keto group). Such a difference in the chemical shifts of the α and β -protons of the C_{12} methylene group adjacent to $>C=O$ group have already been observed in the case of some steroidal compounds in lit.⁴⁸. The NMR spectrum indicates the absence of olefinic protons which is supported by the fact that the compound shows -ve TMM test. The additional oxygen may be present as an ether or oxide. The possibility of an epoxyketone (epoxide at C_{12} , C_{13}); is ruled out due to the absence of any signal in the region between 6 to 7 τ . Structure (XXI) is considered as one of the possibilities, which may be resulting from (XIX) by oxidation at C_9 ,

followed by ring closure. The fourth compound could not be isolated pure.

Jones' chromic acid oxidation of β -amyrinacetate (XII) (5 hr, in refluxing benzene and acetone) leads to a mixture of four compounds (TLC). These have been separated by chromatography (alumina and silicic acid) and purified by crystallisation. The major product (least polar), $C_{32}H_{50}O_3$, m.p. 263-64°, (ethyl acetate), $(\alpha)_D + 108^\circ$ (c, 1.2); UV λ_{max} 248 m μ , ϵ_{max} 14,300 has been identified³⁶ as 11-keto β -amyrinacetate (XIII). The other ketoacetate eluted in the later fractions, $C_{32}H_{50}O_3$, M^+ 482, m.p. 285° (ethyl acetate) is assigned structure (XXII) on the basis of spectral data given below. It shows IR (Fig. 5) bands at 1725, 1250, (acetate), 1690 (conj. $>C=O$), 1650 (conj. $>C=C<$) and 1410 cm^{-1} ($-C-CH_2-$). The relative intensity³⁷ of bands at 1690 and 1650 cm^{-1} suggests the conjugated ketone chromophore to be in cisoid configuration. This is supported by UV λ_{max} 254 m μ , ϵ_{max} 4000. Its NMR (CCl_4) spectrum shows signals at 9.13, 9.08, 8.88 (24H, methyls at C_4 , C_8 , C_{10} , C_{14} , C_{17} and C_{20}), 8.02 (3H, s, acetate methyl at C_3), 7.5 (2H, m, C_{11} methylene protons adjacent to $>C=O$) and at 5.67 (1H, m, C_3 -H). Since the NMR spectrum shows the absence of olefinic protons, the conjugated double bond must be tetrasubstituted, which ultimately suggests that the original trisubstituted double bond in (XII) has migrated to the tetrasubstituted position

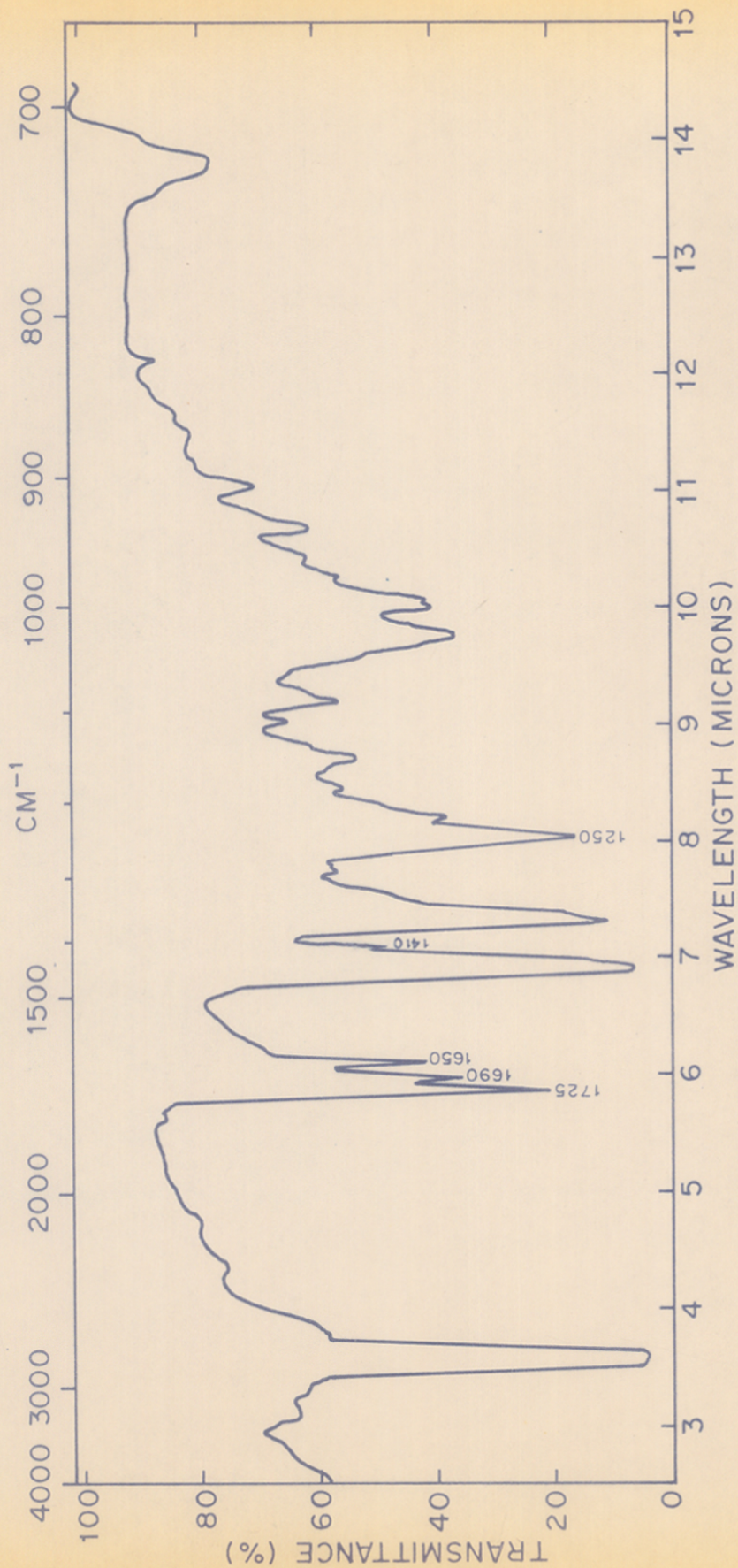
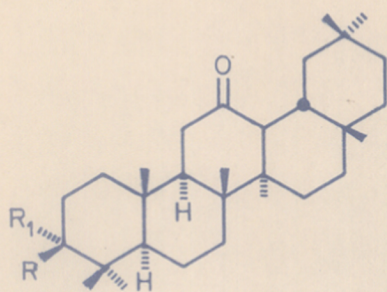
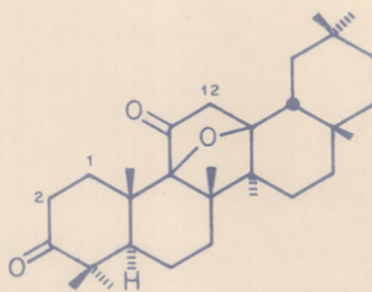


FIG. 5. IR SPECTRUM OF 12-KETOACETATE (XXII) (NUJOL)

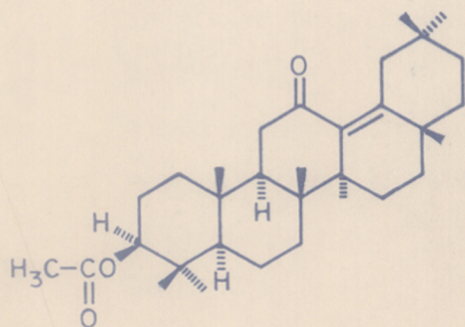


XX $R_1 = H$, $R = -OC(=O)CH_3$

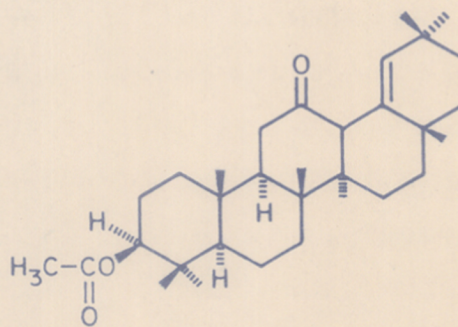


XXI

XXIII $R, R_1 = O$



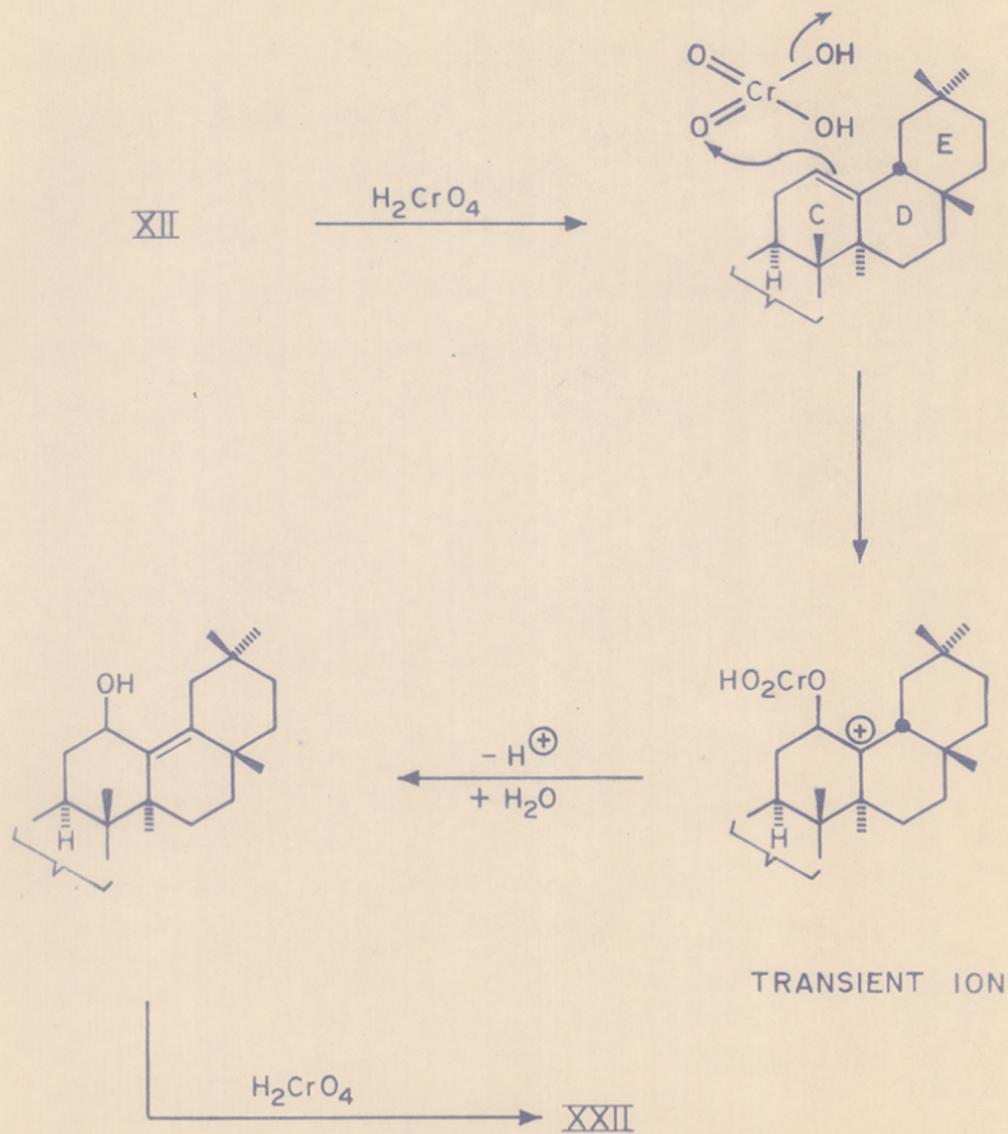
XXII



XXIV

during the formation of (XXII). Though the spectral data of (XXII) indicate the presence of $-\text{COCH}_2$ -group, it fails to give any semicarbazone or oxime. The 3,12-dione (XXIII) $\text{C}_{30}\text{H}_{48}\text{O}_2$, m.p. 218-220°, obtainable from β -amyrone (XVIII) by oxidation with *m*-chloroperbenzoic acid gives only a monosemicarbazone $\text{C}_{31}\text{H}_{51}\text{O}_2\text{N}_3$, m.p. 255-56°, the semicarbazone formation taking place only at the C_3 carbonyl group (a monoxime m.p. 247-48° is also reported³⁸). These data indicate that the keto group at C_{12} is in a hindered position and does not take part in the formation of derivatives. This explains the failure of the -12-ketoacetate (XXII) to give solid derivatives. The ketoacetate is thus assigned the structure (XXII) and its formation³⁹ from (XII) is shown in scheme A.

A minor quantity of a third ketoacetate (XXIV), $\text{C}_{32}\text{H}_{50}\text{O}_3$, m.p. 237-88° (ethanol), IR bands at (1715, broad $>\text{C}=\text{O}$ of acetate and keto groups), 1250 (acetate) and 1408 cm^{-1} ($-\overset{\text{O}}{\underset{\text{O}}{\text{C}}}-\text{CH}_2$), has been isolated from the middle fractions of the chromatography. Its NMR (CCl_4) spectrum shows the presence of an olefinic proton (4.5 τ , 1H) in addition to the signals due to methyl groups, acetate methyl and protons at C_3 and $-\text{CH}_2-$ at C_{11} . The UV spectrum indicates the absence of conjugation. It is assigned structure (XXIV), which is expected to be formed along with (XXII) under the acidic conditions of the reaction^{40,41}.

SCHEME A

The ketoacetate (XX) (β -amyranonylacetate), $C_{32}H_{52}O_3$ m.p. $312-16^\circ$ (ethyl acetate) (Lit.^{32,33} records for (XX) m.p. $300-301^\circ$) obtainable from (XII) by oxidation with m-chloroperbenzoic acid gives as per known procedure⁴² an enol diacetate $C_{34}H_{54}O_4$ (XXV) m.p. $233-34^\circ$. Compound (XXII), under these conditions, however, fails to give any enol acetate but gives a mixture of (XXII) and (XXIV), the latter predominating. Compound (XXIV) has been isolated by crystallisation and identified by IR and m.m.p.

EXPERIMENTAL

1. Extraction of costus leaves and stalks by pet.ether

Costus leaves and stalks were dried and finely powdered. Dried powder (5 kg) was taken in an aspirator bottle and repeatedly extracted with pet.ether. Each time the pet.ether (10 L) was allowed to remain in contact with the powder for 24 hr. Pet.ether was distilled from the total extract when crude concentrate (170 g) was obtained. A solution of the crude concentrate (170 g) in pet.ether (300 ml) was cooled to -18° when a solid (5 g) was separated. The solid (5 g) was chromatographed over alumina (Gr. III, 125 g, 1:25 ratio). The pet.ether fractions of the chromatography on repeated crystallisation from hexane + ethylacetate gave a crystalline solid (1.2 g), m.p. $252-54^{\circ}$ (α)_D + 99.1° . This was identified as taraxasteryl acetate (VII) by m.m.p. and IR [Lit.⁴³ records for taraxasteryl-acetate $256-57^{\circ}$ (α)_D + 100°].

The fraction eluted with benzene gave a solid which on repeated crystallisation with hexane + ethyl acetate followed by acetone gave taraxasterol (VI, 0.95 g), m.p. and m.m.p. 222° , (α)_D + 90° [Lit.⁴³ records for taraxasterol m.p. $226-27^{\circ}$, (α)_D + 102°].

The mother liquor after removal of the solid weighed 162 g.

2. Chromatography of the mother liquor of (1)

The mother liquor from (1), (100 g) was chromatographed over alumina (Gr. I, 1 kg, 1:10 ratio) and eluted as follows:

Chromatogram (Table 1)

Fr.No.	Solvent	Volume in ml	Wt. in g	TLC pattern
1.	Pet.ether	200	5.1	three spots
2.	"	200	6.0	"
3.	"	2500	30.0	"
4.	Pet.ether + benzene (1:1)	1500	13.0	two spots
5.	Pet.ether + benzene (1:2)	1500	11.0	two spots
6.	Benzene	2000	3.0	five spots
7.	Chloroform	1000	4.0	"

Fractions 1 to 3 (TLC similar) were mixed together and rechromatographed over silicic acid impregnated with 10% silver nitrate as in chromatogram (Table 2).

Fraction 4 was repeatedly crystallised from hexane + ethylacetate when taraxsterylacetate (VII, 6.8 g, TLC pure) was obtained m.p. and m.m.p. 252-54°, (α)_D + 99.0°.

Fraction 5 was rechromatographed over alumina (Gr. I, 330 g, 1:30 ratio) and eluted with pet.ether + benzene (1:1), benzene and chloroform respectively. The fraction eluted with chloroform was repeatedly crystallised from hexane +

ethylacetate followed by acetone to furnish taraxasterol (VI, 5.7 g, TLC pure), m.p. and m.m.p. 222-223° (α)_D + 90°. Fractions 6 and 7 being complicated mixtures were not examined further.

3. Rechromatography of fractions 1 to 3 of chromatogram Table 1) and mother liquor of Fraction 4

Fractions 1 to 3 and mother liquor of fraction 4 (chromatogram, table 1, 40 g) were rechromatographed over silicic acid impregnated with 10% silver nitrate (silicic acid 800 g + AgNO₃ 80 g) and eluted as follows:

Chromatogram (Table 2)

Fr.No.	Solvent	Volume in ml	Wt.in g	TLC pattern
1.	Pet.ether	500	0.2	one spot
2.	"	750	10.5	two spots
3.	"	1500	6.1	three spots (one major)
4.	Pet.ether + benzene (12:1)	250	0.5	one spot
5.	Pet.ether + benzene (2:1)	2000	10.3	one spot
6.	Benzene	1500	2.6	five spots
7.	Chloroform	500	0.5	five spots

IR of fraction 1 showed it to be a hydrocarbon. Being in small quantity it was not examined further.

Fraction 2 was rechromatographed as described in chromatogram (table 3).

Fraction 3 was found to contain mainly one compound. It was mixed with fractions 4 and 5 and rechromatographed over silicic acid (340 g) and eluted with pet.ether, pet.ether + benzene (12:1), pet.ether + benzene (8:1) and benzene respectively. The fraction eluted with pet.ether + benzene (8:1) was repeatedly crystallised from acetone to furnish lupeol palmitate (ester c, 9.2 g; TLC pure), m.p. 77-78°, (α)_D + 26 (benzene c, 2.8) (Lit.^{21c} records for ester c, m.p. 79°).

Analysis

Found: C, 83.27; H, 12.30.

$C_{46}H_{80}O_2$ requires C, 83.06; H, 12.13%.

IR bands at 1730, 1632, 1379, 1325, 1309, 1266, 1247, 1221, 1200, 1175, 1149, 1110, 1040, 1010, 981, 887 and 718 cm^{-1} .

Fractions 6 and 7 being complicated mixtures were not examined further.

4. Rechromatography of fraction 2 of chromatogram, table 2

Fraction 2 of chromatogram (table 2) (10.5 g) was rechromatographed over silicic acid (260 g, 1:25 ratio) and eluted as follows:

Chromatogram (Table 3)

Fr.No.	Solvent	Volume in ml	Wt. in g	TLC pattern
1.	Pet. ether	500	-	-
2.	Pet. ether + benzene (12:1)	800	4.2	mainly one spot
3.	Pet. ether + benzene (10:1)	800	3.4	"
4.	Pet. ether + benzene (1:1)	500	0.2	two spots
5.	Benzene	500	0.1	two spots
6.	Chloroform	500	0.1	"

Fraction 2 was repeatedly crystallised from ethanol + acetone (1:4) to furnish a crystalline solid (TLC pure) identified as α -amyrin stearate (ester A, 2.8 g), m.p. 46-48°, (α)_D + 61°, benzene c, 1.84). (Lit.^{21a} records for ester A, m.p. 45°).

Analysis

Found: C, 82.64; H, 12.54

$C_{48}H_{84}O_2$ requires C, 83.17; H, 12.22%

IR bands at 1732, 1645, 1387, 1253, 1181, 1153, 1101, 997, 978, 833 and 720 cm^{-1} .

Fraction 3 was repeatedly crystallised from acetone containing 10% hexane to furnish a crystalline solid (TLC pure) identified as β -amyrin palmitate (ester B, 2.3 g), m.p. 76-78° (α)_D + 57° (benzene, c, 2.8), M^+ 664 (Lit.^{21b} records for ester B, m.p. 77-78°).

Analysis

Found: C, 82.60; H, 12.16

 $C_{46}H_{80}O_2$ requires C, 83.06; H, 12.13%.

IR bands at 1736, 1646, 1248, 1176, 1148, 1100, 995, 972, 835, 819 and 720 cm^{-1} .

Fractions 4, 5 and 6 being in small quantity were not examined further.

5. Saponification of α -myristate (ester A)

To a solution of ester A (1 g) in benzene (25 ml), potassium hydroxide (2.5 g dissolved in 50 ml of ethanol) was added. The reaction mixture was refluxed on a water bath for 8 hr. After distillation of the benzene and alcohol, the reaction mixture was diluted with water and extracted thrice with chloroform (3 x 100 ml). The chloroform layer was washed with water and dried. Removal of chloroform gave a crude solid (0.7 g) which was purified by chromatography over alumina (Gr. II, 8 g). The fraction eluted with benzene was crystallised from hexane containing 10% benzene to furnish α -myrin (VIII, 0.38 g, TLC pure), m.p. $183-84^\circ$ (α)_D + 83° (c, 1) n_D^{20} 1.426 [Lit.⁴⁴ records for VIII, m.p. $186.5 - 187^\circ$, (α)_D + 91.6° (benzene)].

Analysis

Found: C, 84.86; H, 11.72

 $C_{30}H_{50}O$ requires C, 84.44; H, 11.91%.

IR bands at 3360, 1653, 1041, 995, and 835 cm^{-1} .

The aqueous layer was acidified with conc. hydrochloric acid and extracted thrice with chloroform. The chloroform extract was washed with water and dried. Removal of chloroform gave a crude solid which was crystallised from ethanol to furnish stearic acid m.p. 69° , M^+ 284 (Lit.²⁰ records for stearic acid m.p. 69.5°).

Analysis

Found: C, 75.56; H, 12.61

$\text{C}_{18}\text{H}_{36}\text{O}_2$ requires C, 75.99; H, 12.76%.

6. Preparation of α -amyrin acetate (IX)

To a solution of α -amyrin (VIII, 0.1 g) in pyridine (3 ml), acetic anhydride (2 ml) was added. The reaction mixture was kept at room temperature for 48 hr. and then heated on a water-bath for 2 hr. It was diluted with water and extracted thrice with chloroform. The chloroform extract was washed with dil. hydrochloric acid (5%), followed by water and dried. Removal of chloroform gave a crude solid (0.11 g) which was chromatographed over alumina (Gr. II, 5 g). The fraction eluted with pet.ether was crystallised from ethylacetate to furnish α -amyrin acetate (IX, 0.065 g), m.p. $220-222^{\circ}$, $(\alpha)_D + 81^{\circ}$ (c, 1), (Lit.⁴⁴ records for IX, m.p. 227° , $(\alpha)_D + 83.3^{\circ}$).

Analysis

Found: C, 81.61; H, 11.22

$C_{32}H_{52}O_2$ requires C, 81.99; H, 11.18%.

IR bands at 1740, 1667, 1252, 1105, 1032, 1013, 994, 978, 912, 836, 820 and 813 cm^{-1} .

7. Preparation of 11-keto α -amyrin acetate (X)

To a solution of α -amyrin acetate (IX, 0.15 g) in acetic acid (15 ml), sodium dichromate (0.6 g) was added. The reaction mixture was refluxed on a heating mantle for 24 hr. After dilution with water, it was extracted thrice with chloroform. The chloroform layer was washed with water and dried. Removal of chloroform gave a crude solid (0.16 g) which was chromatographed over alumina (Gr. II, 5 g). The fraction eluted with pet.ether + benzene (1:1) was crystallised from ethyl acetate to furnish 11-keto α -amyrinacetate (X, 0.08 g, TLC pure), m.p. $274-76^{\circ}$, $(\alpha)_D + 97^{\circ}$ (c, 0.6). (Lit.⁴⁵ records for X m.p. 278°).

Analysis

Found: C, 79.76; H, 10.61

$C_{32}H_{50}O_3$ requires C, 79.62; H, 10.44%.

IR bands at 1730, 1670, 1626, 1323, 1250, 1215, 1148, ~~1099~~, 1099, 1030, 990, 908, 894, 862 and 837 cm^{-1} .

8. Saponification of β -amyirin palmitate (ester B)

To a solution of β -amyirin palmitate (ester B, 2.0 g), in benzene (30 ml), potassium hydroxide (5 g, dissolved in ethanol 40 ml) was added. The reaction mixture was refluxed on a water bath for 8 hr. The reaction product was worked up as in (5) to separate the alcohol and acid portions. The crude alcohol was purified by chromatography as in (5) and crystallised twice from hexane containing 10% benzene to furnish β -amyirin (XI, 0.8 g, TLC pure), m.p. $195-96^{\circ}$ ($[\alpha]_D + 91^{\circ}$ (c, 2), $n_D^{20} 1.426$ (Lit.⁴⁶ records for XI, m.p. $199-200^{\circ}$, ($[\alpha]_D + 89^{\circ}$).

Analysis

Found: C, 84.47; H, 11.74

$C_{30}H_{50}O$ requires C, 84.44; H, 11.81%.

IR bands at 3400, 1653, 1381, 1361, 1041, 998 and 818 cm^{-1} .

The crude acid obtained after acidification of aqueous layer, after work up as indicated in 5, was crystallised from ethanol to furnish palmitic acid (0.27 g), m.p. 62° , $n_D^{20} 1.423$ (Lit.²³ records for palmitic acid m.p. 62.5°).

Analysis

Found: C, 74.56; H, 12.71

$C_{16}H_{32}O_2$ requires C, 74.94; H, 12.58%.

9. Preparation of β -amyrinacetate (XII)

To a solution of β -amyrin (XI, 0.1 g) in pyridine (2 ml), acetic anhydride (2 ml) was added. The reaction mixture was kept at room temperature for 48 hr. and then heated on a water bath for 2 hr. It was worked up as in 6. The crude product (0.105 g) was chromatographed over alumina (Gr. II, 5 g). The fraction eluted with pet.ether was crystallised twice from ethylacetate to furnish β -amyrinacetate (XII, 0.068 g, TLC pure), m.p. 236-38°, $(\alpha)_D^{25} + 82^\circ$ (c, 1.5). [Lit.⁴⁶ records for XII, m.p. 241°, $(\alpha)_D^{25} + 85.1^\circ$]

Analysis

Found: C, 82.31; H, 11.30

$C_{32}H_{52}O_2$ requires C, 81.99; H, 11.18%.

IR bands at 1739, 1661, 1253, 1156, 1143, 1107, 1086, 1033, 1010, 999, 980, 943, 912, 831 and 817 cm^{-1} .

10. Preparation of 11-keto β -amyrin acetate (XIII)

To a solution of β -amyrin acetate (XII, 0.1 g), in acetic acid (15 ml), sodiumdichromate (0.6 g) was added. The reaction mixture was refluxed for 24 hr. It was then worked up as in 7. The crude product (0.09 g) was chromatographed over alumina (Gr. II, 5 g). The fraction eluted with pet.ether + benzene (1:1), was crystallised twice from ethylacetate to furnish 11-keto β -amyrinacetate (XIII, 0.058 g, TLC pure), m.p. 264°, $(\alpha)_D^{25} + 110^\circ$ (c, 0.9), (Lit.³⁶ records

for XIII, m.p. 264-65°, (α)_D + 117°).

Analysis

Found: C, 79.96; H, 10.73

$C_{32}H_{50}O_3$ requires C, 79.62; H, 10.44%.

IR bands at 1733, 1666, 1613, 1250, 1133, 1075, 1020, and 990 cm^{-1} .

11. Preparation of 11-keto β -amyrin palmitate (XIV)

To a solution of β -amyrin palmitate (ester B, 0.25 g) in acetic acid (20 ml), sodiumdichromate (1 g) was added. The reaction mixture was refluxed for 24 hr. It was worked up as in 7. The crude product (0.24 g) was purified by chromatography over alumina (Gr. II, 8 g). The fraction eluted with pet.ether + benzene (2:1) was crystallised twice from ethylacetate to furnish 11-keto- β -amyrin palmitate (XIV, 0.13 g, TLC pure), m.p. 148°, M^+ 678.

Analysis

Found: C, 81.78; H, 11.71

$C_{46}H_{78}O_3$ requires C, 81.35; H, 11.58%.

IR bands at 1736, 1660, 1613, 1205, 1030, 1121, 1099, 1027, 991, 886 and 720 cm^{-1} .

12. Saponification of lupeol palmitate (ester C)

To a solution of lupeol palmitate (ester C, 1 g) in benzene (25 ml), potassiumhydroxide (5 g dissolved in ethanol 50 ml) was added. The reaction mixture was refluxed on a water

bath for 6 hr. It was worked up as in 5. The crude solid (0.46 g) obtained in the neutral portion was purified by chromatography over alumina (Gr.II, 5 g). The fraction eluted with benzene was crystallised twice from methanol + acetone (3:1) to furnish lupeol (XV, 0.25 g. TLC pure) m.p. 214-16°, (α)_D + 28° (c, 2.5), n_D^{25} 1.426 [Lit.⁴⁷ records for (XV), m.p. 215-16°, (α)_D + 27.2°].

Analysis

Found: C, 83.97; H, 11.93

$C_{30}H_{50}O$ requires C, 84.44; H, 11.81%.

IR bands at 3400, 1639, 1364, 1295, 1183, 1103, 1037, 1012, 981, 943, 917 and 887 cm^{-1} .

The crude acid (0.2 g) obtained after acidification of aqueous layer and work up was crystallised from ethanol to furnish palmitic acid (0.09 g), m.p. 62°, n_D^{25} 1.426.

Analysis

Found: C, 74.97; H, 12.74

$C_{16}H_{32}O_2$ requires C, 74.94; H, 12.58%.

13. Preparation of lupeol acetate (XVI)

To a solution of lupeol (XV, 0.2 g) in pyridine (2 ml), acetic anhydride (3 ml) was added. The reaction mixture was kept at room temperature for 48 hr. and then heated on a water bath for 2 hr. It was worked up as in 6. The crude product (0.21 g) was purified by chromatography over alumina

(Gr. II, 6 g). The fraction eluted with pet.ether was crystallised from ethylacetate to furnish lupeolacetate (XVI, 0.13 g, TLC pure) m.p. 217-18°, (α)_D + 43° (c, 1.2). (Lit.⁴⁷ records for XVI m.p. 220, (α)_D + 47.3).

Analysis

Found: C, 82.26; H, 11.38

$C_{32}H_{52}O_2$ requires C, 81.99; H, 11.18%.

IR bands at 1740, 1642, 1253, 1156, 1112, 1034, 1020, 989, 909 and 885 cm^{-1} .

14. Jones' chromic acid oxidation of lupeol (XV) to lupenone (XVII)

To a cooled solution of lupeol (XV, 0.1 g) in acetone (10 ml), Jones' chromic acid reagent (3 ml) was added slowly with stirring, till the brown colour persisted. The reaction mixture was stirred for 2 hr. It was diluted with water and extracted thrice with chloroform. The chloroform layer was washed with sodiumbicarbonate solution (5%), followed by water and dried. Evaporation of chloroform furnished a crude product (0.08 g) which was purified by chromatography over alumina (Gr. II, 5 g). The fraction eluted with pet.ether was crystallised from acetone to furnish lupenone (XVII, 0.05 g), m.p. 169-71°, (α)_D + 57° (c, 1.5), M^+ 424, (Lit.⁴⁷ records for XVII, m.p. 170.5 - 171.5°, (α)_D + 57.6°).

Analysis

Found: C, 84.41; H, 11.63

$C_{30}H_{48}O$ requires C, 84.84; H, 11.39%.

IR bands at 1704, 1645, 1429, 1148, 991, 896 and 878 cm^{-1} .

It gave a 2:4 DNP derivative m.p. 212-14°, (Lit.⁴⁷ records for 2:4 DNP of XVII m.p. 214°).

Analysis

Found: C, 71.78; H, 8.86; N, 9.75

$\text{C}_{36}\text{H}_{52}\text{O}_4\text{N}_4$ requires C, 71.49; H, 8.76; N, 9.26%.

15. Jones' chromic acid oxidation of β -amyrin (XI)

To a cooled solution of β -amyrin (XI, 0.7 g) in acetone (30 ml), Jones' chromic acid reagent (8 ml) was added slowly with shaking till the brown colour persisted. The reaction mixture was kept at room temperature for 1 hr. It was then diluted with water (100 ml) and extracted thrice with chloroform. The chloroform layer was washed with sodium-bicarbonated solution (5%), followed by water and dried. Removal of chloroform furnished a crude solid (0.6 g) which was chromatographed over silicic acid (9 g, 1:15 ratio) and eluted as follows:

Chromatogram (Table 4)

Fr.No.	Solvent	Volume in ml	Wt. in g	TLC pattern
1	Pet.ether + benzene (4:1)	400	0.25	one spot
2	Pet.ether + benzene (1:1)	200	0.01	two spots
3	Pet.ether + benzene (1:2)	110	0.09	one spot
4	Pet.ether + benzene(1:2)	110	0.02	one spot
5	Benzene	150	0.09	one spot
6	Benzene	150	0.01	three spots
7	Benzene + chloroform(6:1)	225	0.03	"
8	Chloroform	200	traces	"

Fraction 1, (TLC one spot) was crystallised from acetone to furnish β -amyrene (XVIII, 0.14 g, TLC pure), m.p. 120-82°, (α)_D + 114° (c, 1.2), M⁺ 424 (Lit.^{27,28} records for XVIII m.p. 172-80°, (α)_D + 105.6°).

Analysis

Found: C, 84.40; H, 11.55

C₃₀H₄₈O requires C, 84.84; H, 11.39%.

IR bands at 1710, 1653, 1424, 1266, 1244, 1202, 1143, 1117, 1078, 1031, 1005, 972 and 816 cm⁻¹.

It gave a semicarbazone C₃₁H₅₁ON₃ m.p. 248-50° (Lit.^{27,28} records for semicarbazone of XVIII m.p. 243-49°).

Analysis

Found: C, 76.85; H, 11.00; N, 9.08

C₃₁H₅₁ON₃ requires C, 77.28; H, 10.67; N, 8.72%.

Fraction 2 being in small quantity was not examined further.

Fraction 3 (TLC one spot) was crystallised from hexane to furnish β -amyrene 3, 11-dione (XIX, 0.06 g, TLC pure), m.p. 235-37°, (α)_D + 140° (c, 0.8), M⁺ 438 (Lit.³¹ records for XIX m.p. 237°).

Analysis

Found: C, 81.94; H, 10.89

C₃₀H₄₆O₂ requires C, 82.13; H, 10.57%.

IR bands at 1715, 1666, 1613, 1426, 1376, 1355, 1239, 1104, 1070, 1020, 973, 882 and 868 cm⁻¹

It gave a monosemicarbazone $C_{31}H_{49}O_2N_3$ m.p. $244-46^\circ$ (Lit.³¹ records for monosemicarbazone of XIX m.p. 232°).

Analysis

Found: C, 74.82; H, 9.81; N, 8.18

$C_{31}H_{49}O_2N_3$ requires C, 75.10; H, 9.96; N, 8.48%.

Fractions 4 and 5 (TLC one spot, similar) were mixed together and crystallised from pet. ether to furnish compound (XXI, 0.07 g), m.p. $232-35^\circ$, M^+ 454.

Analysis

Found: C, 79.29; H, 10.13

$C_{30}H_{46}O_3$ requires C, 79.24; H, 10.20%.

IR bands at 1700, 1403, 1389, 1332, 1015 and 922 cm^{-1} .

Fractions 6, 7 and 8 being in minor quantity were not examined further.

16. Jones' chromic acid oxidation of β -amyrin acetate (XII)

To a cooled solution of β -amyrinacetate (XII, 1 g) in acetone (25 ml) and benzene (5 ml), Jones' chromic acid reagent (8 ml) was added slowly under stirring till the brown colour persisted. The reaction mixture was refluxed on a water bath for 5 hr with stirring. It was then diluted with water (100 ml) and extracted thrice with chloroform. The chloroform extract was washed with sodium bicarbonate solution, followed by water and dried. Removal of chloroform

furnished a crude product (0.8 g) which was chromatographed over alumina (Gr. II, 32 g, 1:40 ratio) and eluted as follows:

Chromatogram (Table 5)

Fr.No.	Solvent	Volume in ml	wt.in g	TLC pattern
1.	Pet. ether	250	0.05	one spot
2.	Pet.ether + benzene (5:1)	750	0.31	mainly one spot
3.	Pet.ether + benzene (3:1)	500	0.17	"
4.	Pet.ether + benzene (1:1)	500	0.09	"
5.	Benzene	500	0.02	one spot
6.	Chloroform	250	0.01	three spots

Fraction 1 was crystallised from ethylacetate to furnish unreacted β -amyrinacetate (XII, 0.03 g), m.p. and m.m.p. 236-38°. IR superimposable with that of authentic (XII).

Fraction 2 was rechromatographed over silicic acid, (5 g, 1:15 ratio) and the fraction eluted with pet.ether + benzene (1:2) was crystallised twice from ethylacetate to furnish 11-keto β -amyrinacetate (XIII, 0.13 g, TLC pure), m.p. 263-64°, $(\alpha)_D + 103^\circ$ (c, 1.2). (Lit.³⁶ records for XIII m.p. 264-65°, $(\alpha)_D + 117^\circ$).

Analysis

Found: C, 79.31; H, 10.22

$C_{32}H_{50}O_3$ requires C, 79.62; H, 10.44%.

IR bands at 1732, 1666, 1613, 1250, 1133, 1075, 1020, and 990 cm^{-1} .

Fraction 3 was crystallised from ethylacetate to furnish ketoacetate (XXII), m.p. 285° , M^+ 482.

Analysis

Found: C, 79.79; H, 10.62

$\text{C}_{32}\text{H}_{50}\text{O}_3$ requires C, 79.62; H, 10.44%.

IR bands at 1725, 1690, 1650, 1410, 1250, 1220, 1149, 1110, 1086, 1025, 998, 935 and 903 cm^{-1} .

Fractions 4 and 5 (TLC similar) were mixed together and crystallised from ethanol to furnish ketoacetate (XXIV, 0.06 g, TLC pure), m.p. $287-88^{\circ}$.

Analysis

Found: C, 79.41; H, 10.32

$\text{C}_{32}\text{H}_{50}\text{O}_3$ requires C, 79.62; H, 10.44%.

IR bands at 1715 (broad), 1408, 1250, 1210, 1110 and 995 cm^{-1} .

17. Preparation of 3,12-dione (XXIII)

To a solution of β -amyrone (XVIII, 0.1 g) in chloroform (15 ml), *m*-chloroperbenzoic acid (0.3 g) in chloroform (20 ml) was added. The reaction mixture was kept at 15° for 24 hr. It was then diluted with chloroform and extracted with sodiumbicarbonate solution (5%). The chloroform layer was washed with water and dried. Removal of chloroform

gave a crude solid which was purified by chromatography over silicic acid. The fraction eluted with benzene was crystallised twice from ethylacetate to furnish β -amyrene, 3,12-dione (XXIII, 0.04 g, TLC pure), m.p. 218-20° (Lit.³⁸ records for XXIII, m.p. 216-18°).

Analysis

Found: C, 81.52; H, 10.81

$C_{30}H_{48}O_2$ requires C, 81.76; H, 10.98%.

IR bands at 1709 (broad), 1418, 1319, 1289, 1144, 1106, 1050, 994 and 910 cm^{-1} .

It gave a monosemicarbazone m.p. 255-56°.

Analysis

Found: C, 74.48; H, 10.12; N, 7.98.

$C_{31}H_{51}O_2N_3$ requires C, 74.80; H, 10.33; N, 8.44%.

18. Preparation of β -amyranonyl acetate (XX)

To a solution of β -amyrinacetate (XII, 0.1 g) in chloroform (15 ml), m-chloroperbenzoic acid (0.3 g) dissolved in chloroform (20 ml) was added. The reaction mixture was kept at 15° for 24 hr. It was worked up as in 17. The crude solid obtained was purified by chromatography over silicic acid and the fraction eluted with pet.ether + benzene (1:2) was crystallised twice from ethylacetate to furnish β -amyranonylacetate (XX, 0.05 g), m.p. 312-16°. (Lit.^{32,33} records for XX m.p. 300-01°).

Analysis

Found: C, 79.25; H, 10.48

$C_{32}H_{52}O_3$ requires C, 79.28; H, 10.81%.

IR bands at 1730, 1704, 1420, 1376, 1248, 1217, 1104, 1081, 1018, 988, and 897 cm^{-1} .

19. Preparation of enoldiacetate (XXV) from (XX)

To β -amyranonylacetate (XX, 0.1 g), acetic anhydride (15 ml), fused sodiumacetate (0.4 g) and catalytic quantity of p-toluenesulphonic acid (0.02 g) were added. The reaction mixture was refluxed for 48 hr. It was then diluted with water (50 ml) and extracted thrice with chloroform. The chloroform extract was washed with water and dried. Evaporation of chloroform furnished a crude solid which was crystallised from ethylacetate to furnish enoldiacetate (XXV, 0.06 g), m.p. $233-34^{\circ}$ (Lit.³⁸ records for XXV, m.p. $233-34^{\circ}$).

Analysis

Found: C, 77.41; H, 10.20

$C_{34}H_{54}O_4$ requires C, 77.52; H, 10.33%

IR bands at 1764, 1748, 1695, 1372, 1337, 1250, 1230, 1200, 1151, 1133, 1116, 1018, 1000, 963, 946, 928, 897, 888, 858, and 845 cm^{-1} .

20. Attempted preparation of enol diacetate of
ketoacetate (XXII)

To ketoacetate (XXII, 0.1 g), acetic anhydride (10 ml), fused sodium acetate (0.4 g) and catalytic quantity of p-toluene sulphonic acid (0.02 g) were added. The reaction mixture was refluxed for 48 hr. It was worked up as in 19. However, the IR of the crude product showed the presence of a keto group. The crude product (TLC, two spots) was chromatographed over silicic acid and eluted with pet. ether, pet. ether + benzene (1:1), benzene, benzene + chloroform (10:1) and chloroform respectively. The fraction eluted with benzene was found to be unreacted (XXII, TLC and m.p.). The fraction eluted with benzene + chloroform (10:1) was crystallised from ethyl acetate to furnish ketoacetate (XXIV, 0.06 g) m.p. and m.m.p. 287-88°. IR superimposable with that of authentic sample.

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CHAPTER - II

OXIDATION STUDIES ON
TARAXASTEROL AND ITS DERIVATIVES

S U M M A R Y

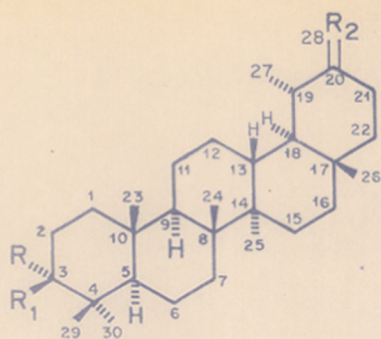
Oxidation of taraxasterol (I), its acetate (IV) and taraxasterone (VI) by *m*-chloroperbenzoic acid gives as the main products, the allylic alcohols (XX), (XVI) and (XXIV), formed by the rearrangement of initially formed epoxides, under the acidic conditions of the reactions, along with the C₂₀-nor-compounds, viz. (XIX), (XV) and (VII) as the minor products. Jones' chromic acid oxidation of (IV) and (I) gives, in addition to the C₂₀-nor-compounds (XV) and (XIX), the conjugated aldehydes (XVII) and (XXI) which are formed by initial migration of the exocyclic double bond to trisubstituted position, followed by allylic oxidation at the C₂₀-methyl.

Oxidation of taraxasterol (I) and its acetate (IV) by seleniumdioxide gives, the selenoxides (XXX) and (XXVII) respectively, in addition to the normally expected allylic oxidation products, viz. conjugated ketoalcohol (XXXI) and conjugated ketoacetate (XXVIII).

INTRODUCTION

Taraxasterol (I), $C_{30}H_{50}O$, is a pentacyclic triterpene alcohol belonging to the 13 α H, 19 β H ursane group (taraxastane group). It contains an exocyclic double bond at C₂₀(28) and a β -hydroxy group at C₃. Its structure has been established by converting it into taraxastene, identical with heterolupene (II). Taraxasterol and some of its esters viz. acetate, isovalerate etc. have been isolated from a number of natural sources, some of which are mentioned below.

- 1) The roots of Taraxacum^{1,2} officinale T. (ii) from the extract of the flowers of Roman or English Camomile³ (Anthemis nobilis L), (iii) as acetate, isovalerate and pyroterebate in the ethanolic extract of the latex or the stem bark of Calotropis Procera⁴ (family Asclepiadaceae), (iv) in the latex and root bark of calotropis gigantes^{5,6,7} (v) as acetate in the resin of Lactuca Vivosa⁸, (vi) in the white rays of Marguerite⁹ flowers, (vii) in the thistle flowers¹⁰, (viii) in the resin from Euphorbia Tirucalli L.¹¹, (ix) in the latex of Andryala Canariensis¹², (x) and along with ψ -taraxasterol (III) from the unsaponifiable fraction of an ethanolic extract of artichokes¹³ (Cyanara Scolymus). Both taraxasterol (I) and its acetate (IV) have also been isolated from the hexane extract of the leaves and stalks



I $R=H, R_1=OH, R_2=CH_2$.

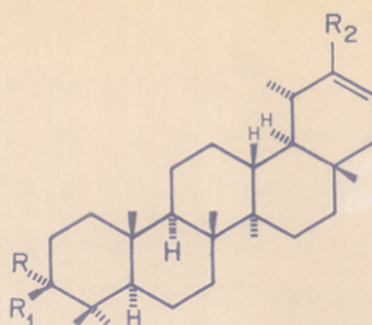
IV $R=H, R_1=-O-C(=O)-CH_3, R_2=CH_2$.

VI $R, R_1=O, R_2=CH_2$.

VII $R, R_1=O, R_2=O$.

XV $R=H, R_1=-O-C(=O)-CH_3, R_2=O$.

XIX $R=H, R_1=OH, R_2=O$.



II $R=R_1=H, R_2=CH_3$.

III $R=H, R_1=OH, R_2=CH_3$.

VIII $R=H, R_1=-O-C(=O)-H, R_2=CH_3$.

IX $R, R_1=O, R_2=CH_3$.

XII $R=H, R_1=-O-C(=O)-CH_3, R_2=CH_3$.

XVI $R=H, R_1=-O-C(=O)-CH_3, R_2=CH_2OH$.

XVII $R=H, R_1=-O-C(=O)-CH_3, R_2=-CHO$.

XXVIII $R=H, R_1=-O-C(=O)-CH_3, R_2=-CH_2-O-C(=O)-CH_3$.

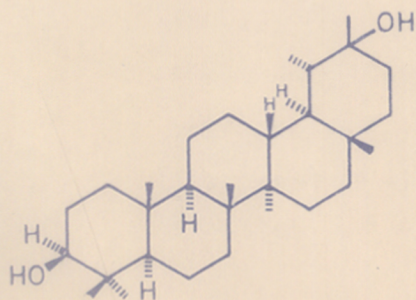
XX $R=H, R_1=OH, R_2=CH_2OH$.

XXI $R, R_1=O, R_2=-C(=O)-H$.

XXIV $R, R_1=O, R_2=-CH_2OH$.

XXV $R, R_1=O, R_2=-CH_2-O-C(=O)-CH_3$.

XXVI $R=R_1=H, R_2=-CHO$.



V

of the costus plant, Saussurea Lappa clarke¹⁴.

A diol belonging to this group viz. taraxastane 3 β -20 diol (V) C₃₀H₅₀O₂, m.p.270-272°, has been isolated¹⁵ from the Manila Elmi resin. The corresponding C₂₀ epimer has been isolated in our Laboratory from the black dammer¹⁶ resin m.p.261-3°.

On oxidation with chromic acid or on heating at 300°C with copper powder, compound (I) has been reported¹⁷ to give the ketone, taraxasterone (VI), C₃₀H₄₈O, m.p.184-185° (α)_D + 110°, along with another neutral compound, C₂₉H₄₆O₂, m.p.195°, which was tentatively assigned the diketone structure (VII)¹⁸.

When (I) was heated with formic acid (90%), the formate ester of ψ -taraxasterol was obtained, (VIII) m.p.221°, (α)_D + 57°, which on hydrolysis¹⁹ gave ψ -taraxasterol (III), C₃₀H₅₀O, m.p.220-221°, (α)_D + 50°; acetate m.p. 246-7°.

Compound (I) could be rearranged to (III), by heating it with 10% H₂SO₄ in benzene or ethanol solution. Compound (III) on chromic acid oxidation gave the ketone (IX), ψ -taraxasterone, which on Wolff-Kishner reduction gave, taraxastene^{20,21} which was found to be identical with heterolupene (II).

Both (I) and (III) on catalytic hydrogenation gave the same dihydro compound (X), indicating that both possess the same carbon-skeleton. From these evidences, the structure

of taraxasterol was confirmed as (I).

On treatment with hydrogen peroxide in acetic acid solution, compound (I) has been reported to give an oxide¹⁹ (XI), m.p. 152-154°, acetate m.p. 208-210°.

Ψ -Taraxasterylacetate (XII) on treatment with perbenzoic acid gave the corresponding epoxide which on LAH reduction in the presence of sodium ethoxide gave epi- Ψ -taraxastane-diol²² (V, one of the C₂₀ epimers).

Epoxidation²³ of taraxasteryl acetate (IV) by perbenzoic acid at 0° gave in addition to the mixture of stereoisomeric epoxides (XIII and XIV), three more compounds, which are probably produced as a result of the rearrangement of the epoxides under the acidic conditions of the reaction. The major of the two epoxides, C₃₂H₅₂O₃, m.p. 236-238°, M⁺ 484, on LAH reduction gave the taraxastane 3,3 β ,20 diol (V), m.p. 270-272° identical with the one isolated from Manila, Elmiresia. The other products of epoxidation include (1) a nor-ketoacetate, m.p. 270-272° M⁺ 470, C₃₁H₅₀O₃ and (2) a hydroxy acetate C₃₂H₅₂O₄, m.p. 256-260°, M⁺ 500.

Since the structural elucidation work on norketoacetate and the hydroxy acetate was not taken up in detail, it was felt desirable to obtain these compounds in quantity and study their structures. In addition, we were interested in ascertaining whether the epoxidation products of (I) and

(IV) by using perbenzoic acid were essentially the same as those obtained ^{by using} ~~from~~ m-chloroperbenzoic acid. For these reasons, the oxidation of (I), (IV) and (VI) was carried out by using m-chloroperbenzoic acid.

PRESENT WORK

Epoxidation of Taraxasteryl acetate (IV)

Taraxasteryl acetate (IV) on oxidation with m-chloroperbenzoic acid (1.1 moles, 85% chloroform, 96 hrs at 15°) gives the normally expected epoxides only in small proportion, the rearranged products of the epoxides predominating. Chromatography of the oxidation product on silicic acid, followed by crystallisation gives two compounds in the TLC pure state. The less polar of these, $C_{31}H_{50}O_3$, M^+ 470, m.p. 268-70° (ethanol), has been identified as the nor-keto-acetate (XV) from spectral data; IR (Fig. 1), 1740, 1250 (acetate), 1700 ($>C=O$); 1412 ($-C-\overset{\overset{O}{\parallel}}{CH_2}-$);

NMR(CCl_4); 9.24, 9.17, 9.10, 9.04, 8.97, 8.88 (21H, methyls at C-4, C-8, C-10, C-14, C-17 and C-19); 8.04 (3H, s, acetate methyl at C-3); at 5.54 (1-H, brt, C-3 proton); 2,4-DNP derivative $C_{37}H_{54}O_6N_4$, m.p. 262°. The more polar compound, $C_{32}H_{52}O_3$, M^+ 484, m.p. 310° (ethyl acetate), $(\alpha)_D + 58^\circ$ (c, 0.8), has been identified as the hydroxyacetate (XVI)

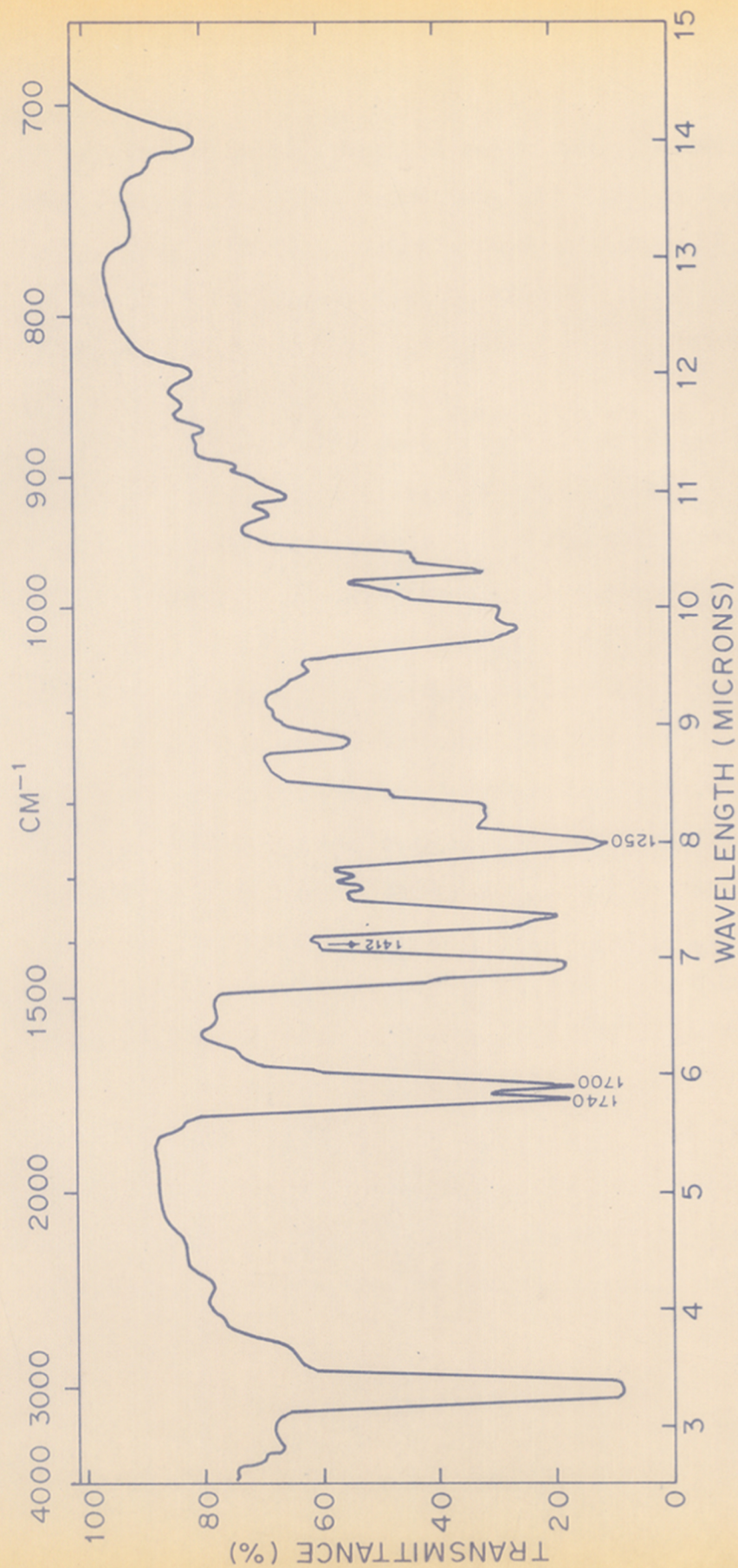


FIG. 1. IR SPECTRUM OF NORKETOACETATE (XV) (NUJOL)

by spectral data and chemical reactions. IR (Fig. 2); 3600 (OH), 1724, 1250, (acetate); NMR (CCl_4); 9.24, 9.2, 9.17, 9.05, 8.94 (21H, methyls at C-4, C-8, C-10, C-14, C-17 and C-19); 8.04 (3H, s, $-\text{OCOCH}_3$ at C-3); 6.08 (2H, s, C-28 methylene protons); 5.57 (1H, brn, C-3 proton); 4.54 (1H, brn, olefinic proton at C-21).

On oxidation with Jones' chromic acid reagent, (XVI) furnished a conjugated aldehyd-acetate (XVII) $\text{C}_{32}\text{H}_{50}\text{O}_3$, M^+ 482, m.p. 258-60° (hexane), IR (Fig. 3); 1754, 1245 (acetate), 1681, 2817 (conjugated aldehyde) and 1645 (conj. $-\text{CH} = \text{C} <$); NMR (CCl_4); 9.3, 9.17, 9.14, 9.07, 8.97 (21H, methyls at C-4, C-8, C-10, C-14, C-17 and C-19), 8.07 (3H, s, $\text{C}_3-\text{OCOCH}_3$); 5.6 (1H, brn C-3 proton); 3.5 (1H, brs, conj. olefinic proton at C-21); 0.77 (1H, $\text{C}_{28}-\text{CHO}$); UV λ_{max} 232.5 m μ (ϵ_{max} 11,000); 2,4-DNP derivative, $\text{C}_{38}\text{H}_{54}\text{O}_6\text{N}_4$, m.p. 263-5°. Compound (XVI) on acetylation ($\text{AC}_2\text{O/Py}$) gives the diacetate (XVIII) $\text{C}_{34}\text{H}_{54}\text{O}_4$, M^+ 526 m.p. 240-42° (ethyl acetate), $(\alpha)_D^{25} + 63^\circ$ (c, 0.2); IR 1754, 1250, 1235 (acetates); NMR (CCl_4); 9.25, 9.13, 9.11, 9.05, 8.94 (21H, methyls at C-4, C-8, C-10, C-14, C-17 and C-19); 8.04, 7.97 (6H, s, C_3 and C-28 $-\text{OCOCH}_3$); 5.54 (3H, m, C_3 - and C_{28} -protons); 4.44 (1H, C_{21} -olefinic proton).

In addition to (XV) and (XVI), a minor compound more polar than (XVI) was also formed which could not be isolated pure.

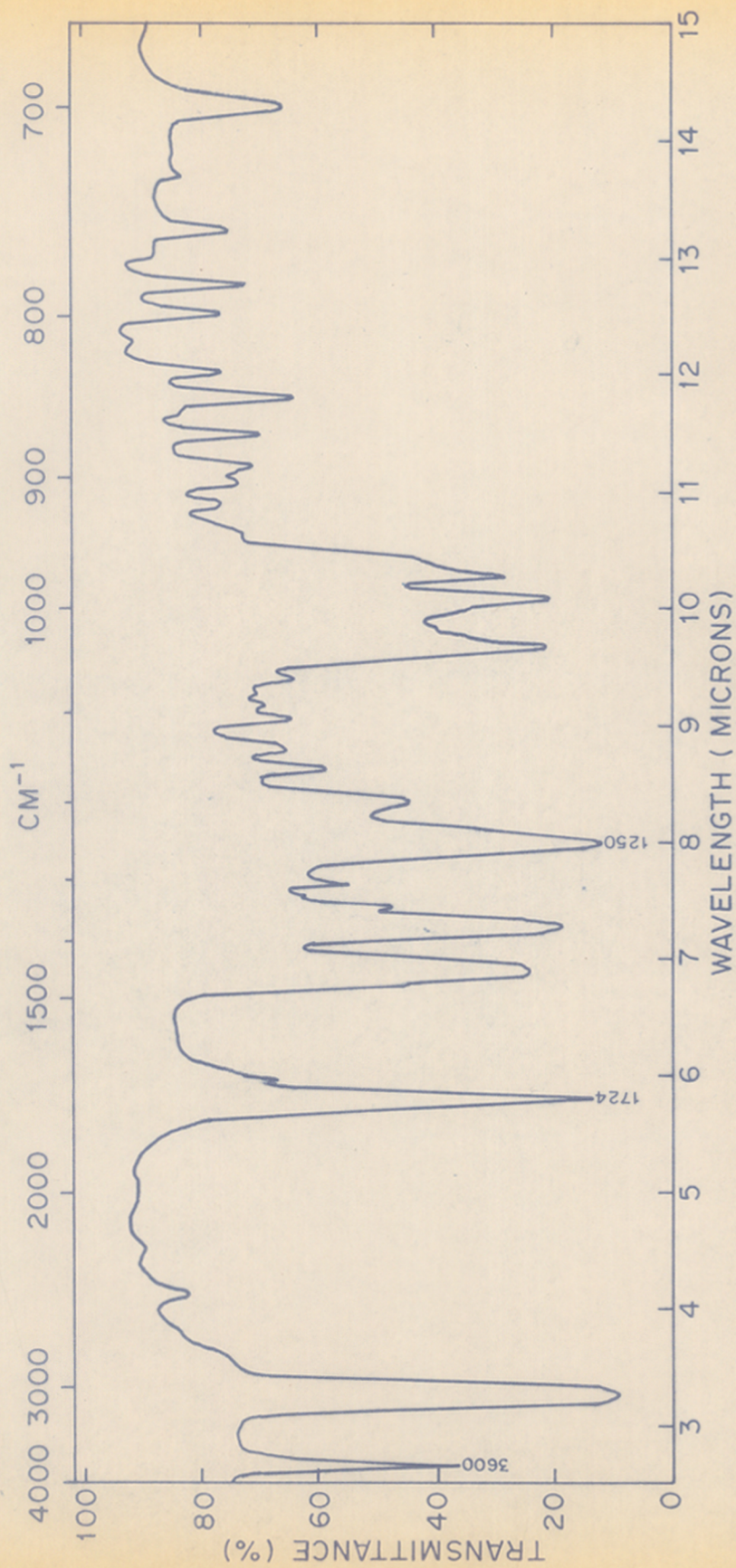


FIG. 2. IR SPECTRUM OF HYDROXYACETATE (XVI) (NUJOL)

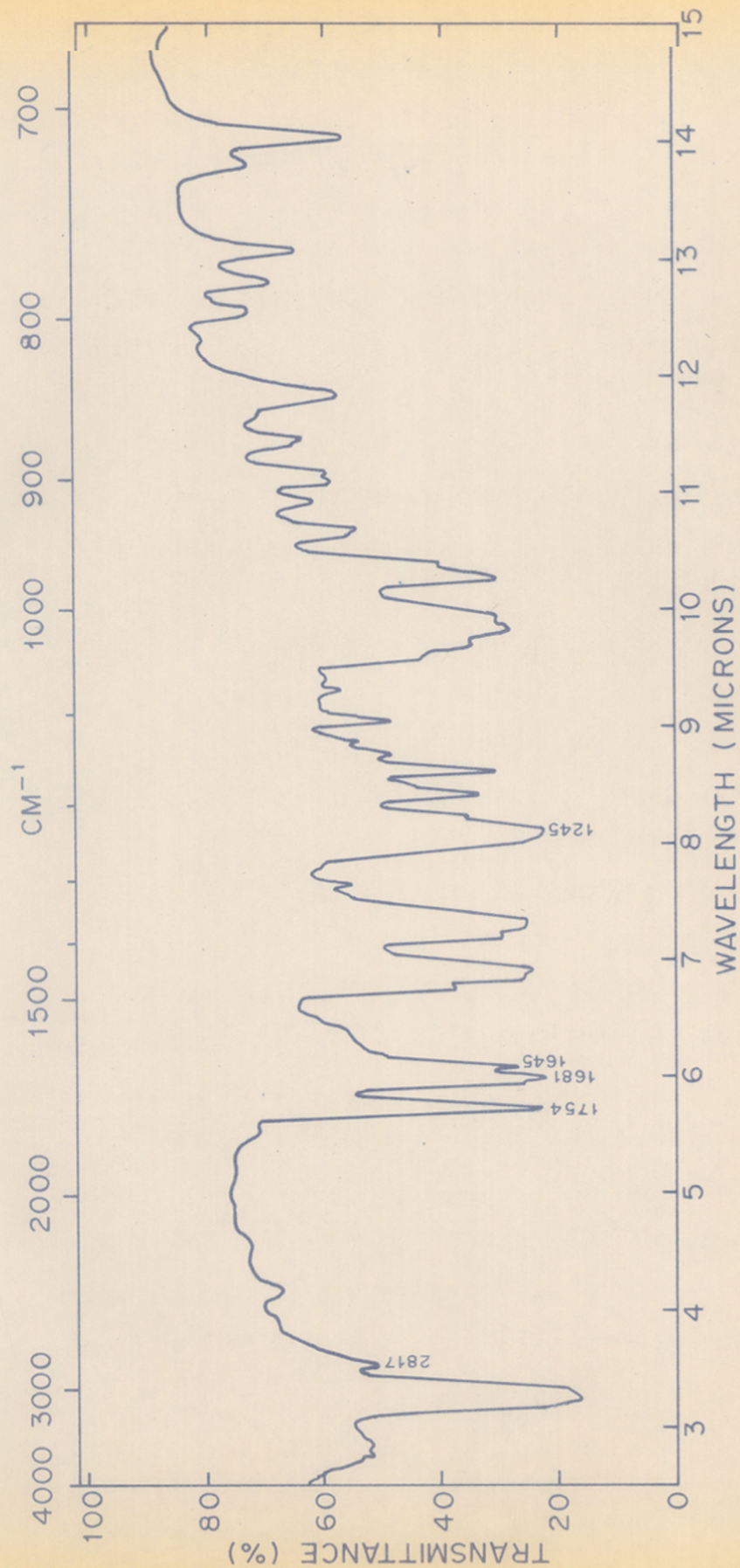


FIG. 3. IR SPECTRUM OF ALDEHYDOACETATE (XVII) (NUJOL)

Epoxidation of Taraxasterol (I)

Oxidation of taraxasterol (I) with *m*-chloroperbenzoic acid under similar conditions gives a mixture of four compounds of which three have been isolated by chromatography over alumina and silicic acid in the pure state. The least polar compound has been identified as the nor-ketoalcohol (XIX) $C_{29}H_{48}O_2$, M^+ 428, m.p. 236-38° (benzene + 20% hexane); IR: 3600 (OH), 1695 ($>C=O$), 1405 ($-C-CH_2$). On acetylation (Ac_2O/Py) it gives the nor-ketoacetate (XV), m.p. and m.m.p. 268-70°.

The compound eluted next to (XIX) has been identified as the diol (XX) by spectral properties and chemical reactions; $C_{30}H_{50}O_2$, M^+ 442, m.p. 223-25° (benzene + 10% hexane), $(\alpha)_D + 21^\circ$ (c, 0.6); IR: 3560, 1030 ($-CH_2OH$); NMR (CCl_4): 9.25, 9.12, 9.05, 8.95 (21 H, methyls at C-4, C-8, C-10, C-14, C-17 and C-19); 6.05 (2H, s, C-28-methylene protons); 5.57 (1H, brt, C-3 proton) and 4.47 (1H, C-21-olefinic proton). Acetylation of (XX) (Ac_2O/Py) gives the diacetate (XVIII) m.p. and m.m.p. 240-42°.

Oxidation of diol (XX) by Jones' chromic acid reagent gives the ketoaldehyde $C_{30}H_{48}O_2$ (XXI) m.p. 168-70° (ethanol); IR (Fig. 4); 1700 ($>C=O$), 1672, 2778 (conj. $-CHO$), 1639 (conj. $-CH=C<$); NMR (CCl_4): 9.3, 9.0, 8.97, 8.9 (21H, methyls at C-4, C-8, C-10, C-14, C-17 and C-19); 3.4 (1H,

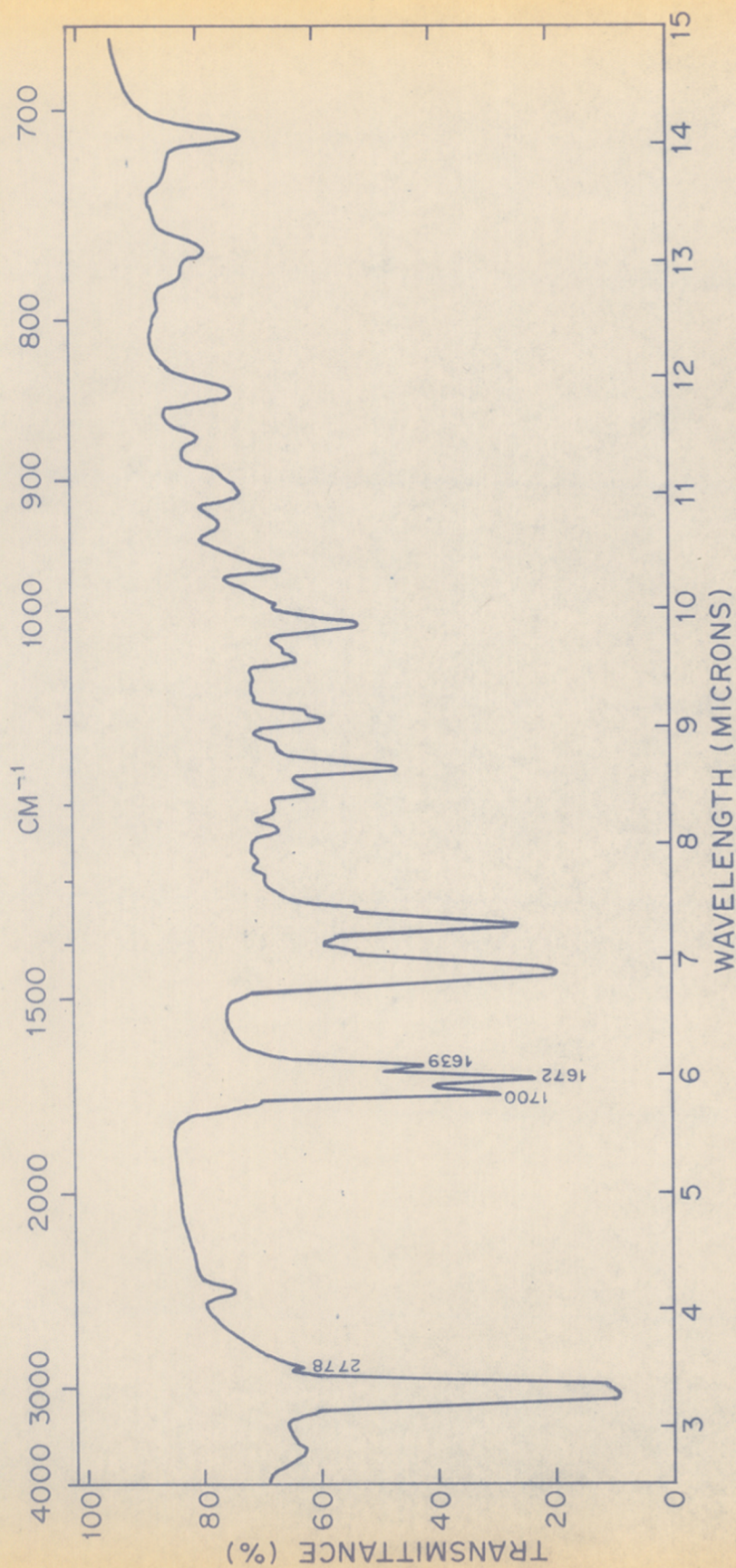


FIG. 4. IR SPECTRUM OF KETOALDEHYDE (XXI) FROM DIOL (XX) (NUJOL)

brn, conj. olefinic proton at C-21); 0.6 (1H, C-28-CHO);
 UV λ_{max} 232 m μ (ϵ_{max} 10,500); 2,4-DNP derivative $\text{C}_{42}\text{H}_{54}\text{O}_8\text{N}_2$,
 m.p. 260-62°.

The compound eluted (next to XX) in the later fractions gives a solid identified as the epoxydiol (XXII) $\text{C}_{30}\text{H}_{50}\text{O}_3$, m.p. 198-200° (benzene + chloroform, 1:4); IR: 3640, 3546 (OH). On acetylation, ($\text{Ac}_2\text{O}/\text{Py}$), (XXII) gives the epoxy diacetate (XXIII), $\text{C}_{34}\text{H}_{54}\text{O}_5$; M^+ 542, m.p. 264-66° (ethanol); IR bands 1745, 1727, 1250, 1235 (acetates); NMR (CDCl_3) (Fig. 5); 9.2, 9.15, 9.1, 8.97, 8.94 (21H, methyls at C-4, C-8, C-10, C-14, C-17 and C-19); 8.0, 7.95 (6H, s, C_3 and $\text{C}_{28}\text{-OCO-CH}_3$); 5.48 (1H, t, $J = 7.8$ Hz, C_3 -proton); 6.8 (1H, m, C_{21} -proton); 6.05, 5.72 (2H, d each, $J = -12$ Hz, each, C-28 protons). It is interesting to note that the C-28 methylene protons being non-equivalent by virtue of the restricted rotation^{24,25} around $\text{C}_{20}\text{-C}_{28}$ bond are observed as a pair of doublets, with geminal coupling constant -12 Hz. From these data, the epoxydiol and its acetate have been assigned structures (XXII) and (XXIII) respectively.

Epoxidation of taraxasterone (VI)

Oxidation of taraxasterone (VI) with *m*-chloroperbenzoic acid under similar conditions gives mainly two compounds (TLC) which are separated by chromatography over alumina. The less polar compound has been identified as the diketone (VII),

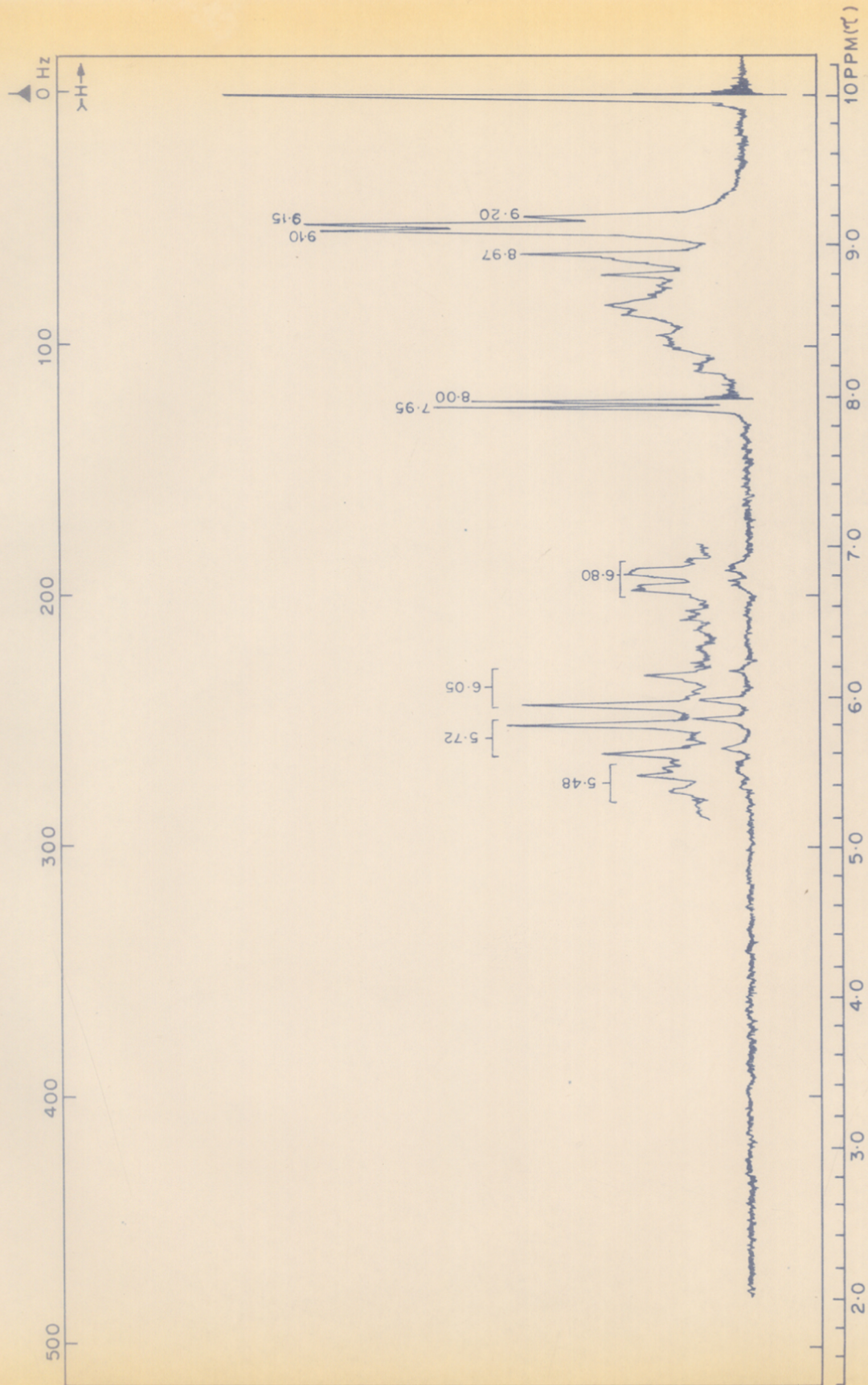
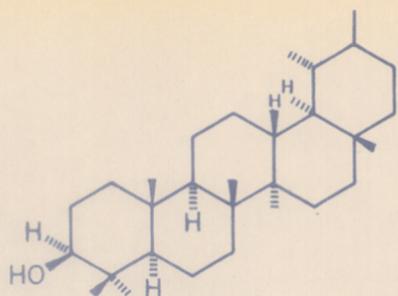
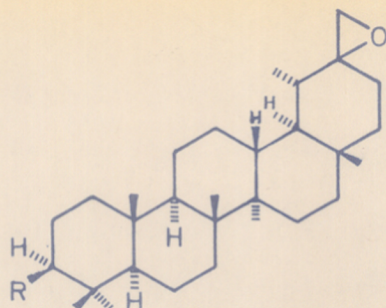


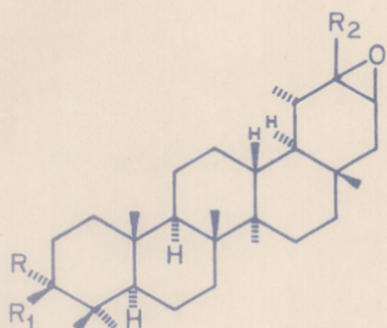
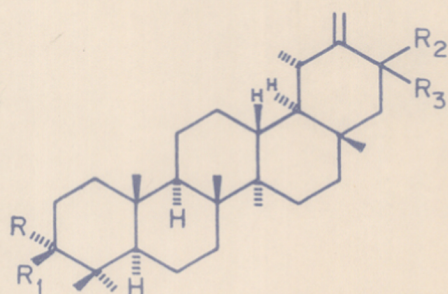
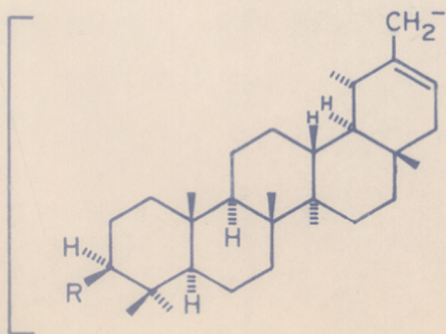
FIG. 5. NMR SPECTRUM OF EPOXY DIACETATE (XXIII) (CDCl_3)



X



XI R = OH

XIII & XIV R = $-\text{O}-\text{C}(=\text{O})-\text{CH}_3$ XXII R = H, R₁ = OH, R₂ = CH₂OHXXIII R = H, R₁ = $-\text{O}-\text{C}(=\text{O})-\text{CH}_3$,R₂ = $-\text{CH}_2-\text{O}-\text{C}(=\text{O})-\text{CH}_3$ XXVIII R = H, R₁ = $-\text{O}-\text{C}(=\text{O})-\text{CH}_3$, R₂, R₃ = OXXIX R₁ = $-\text{O}-\text{C}(=\text{O})-\text{CH}_3$, R = R₂ = H, R₃ = OHXXXI R = H, R₁ = OH, R₂, R₃ = O

-Se = O

XXXVII R = $-\text{O}-\text{C}(=\text{O})-\text{CH}_3$

XXX R = OH

$C_{29}H_{46}O_2$ M^+ 426, m.p. 200° (ethanol), (lit.¹⁸ m.p. 195°); IR bands 1700 ($>C=O$); 1408 ($-C-\overset{\overset{O}{\parallel}}{CH_2}$); NMR (CCl_4): 9.25, 9.04, 8.98, 8.94, 8.88 (2H, methyls at C-4, C-8, C-10, C-14 C-17 and C-19), 7.7 (4H, m, methylene protons at C-2 and C-21); 2,4-DNP derivative, $C_{41}H_{54}O_8N_8$, m.p. 260° (d). The more polar compound $C_{30}H_{48}O_2$, M^+ 440, m.p. 203° (ethanol) has been identified as the ketoalcohol (XXIV) by spectral data; IR: 3640, 1000 (primary OH), 1704 ($>C=O$); NMR (CCl_4): 9.24, 9.0, 8.95, 8.9 (2H, methyls at C-4, C-8, C-10, C-14, C-17 and C-19); 7.6 (2H, m, protons at C-2, adjacent to carbonyl); 6.02 (2H, s, C_{28} -methylene) and 4.47 (1H, olefinic protons at C-21). It gives on acetylation (Ac_2O/Py), the ketoacetate (XXV), $C_{32}H_{50}O_3$, M^+ 482, m.p. $166-68^\circ$ (ethanol) IR: 1754, 1235 (acetate), 1705 ($>C=O$).

It appears from the above observations that during oxidation of (IV), (I) and (VI) by *m*-chloroperbenzoic acid at $15^\circ C$, the epoxides initially formed get rearranged to the allylic alcohols (XVI), (XX) and (XXIV) respectively, under the acidic conditions of the reaction. However, the formation of nor-compounds such as (XV), (XIX) and (VII) from compounds containing exomethylene double bonds like (IV), (I) and (VI) by *m*-chloroperbenzoic acid or any other peracid appears to have been not reported.

The rearrangement of taraxasterol (I) to ψ -taraxasterol²⁶ (III) and also the allylic oxidation of ψ -taraxastene

(II) to the conjugated aldehyde (XXVI) by selenium dioxide²⁷ have been reported. When (I) is oxidized with m-chloroperbenzoic acid at room temperature, in addition to the products reported above, some ψ -taraxasterol (III) can also be isolated, characterized through its acetate (XII).

It is well known that taraxasterol (I) rearranges²⁶ to ψ -taraxasterol (III) in the presence of acids. So both (I) and its acetate (IV) were subjected to oxidation with Jones' chromic acid reagent in order to find out whether the exocyclic double bond in (I) and (IV) migrates to the trisubstituted position during oxidation under acidic conditions.

Oxidation of taraxasteryl acetate (IV) by Jones' chromic acid reagent

Jones' chromic acid oxidation of (IV) $\angle$ benzene + acetone (1:1), room temperature 8 hr.,⁷ gives mainly a mixture of two compounds, which are separated by chromatography. These have been identified (m.m.p., IR and NMR spectra) as (i) the aldehydoacetate (XVII) $C_{32}H_{50}O_3$ m.p. 258-60° (hexane) and (ii) nor-ketoacetate (XV) $C_{31}H_{50}O_3$ m.p. 268° (ethanol).

Oxidation of taraxasterol (I) by Jones' chromic acid reagent

Jones' chromic acid oxidation of (I) $\angle$ benzene + acetone (1:1), 3 hr. at room temperature) furnished mainly

three compounds (TLC), which have been separated by chromatography over alumina and isolated in the pure state. The least polar of these, $C_{30}H_{48}O$ m.p. 182-83° (acetone) has been identified as taraxasterone (VI); IR: 1700 ($>C=O$), 1653, 885 ($>C=CH_2$) [Lit.²⁸ records m.p. 184-85° for (VI)]. The compound eluted next to (VI) has been identified (m.m.p. IR and NMR spectra) as the conjugated keto-aldehyde (XXI), $C_{30}H_{46}O_2$, m.p. 168-70° (ethanol). The compound which is eluted in the later fractions has been identified (m.m.p., IR and NMR spectra) as the diketone (VII) $C_{29}H_{46}O_2$, m.p. 200° (ethanol).

The formation of diketone (VII) from (I) by excess chromic acid in acetic acid is already reported¹⁸. The formation of norketoacetate (XV) and diketone (VII) from (IV) and (I) respectively by Jones' chromic acid reagent is therefore as per expectation. In the formation of conjugated aldehydes (XVII) and (XXI) from (IV) and (I) respectively, by Jones' chromic acid reagent, the exocyclic double bond at C_{20-23} is rearranging to the trisubstituted position (C_{20-21}) under strong acidic conditions and the rearranged product then undergoes allylic oxidation to give the conjugated aldehydes.

Oxidation of taraxasterol and its acetate by selenium dioxide

As described above, in the formation of conjugated aldehydes XVII and XXI from IV and I respectively by Jones chromic acid reagent the exocyclic double bond present in IV and I migrates to the trisubstituted position under acidic conditions of the reaction. This is followed by allylic oxidation of the vinyl methyl at C-20. As a result, the normally expected allylic oxidation product with the oxygen function at C-21 cannot be obtained. With a view to introduce an oxygen function at C-21, without rearrangement of the exocyclic methylenic double bond at C₂₀₋₂₈, the selenium dioxide oxidation of IV and I has been carried out under mild conditions.

When IV is stirred in benzene solution with one equimolar proportion of selenium dioxide at room temperature (14 hrs) a mixture containing four compounds (TLC, benzene + 4% ethyl acetate) results, of which two compounds can be isolated in the pure state, by chromatography, followed by crystallisation. (The reaction of IV and I with selenium dioxide in ethanol at reflux temperature gives a complex mixture). The less polar compound (polarity slightly more than IV) m.p. 188-90° (pet. ether + 5% benzene), has been assigned the selenoxide structure XXVII, on the basis of elemental analysis and spectral data; IR: 1739, 1235 (acetate),

970 (ref. 29), 840 ($\text{Se} = \text{O}$), NMR (CCl_4): 9.28, 9.18, 9.14, 9.05, 8.95 (21H, methyls at C-4, C-8, C-10, C-14, C-17 and C-19); 8.07 (3H, s, acetate methyl at C-3), 5.5 (1H, t, $J = 7.2 \text{ Hz}$, $\text{C}_3\text{-H}$), 4.4 (1H, brt, olefinic proton at C-21) and 6.27 (2H, m, probably due to $\text{CH}_2\text{-Se}$ at C-20). The two protons signal at 6.27 can be usually assigned to either $-\text{CH}_2\text{OH}$ or $-\text{CH}_2\text{-O-alkyl}$. Since the IR spectrum of (XXVII) shows the absence of hydroxyl band, the signal can arise only from an ether (or oxide) of the type $-\text{CH}_2\text{-O-alkyl}$. But the elemental analysis is not in agreement with that of a C-32 compound containing three oxygen functions. It is therefore, thought that the signal may be arising due to $-\text{CH}_2\text{-Se=O}$, which is supported by the presence of bands at 970 (ref. 29) and 840 cm^{-1} in its IR spectrum. The elemental analysis of (XXVII) is in agreement with the molecular formula $\text{C}_{64}\text{H}_{102}\text{SeO}_5$. Similar type of selenoxides have been reported^{30,31} during selenium dioxide oxidation of cyclohexene and 1,3-diphenyl propene.

The more polar compound $\text{C}_{32}\text{H}_{50}\text{O}_3$, $M^+ 482$, m.p. $280-82^\circ$ (ethanol) has been assigned structure XXVIII; IR (Fig. 6), 1724, 1242 (acetate), 1690 (conj. $>\text{C}=\text{O}$), 1603, (conj. $>\text{C}=\text{CH}_2$); NMR (CCl_4): 9.15, 9.04, 8.97, 8.9 (21H, methyls at C-4, C-8, C-10, C-14, C-17 and C-19), 8.05 (3H, s, acetate methyl at C-3), 5.6 (1H, t, $J = 9 \text{ Hz}$, $\text{C}_3\text{-H}$), 7.5, 7.33 (2H, ds, $J = 8.4 \text{ Hz}$ each, α^{32} and β protons at C-22) and a pair of doublets

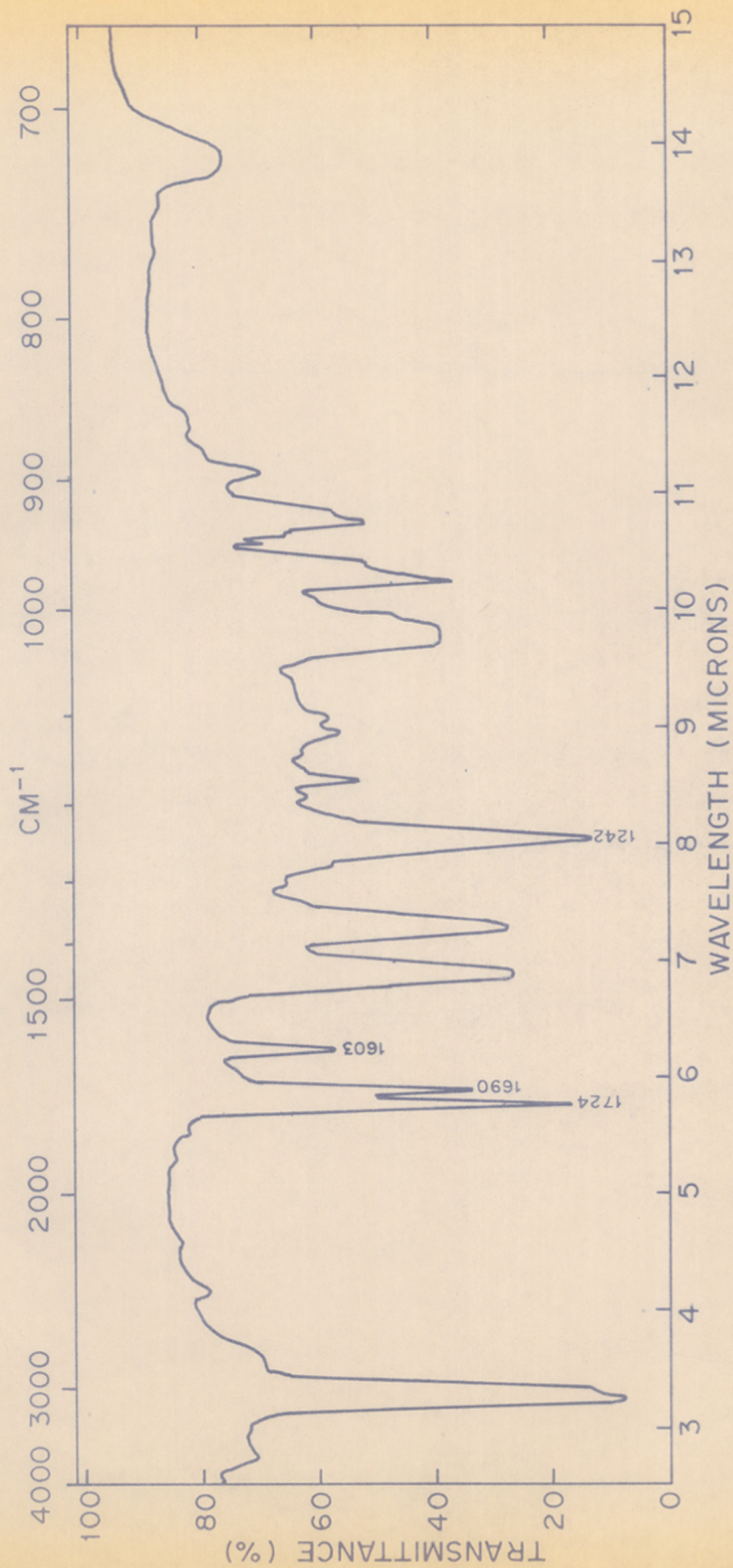


FIG. 6. IR SPECTRUM OF CONJUGATED KETOACETATE (XXVIII) (NUJOL)

centred at 5.0 and 4.2 (2H, $J = 3.6$ Hz each, conj. $>C = CH_2$ at C-20); UV λ_{max} , 235 m μ (ϵ_{max} 4700); 2,4-DNP, $C_{38}H_{54}O_6N_4$ m.p. 256-58°.

On sodium borohydride reduction the ketoacetate XXVIII gives the hydroxy acetate XXIX $C_{32}H_{52}O_3$, m.p. 252-54° (pet.ether + 10% benzene); IR (Fig. 7) 3650 (OH), 1724, 1242 (acetate), 1650, 885 ($>C = CH_2$); NMR(CCl_4); 9.15, 9.1, 9.02, 8.92 (21H, methyls at C-4, C-8, C-10, C-14, C-17 and C-19), 8.05 (3H, acetate methyls at C-3), 5.67 (1H, t, $J = 7.8$ Hz, C_3 -H), 6.0 (1H, t, $J = 6$ Hz, C_{21} -H) and multiplet centred at 5.27 (2H, $>C = CH_2$ at C-20).

Taraxasterol (I), when treated with selenium dioxide under identical conditions followed by chromatographic separation of the products gives two compounds, identified as selenoxide (XXX) and conjugated ketoalcohol (XXXI) on the basis of spectral evidence and elemental analysis.

Compound XXX, $C_{60}H_{92}SeO_3$, m.p. 177-79° (pet.ether + 5% benzene) shows IR bands at 3650 (OH), 965 (ref. 29), 840 (Se = O) and NMR (CCl_4) signals at 6.0 (2H, m, CH_2 -Se = O), 4.37 (1H brt C_{21} -olefinic proton) in addition to the methyl signals at 9.24, 9.23, 9.15, 9.06 and 8.95. Compound XXXI $C_{30}H_{48}O_2$, $M^+ 440$, m.p. 255° (pet.ether + 10% benzene) shows IR bands at 3534 (OH), 1690 (conj. $>C = O$), 1613 (conj. $>C = CH_2$) and NMR (CCl_4) signals at 9.3, 9.27, 9.17, 9.08, 9.02, 8.95 (21H, methyls at C-4, C-8, C-10, C-14, C-17 and C-19); 7.4

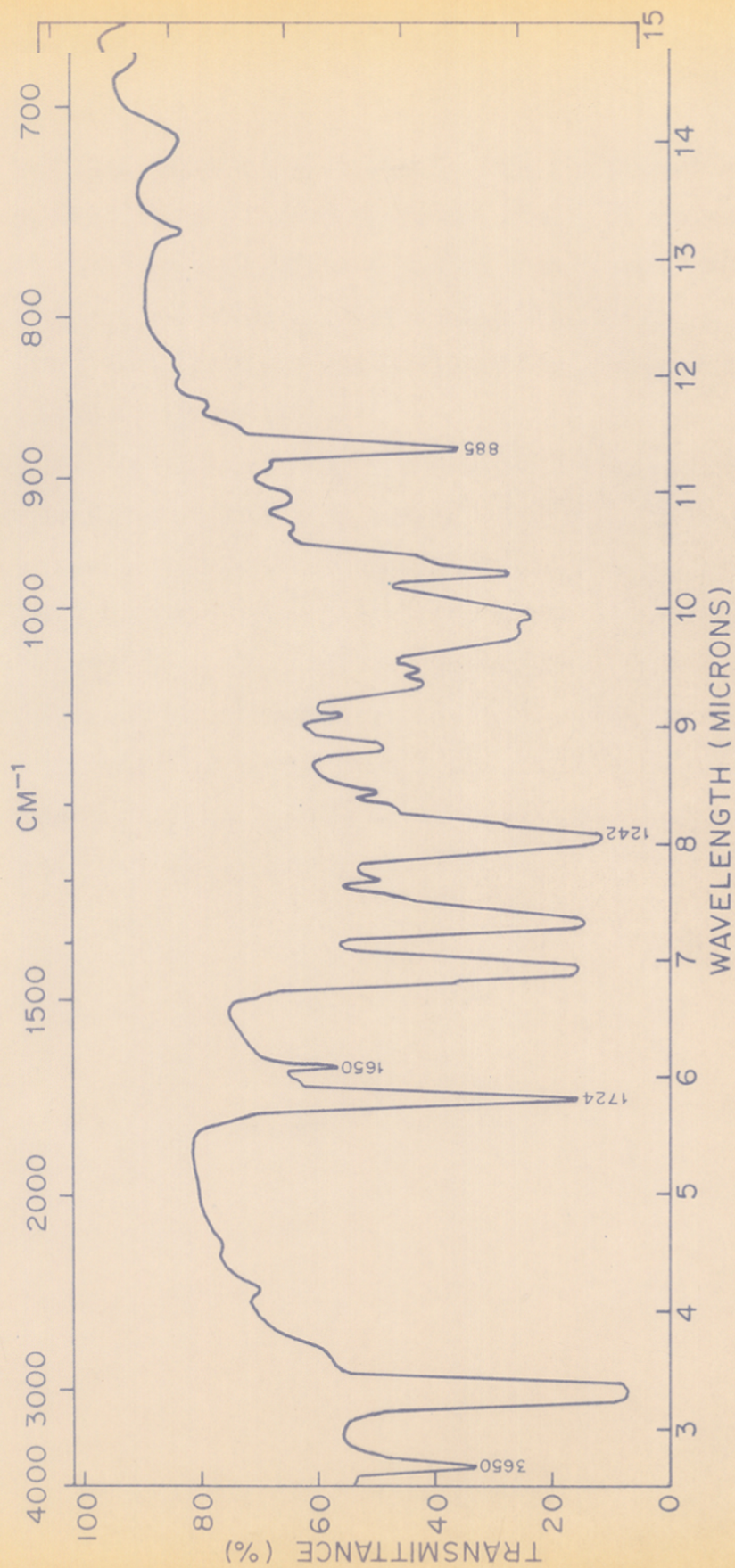


FIG. 7. IR SPECTRUM OF HYDROXYACETATE (XXIX) (NUJOL)

7.6 (2H, ds, $J = 8.4$ Hz each, α and β -protons³² at C-22), 5.8 (1H, t, $J = 7.8$ Hz C₃-H) and a pair of doublets centred at 4.94 and 4.13 (2H, $J = 3.6$ Hz each, conj. $>C=CH_2$ at C-20); UV λ_{max} : 238 m μ (ϵ_{max} 4900). On acetylation (Ac₂O/Py) it gives the acetate XXVIII m.p. and m.m.p. 280-82° (superimposable IR and NMR).

The formation of compounds XXVII and XXX from IV and I respectively, can be explained, according to the mechanism³³ suggested for the formation of selenoxides from cyclohexene and 1,3-diphenylpropene. Since in the present case, benzene was used as the medium, the intermediate carbonium ion (at C-20) formed after the addition of $HO-Se^+=O$ to the double bond at C-20, rearranges by the abstraction of a proton from the C₂₁-CH₂-creating a double bond between C-20 and C-21.

EXPERIMENTAL1. Epoxidation of taraxasteryl acetate by m-chloro-perbenzoic acid

To a cooled solution of taraxasteryl acetate (IV; 0.5 g) in chloroform (20 ml), a solution of m-chloroperoxybenzoic acid (0.3 g; 1.1 mole, 85%) in chloroform (50 ml) was added. The reaction mixture was kept at 15° for 96 hr. It was diluted with chloroform (100 ml) and washed with aqueous sodium carbonate solution (5%) (to extract m-chlorobenzoic acid), followed by water and dried. Removal of chloroform gave the crude product (0.41 g; TLC - 3 spots). It was chromatographed over silicic acid (6 gms, 1:15 ratio) and eluted as follows:

Chromatogram (Table 1B)

Fr.No.	Solvent	Volume in ml	Wt in g.	Remarks
1.	Pet.ether	150	-	-
2.	Pet.ether + benzene(3:1)	180	0.01	TLC two spots
3.	Pet.ether+benzene (2:1)	120	0.120	TLC two spots
4.	Pet.ether+benzene (2:1)	120	0.110	"
5.	Pet.ether+benzene (1:1)	200	0.150	TLC single spot
6.	Benzene	200	-	-
7.	Chloroform	200	-	-

Fraction 2 showing mainly unreacted taraxasteryl acetate, (TLC) was not examined further.

Fraction 3 eluted with pet.ether + benzene (2:1) showed mainly one spot with a small impurity of a more polar compound in TLC analysis. It was repeatedly crystallised from ethanol to give the solid nor-ketoacetate (XV; 0.07 g, TLC pure), m.p. 268-70°, M^+ 470.

Analysis

Found: C, 78.96; H, 10.62

$C_{31}H_{50}O_3$ requires C, 79.10; H, 10.71%.

IR bands at: 2857, 1740, 1700, 1450, 1412, 1355, 1316, 1250, 1220, 1144, 1076, 1036, 1016, 980 and 905 cm^{-1} .

It gave the 2:4 DNP derivative m.p. 262°.

Analysis

Found: C, 67.61; H, 8.08; N, 8.3

$C_{37}H_{54}O_6N_4$ requires C, 68.28; H, 8.36; N, 8.61%.

Fraction 4 eluted with pet.ether + benzene (2:1) showed two spots in TLC analysis the major spot corresponding to the compound present in fraction 5. It was rechromatographed over alumina (Gr. II, 5.5 g, 1:50 ratio) and eluted with pet.ether; pet.ether + benzene (4:1); pet.ether + benzene (1:1) and benzene respectively. The fraction eluted with pet.ether + benzene (1:1) (TLC single spot) of this chromatography was mixed with fraction 5 of chromatogram (Table 1) and crystallised twice from ethyl acetate to give the solid hydroxy acetate (XVI; 0.110 g, TLC pure), m.p. 310°, $(\alpha)_D = +58^\circ$ (c, 0.80) M^+ 484.

Analysis

Found: C, 79.09; H, 10.59

$C_{32}H_{52}O_3$ requires C, 79.23; H, 10.81%.

IR bands at: 3600, 1724, 1460, 1412, 1379, 1350, 1326, 1314, 1203, 1162, 1143, 1105, 1032, 991, 972, 917, 904, 892, 870, 847, 833, 799, 784, 755 and 699 cm^{-1} .

2. Jones' chromic acid oxidation of hydroxyacetate (XVI) to conjugated aldehyde acetate (XVII)

To an ice cooled solution of hydroxyacetate (XVI; 0.05 g) in acetone (10 ml), Jones' chromic acid reagent (3 ml) was added dropwise till brown colour persisted. The reaction mixture was kept at room temperature for 1 hr. It was then diluted with water (100 ml) and extracted thrice with chloroform. The chloroform layer was washed with aq. sodium-bicarbonate solution (5%), followed by water and dried. Removal of chloroform furnished the crude solid product (0.042 g), which was crystallised twice from hexane to give the conjugated aldehyde acetate (XVII; 0.025 g, TLC pure), m.p. 258-60°, M^+ 482.

Analysis

Found: C, 79.81; H, 10.47

$C_{32}H_{50}O_3$ requires C, 79.62; H, 10.44%.

IR bands at 2817, 1754, 1681, 1645, 1490, 1449, 1399, 1370, 1312, 1245, 1190, 1163, 1143, 1130, 1106, 1075, 1045, 1031, 1015, 1005, 974, 962, 935, 915, 903, 894, 873, 845, 797, 781, 765, 725 and 712 cm^{-1} .

a

It gave 2:4 DNP derivative m.p. 263-5°.

Analysis

Found: C, 68.69; H, 8.41; N, 8.35



requires C, 68.85; H, 8.21; N, 8.45%.

3. Acetylation of hydroxy acetate (XVI) to diacetate (XVIII)

To a solution of hydroxyacetate (XVI; 0.050 g) in pyridine (5 ml) acetic anhydride (5 ml) was added dropwise. The reaction mixture was kept at room temperature for 24 hrs and then heated on a water bath for 2 hrs. It was then poured into ice-water mixture (100 ml) and extracted thrice with chloroform. The chloroform layer was washed with dilute hydrochloric acid (5%), followed by water and dried. Removal of chloroform furnished the crude solid product (0.04 g). It was crystallised twice from ethylacetate to give the solid diacetate (XVIII; 0.021 g, TLC pure), m.p. 240-42°, (α)_D + 63°, (c, 0.2), M⁺ 526.

Analysis

Found: C, 77.46; H, 10.58

$C_{34}H_{54}O_4$ requires C, 77.52; H, 10.33%

IR bands at 1750, 1462, 1370, 1314, 1284, 1254, 1235, 1160, 1105, 1070, 1024, 974, 942, 918, 894, 872, 854 and 781 cm⁻¹.

4. Epoxidation of taraxasterol by m-chloroperbenzoic acid

To a cooled solution of taraxasterol (I, 3.8 g) in chloroform (25 ml), a solution of m-chloroperbenzoic acid (1.6 g) in chloroform (10 ml) was added. The reaction mixture was kept at 15° for 96 hrs. It was then diluted with chloroform (200 ml) and extracted twice with aq. sodium-bicarbonate solution (5%). The chloroform layer was washed with water and dried. Removal of chloroform furnished a crude product (3.5 g, TLC - four spots). It was chromatographed over alumina (Gr. II, 52.5 g, 1:15 ratio) and eluted as follows:

Chromatogram (Table 2)

Fr.No.	Solvent	Volume in ml	Wt. in g	Remarks
1.	Pet.ether	250	-	-
2.	Pet.ether + benzene (3:1)	250	-	-
3.	Pet.ether + benzene (1:1)	250	0.260	TLC two spots
4.	Pet.ether + benzene (1:1)	250	0.945	"
5.	Benzene	250	0.610	TLC single spot
6.	Chloroform	250	1.12	TLC two spots

Fraction 3, showing mainly unreacted taraxasterol (I) was not examined further.

Fraction 4 showed two spots according to TLC analysis. It was rechromatographed over alumina (Gr. II, 40 g; 1:40 ratio) and eluted with pet.ether + benzene (1:1), benzene and chloroform respectively. The fraction eluted with pet.ether + benzene was found to be almost pure. It was crystallised twice from benzene + 20% hexane to give the norketoalcohol (XIX, 0.315 g. TLC pure), m.p.236-38°, M^+ 423.

Analysis

Found: C, 81.56; H, 10.97

$C_{29}H_{48}O_2$ requires C, 81.25; H, 11.29%.

IR bands at 3600, 1695, 1460, 1405, 1370, 1277, 1250, 1224, 1192, 1175, 1130, 1100, 1085, 1064, 1040, 1029, 1010, 980, 962, 932, 910 and 848 cm^{-1} .

Fraction 5 eluted with benzene showed single spot in TLC analysis. It was crystallised from benzene + 10% hexane to give the diol (XX, 0.39 g, TLC pure), m.p.223-25°, $(\alpha)_D^{21}$ (c, 0.6), M^+ 442.

Analysis

Found: C, 80.98; H, 11.41

$C_{30}H_{50}O_2$ requires C, 81.39; H, 11.38%.

IR bands at 3560, 1460, 1376, 1297, 1209, 1176, 1153, 1131, 1090, 1079, 1030, 998, 966, 939, 914, 838, 800 and 758 cm^{-1} .

Fraction 6 eluted with chloroform showed two spots in TLC analysis. It was rechromatographed over alumina (Gr. II, 22 gms,

1:20 ratio) and eluted with pet.ether + benzene (1:1) pet.ether + benzene (1:2), benzene and chloroform. The pet.ether + benzene (1:1) and pet.ether + benzene (1:2) fractions gave pure diol (XX; TLC) m.p. and m.m.p. 224° (superimposable IR spectra). The benzene fraction was a mixture of diol (XX) and epoxydiol (XXII) (TLC). It was mixed with mother liquors obtained in the crystallisation of diol for further purification. The chloroform fraction gave a pure (TLC) solid epoxydiol which was crystallised from benzene + chloroform (1:4) to give epoxydiol (XXII, 0.220 g TLC pure), m.p. $198-200^{\circ}$.

Analysis

Found: C, 78.18; H, 10.81.

$C_{30}H_{50}O_3$ requires C, 78.55; H, 10.99%.

IR bands at 3640, 3546, 1458, 1377, 1353, 1316, 1284, 1245 1227, 1205, 1179, 1149, 1100, 1080, 1064, 1032, 1019, 997, 980, 968, 938, 909, 848, 828, 799, 779 and 746 cm^{-1} .

5. Acetylation of epoxydiol (XXII)

To a solution of epoxydiol (XXII, 0.125 g) in pyridine (10 ml), acetic anhydride (2.5 ml) was added. The reaction mixture was kept at room temperature for 24 hrs. and then heated on a water bath for 2 hrs. It was then poured in cold water (100 ml) and extracted thrice with chloroform. The chloroform extract was washed with dil. HCl (5%), followed by water and dried. Removal of chloroform furnished a solid

product (TLC pure). It was crystallised twice from ethanol to give epoxydiacetate (XXIII; 0.088 g, TLC pure), m.p. 264-66°, M^+ 542.

Analysis

Found: C, 75.61; H, 9.84

$C_{34}H_{54}O_5$ requires C, 75.23; H, 10.03%.

IR bands at 1745, 1727, 1460, 1379, 1364, 1292, 1250, 1235, 1099, 1081, 1031, 1010, 969, 870, 781, 760 and 708 cm^{-1} .

6. Acetylation of norketoalcohol (XIX)

To a solution of nor-ketoalcohol (XIX, 0.105 g), in pyridine (10 ml), acetic anhydride (2 ml) was added. The reaction mixture was kept at room temperature for 24 hrs and then heated for 2 hrs on a water bath. It was then poured in cold water (100 ml) and worked up as in (5). The crude solid product was crystallised from ethylacetate to give norketoacetate (XV, 0.06 g, TLC pure), m.p. and m.m.p. 268-70°.

IR superimposable with the IR of norketoacetate obtained in (1).

7. Acetylation of diol (XX)

To a solution of diol (XX, 0.150 g) in pyridine (10 ml) acetic anhydride (3 ml) was added. The reaction mixture was kept at room temperature for 24 hrs and then heated on a water-bath for 2 hrs. It was then poured in water (100 ml)

and worked up as usual. The crude solid product was crystallised from ethylacetate to give diacetate (XVIII; 0.080 g, TLC pure), m.p. and m.m.p. 240-42°.

IR superimposable with the IR of diacetate obtained in 3.

8. Oxidation of diol (XX) by Jones' chromic acid reagent

To an ice cooled solution of diol (XX; 0.150 g), in acetone (20 ml), Jones' chromic acid reagent (4 ml) was added drop by drop till the brown colour persisted. After the addition, the reaction mixture was kept at room temperature for one hour. It was then poured in ice cold water (150 ml) and extracted thrice with chloroform. The chloroform layer was washed with aq. sodium bicarbonate solution (5%), followed by water and dried. Removal of chloroform furnished a crude solid which was crystallised from ethanol to give the keto-aldehyde (XXI) (0.072 g, TLC pure) m.p. 168-70°.

Analysis

Found: C, 81.70; H, 10.78

$C_{30}H_{46}O_2$ requires C, 82.13; H, 10.57%

IR bands at 2778, 1700, 1672, 1639, 1460, 1355, 1218, 1189, 1156, 1105, 1014, 980, 844, 763 and 705 cm^{-1} .

It gave a 2:4 DNP derivative m.p. 260-62°.

Analysis

Found: C, 63.73; H, 7.10; N, 13.82

$C_{42}H_{54}O_3N_3$ requires C, 63.16; H, 6.77; N, 14.04%.

9. Epoxidation of taraxasterone (VI) by m-chloroperbenzoic acid

To a cooled solution of taraxasterone (VI, 0.5 g) in chloroform (10 ml), a solution of m-chloroperbenzoic acid (0.3 g) in chloroform (10 ml) was added. The reaction mixture was kept at 15° for 96 hrs. It was then diluted with chloroform (100 ml) and washed with aqueous sodium carbonate solution (5%) to extract m-chlorobenzoic acid. The chloroform layer was then washed with water and dried. Removal of chloroform furnished the crude product (0.52 g TLC - three spots). It was chromatographed over silicic acid (5.5 g, 1.10 ratio) and eluted as follows:

Chromatogram (Table 3)

Fr.No.	Solvent	Volume in ml	Wt. in g	Remarks
1.	Benzene	200 ml	-	-
2.	Benzene + chloroform (10:1)	200 ml	0.18	TLC single spot with slight impurity
3.	Benzene + chloroform (7:1)	200 ml	0.26	"
4.	Chloroform	200 ml	0.05	Two spots
5.	Ether	200	-	-

Fraction 2 eluted with benzene + chloroform (10:1), was rechromatographed over alumina (Gr. II, 5 gms, 1:25 ratio) and eluted with pet.ether, pet.ether + benzene (1:1), benzene and

chloroform. The fraction eluted with pet.ether + benzene (1:1, TLC single spot), was crystallised from ethanol to give diketone (VII; 0.060 g. TLC pure), m.p. 200° . (Lit.¹⁸ m.p. 195°), M^+ 426.

Analysis

Found: C, 81.41; H, 10.79.

$C_{29}H_{46}O_2$ requires C, 81.63; H, 10.87%.

IR bands at 1700, 1460, 1408, 1389, 1372, 1220, 1136, 1103, 1064, 1015, 1000, 964, 913 and 839 cm^{-1} .

It gave a 2:4 DNP derivative m.p. 260 (d).

Analysis

Found: C, 63.16; H, 7.24; N, 13.98

$C_{41}H_{54}O_3N_2$ requires C, 62.61; H, 6.87; N, 14.25%.

Fraction 3 eluted with benzene + chloroform (7:1) was rechromatographed over alumina (Gr. II, 5.0 g, 1:20 ratio) and eluted with pet.ether, pet.ether + benzene (1:1), benzene and chloroform. The fraction eluted with pet.ether + benzene (1:1) and benzene (TLC similar) were mixed together and crystallised from ethanol to give the ketoalcohol (XXIV, 0.110 g, TLC pure), m.p. 203° , M^+ 440.

Analysis

Found: C, 81.34; H, 10.98

$C_{30}H_{48}O_2$ requires C, 81.76; H, 10.98%.

IR bands at 3640, 1704, 1462, 1374, 1156, 1000, 966, 844 and 714 cm⁻¹.

10. Acetylation of ketoalcohol (XXIV)

To a solution of ketoalcohol (XXIV, 0.125 g) in pyridine (5 ml), acetic anhydride (2.5 ml) was added. The reaction mixture was kept at room temperature for 24 hrs and then ~~drks~~ heated on a water bath for 2 hrs. It was then poured into ice-water (100 ml) and extracted thrice with chloroform. The chloroform layer was washed with dil.HCl (5%), followed by water and dried. Removal of chloroform furnished a crude solid product. It was crystallised from ethanol to give solid ketoacetate (XXV, 0.062 g, TLC pure) m.p.166-68°; M⁺ 482.

Analysis

Found: C, 79.41; H, 10.28

C₃₂H₅₀O₃ requires C, 79.62; H, 10.44%.

IR bands at 1754, 1705, 1449, 1372, 1295, 1256, 1235, 1156, 1101, 1010, 980, 964, 943 and 775 cm⁻¹.

11. Oxidation of taraxasteryl acetate by Jones' chromic acid reagent

To a cooled solution of taraxasteryl acetate (IV; 1 gm), in acetone (15 ml) and benzene (15 ml), Jones' chromic acid reagent (6 ml) was added drop by drop till the brown colour persisted. After the addition, the reaction mixture was kept

at room temperature for 8 hrs. It was then poured in water (200 ml) and extracted thrice with benzene. The benzene layer was washed with aq. sodiumbicarbonate solution (5%), followed by water and dried. Removal of benzene furnished the crude product (0.9 g). It was chromatographed over alumina (Gr. II, 36 gms, 1:40 ratio) and eluted as follow

Chromatogram (Table 4)

Fr.No.	Solvent	Volume in ml	Wt. in g	Remarks
1.	Pet.ether	250	0.105	TLC one spot
2.	Pet.ether + benzene (5:1)	250	traces	"
3.	Pet.ether + benzene (3:1)	250	0.080	"
4.	Pet.ether + benzene (1:1)	250	0.310	TLC two spots
5.	Benzene	250	0.225	TLC one main spot
6.	Chloroform	200	0.070	TLC three spots

Fraction 1 showed single spot in TLC analysis corresponding to that of taraxasteryl acetate.

Fractions 2 and 3 showing identical TLC pattern (single spot) were mixed together and crystallised twice from hexane to give solid aldehydoacetate (XVII, 0.04 g, TLC pure), m.p. and m.m.p. 258-60°.

IR superimposable with that of aldehydo-acetate obtained in 2.

Fraction 4 showed two spots in TLC analysis one corresponding to aldehydoacetate (XVII, major spot) and the other corresponding to nor-ketoacetate (XV, minor spot). It was rechromatographed over silicic acid (9 gms, 1:30 ratio) and eluted with pet.ether + benzene (1:1), pet.ether + benzene (1:2), benzene, benzene + chloroform (10:1), benzene + chloroform (7:1) and chloroform. The fraction eluted with benzene (TLC one spot) was crystallised from hexane to give aldehydoacetate (XVII, 0.160 g, TLC pure), m.p. and m.m.p. 258-60°.

Fraction 5 eluted with benzene showed mainly one spot in TLC analysis. It was crystallised from ethyl acetate to give nor-ketoacetate (XV, 0.105 g, TLC pure), m.p. and m.m.p. 268°.

IR superimposable with that of (XV) obtained in 1.

Fraction 6 showed three spots in TLC analysis. It being in small quantity was not examined further.

12. Oxidation of taraxasterol by Jones' chromic acid reagent

To an ice cooled solution of taraxasterol (I, 2 gms) in benzene (50 ml) and acetone (50 ml), Jones' chromic acid reagent (30 ml) was added drop by drop till the brown colour persisted. After the addition, the reaction mixture was kept at room temperature for 3 hrs. It was then poured in ice cool water (250 ml) and extracted thrice with chloroform. The chloroform extract was washed with aq. sodium bicarbonate

solution (5%), followed by water and dried. Removal of chloroform furnished a crude oxidation product (1.8 gms, TLC three spots). It was chromatographed over alumina (Gr. II, 72 g; 1:40 ratio) and eluted as follows:

Chromatogram (Table 5)

Fr.No.	Solvent	Volume in ml	Wt. in g	Remarks
1.	Pet.ether	200	0.25	TLC one spot
2.	Pet.ether + benzene (3:1)	200	0.52	TLC two spots
3.	Pet.ether + benzene (1:1)	200	0.55	TLC one spot
4.	Benzene	200	0.15	TLC two spots
5.	Chloroform	200	-	-

Fraction 1 eluted with pet.ether showed a single spot in TLC analysis. It was crystallised twice from acetone to give taraxasterone (VI; 0.12 g TLC pure); m.p. 182-83°.

Analysis

Found: C, 84.29; H, 11.06

$C_{30}H_{48}O$ requires C, 84.84; H, 11.39%.

IR bands at 1700, 1653, 1451, 1381, 1351, 1360, 1258, 1217, 1190, 1168, 1136, 1104, 1075, 1019, 983, 966, 953, 933, 912, 885 and 837 cm^{-1} .

Fraction 2 eluted with pet.ether + benzene (3:1) showed two spots in TLC analysis. It was rechromatographed over alumina

(Gr. II, 11 gms, 1:20 ratio) and eluted with pet.ether, pet.ether + benzene (4:1), pet.ether + benzene (2:1) and benzene respectively. The fraction eluted with pet.ether + benzene (4:1) (TLC one spot) was crystallised from ethanol to give keto-aldehyde (XXI; 0.210 g. TLC pure) m.p. and m.m.p. 168-70°.

IR superimposable with that of (XXI) obtained in 8. Fraction 3 eluted with pet.ether + benzene (1:1) showed one spot in TLC analysis. It was crystallised twice from ethanol to give diketone (VII, 0.34 g, TLC pure), m.p. and m.m.p. 200°.

IR superimposable with that of (VII) obtained in 9.

13. Oxidation of taraxasteryl acetate by selenium dioxide

To a solution of taraxasteryl acetate (IV, 2 gms), in benzene (100 ml), selenium dioxide (2 gms) was added. The reaction mixture was stirred at room temperature for 14 hrs. After removal of the benzene, the crude product was dissolved in ethyl acetate (100 ml), when some selenium metal precipitated after some time. The solution was boiled with activated charcoal and filtered. Removal of ethyl acetate furnished a crude product (2.02 g, TLC four spots). It was chromatographed over silicic acid (30 gms, 1:15 ratio) and eluted as follows:

Chromatogram (Table 6)

Fr.No.	Solvent	Volume in ml	Wt. in g	Remarks
1.	Pet.ether + benzene (1:1)	250	0.11	TLC two spots
2.	Benzene	250	0.755	"
3.	Benzene + chloroform (10:1)	250	0.205	"
4.	Benzene + chloroform (10:1)	250	0.04	"
5.	Benzene + chloroform (6:1)	250	0.460	TLC one spot
6.	Chloroform	250	0.12	TLC one spot
7.	Ethyl acetate	250	-	-

Fraction 1, being in small quantity was not examined further.

Fraction 2 and 3 showed two spots in TLC analysis. They were mixed together and rechromatographed over alumina (Gr. II, 50 gms, 1:50 ratio) and eluted with pet.ether, pet.ether + benzene (3:1), pet.ether + benzene (2:1), benzene and chloroform respectively. The pet.ether + benzene (3:1) fraction (TLC one spot) was crystallised from pet.ether containing 5% benzene to give (XXVII), 0.360 g. TLC pure) m.p. 138-90°.

Analysis

Found: C, 74.32; H, 10.22

$C_{64}H_{102}SeO_5$ requires C, 74.66; H, 9.92%.

IR bands at 1739, 1664, 1460, 1372, 1235, 1145, 1015, 970, and 840 cm^{-1} .

Fraction 4 being in small quantity was not examined further.

Fraction 5 and 6 eluted with benzene + chloroform (6:1) and chloroform respectively (TLC one spot) were mixed together and crystallised from ethanol to give solid keto acetate (XXVIII, 0.280 g, TLC pure) m.p. $280-82^{\circ}$.

M^+ 482.

Analysis

Found: C, 79.27; H, 10.54

$\text{C}_{32}\text{H}_{50}\text{O}_3$ requires C, 79.62; H, 10.44%

IR bands at 1724, 1690, 1602, 1449, 1370, 1242, 1171, 1135, 1020, 975, 929 and 894 cm^{-1} .

It gave a 2:4 DNP derivative m.p. $256-58^{\circ}$.

Analysis

Found: C, 68.46; H, 8.08; N, 8.87

$\text{C}_{38}\text{H}_{54}\text{O}_6\text{N}_4$ requires C, 68.85; H, 8.21; N, 8.45%.

14. Reduction of ketoacetate (XXVIII) by sodium borohydride

To an ice cooled solution of ketoacetate (XXVIII; 0.1 g) in methanol (15 ml), sodiumborohydride (0.2 g) was added slowly in portions. The reaction product was stirred at room temperature for 10 hrs and then kept at room temperature for 48 hrs. It was diluted with cold water (100 ml) and acidified by dil. HCl. It was then extracted thrice with benzene,

Benzene extract was washed with water and dried. Removal of benzene furnished the crude product (0.105 g, TLC three spots). It was chromatographed over alumina (Gr. II; 4 g, 1:40 ratio) and eluted with pet.ether, pet.ether + benzene (5:1), pet.ether + benzene (3:1), pet.ether + benzene (1:1) and benzene respectively. Benzene fraction (TLC one spot) was crystallised from pet.ether containing 10% benzene to give hydroxy acetate (XXIX, 0.03 g, TLC pure); m.p. 252-54°.

Analysis

Found: C, 78.97; H, 11.03

$C_{32}H_{52}O_3$ requires C, 79.28; H, 10.81%.

IR bands at 3650, 1724, 1650, 1445, 1366, 1242, 1008, 970 and 885 cm^{-1} .

15. Oxidation of taraxasterol by seleniumdioxide

To a solution of taraxasterol (I, 0.625 g) in benzene (100 ml) selenium dioxide (0.625 g) was added. The reaction mixture was stirred at room temperature for 14 hrs. It was filtered and evaporated and ethyl acetate (100 ml) was added to the residue. The ethyl acetate solution was boiled and allowed to settle. The separated selenium metal was filtered off. Removal of ethyl acetate furnished the crude product (0.631 g, TLC four spots). It was chromatographed over alumina (Gr. II, 30 gms, 1:50 ratio) and eluted as follows:

Chromatogram (Table 7)

Fr.No.	Solvent	Volume in ml	Wt. in gm	Remarks
1.	Pet.ether + benzene (3:1)	200	traces	TLC one spot
2.	Pet.ether + benzene (1:1)	200	0.22	"
3.	Benzene	200	0.27	"
4.	Benzene + chloroform (10:1)	200	traces	-
5.	Benzene + chloroform (6:1)	200	0.03	TLC two spots
6.	Chloroform	200	0.05	"

Fraction 1 being in small quantity was not examined further.

Fraction 2 eluted with pet.ether + benzene (1:1) (TLC one spot) was crystallised from pet.ether containing 5% benzene to give (selenoid compound (XXX; 0.12 g, TLC pure), m.p.177-79°.

Analysis

Found: C, 76.70; H, 10.71.

$C_{60}H_{98}SeO_3$ requires C, 77.03; H, 10.37%.

IR bands at 3650, 1664, 1462, 1381, 1297, 1253, 1189, 1110, 1078, 1038, 1011, 965, 840 and 757 cm^{-1} .

Fraction 3 eluted with benzene showed single spot in TLC analysis. It was crystallised from pet.ether + 10% benzene to give the keto-alcohol (XXXI, 0.17 g TLC pure), m.p.255°, M^+ 440.

Analysis

Found: C, 81.32; H, 11.21.

$C_{30}H_{48}O_2$ requires C, 81.76; H, 10.98%.

IR bands at 3524, 1695, 1613, 1449, 1370, 1032, 962 and 926 cm^{-1} .

Fraction 4,5 and 6 being in small quantity, were not examined further.

16. Acetylation of keto-alcohol (XXXI)

To a solution of keto-alcohol (XXXI, 0.15 g) in pyridine (5 ml), acetic anhydride (3 ml) was added. The reaction product was kept at room temperature for 24 hrs and then heated on a water bath for 2 hrs. It was then poured in cold water (200 ml) and extracted thrice with chloroform. The chloroform layer was washed with dil. HCl 5%, followed by water and dried. Removal of chloroform furnished a crude solid (0.145 g). It was crystallised from ethanol to give solid keto-acetate (XXVIII, 0.09 g, TLC pure) m.p. and m.m.p. 280-32°.

IR superimposable with that of keto-acetate (XXVIII) obtained in 13.

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CHAPTER - III
OXIDATION STUDIES ON
(+) CAR-3-ENE AND ITS DERIVATIVES

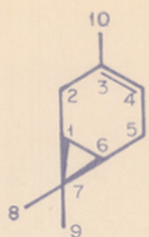
SUMMARY

(+) Car-3-ene on oxidation with sodiumdichromate and acetic acid gives as the main products (1) 8-hydroxy m-cymene (XX) (2) car-3-ene 2,5-dione (XV) (3) 1,1,4-trimethyl cyclohepta, 2,4-diene-6-one (XIX) and (4) care-2-ene, (XXVI) which have been isolated by chromatography on alumina and characterised through spectral data and physical constants.

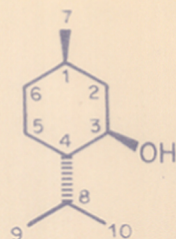
Oxidation of 4-acetyl car-2-ene (XXVII) with perbenzoic and m-chloroperbenzoic acids, gives in addition to the epoxide (XXXI), the rearranged alcohol (XXXII). Under mild alkaline conditions, epoxide (XXXI), rearranges to the conjugated ketoalcohol (XXXVII). However, in the presence of acids, in methanol medium the paramenthane derivatives (XXXII) and (XXXVIII) are formed as the major products of the reaction. The structures are assigned to the rearranged products, on the basis of elemental analysis and spectral data.

INTRODUCTION

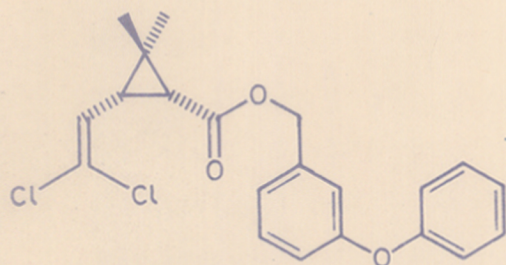
(+) Car-3-ene (I), is an abundantly occurring cheap indigenous by product of pine oil (*Pinus longifolia*). Many interesting transformations of (I) leading to commercially important chemicals like 1-menthol (II) have been carried out. In addition, methods have been developed for its conversion to chrysanthamic acid^{1,2,3} an important component occurring in the form of esters like pyrethrins and cinerins. Synthetic analogues of pyrethrins and cinerins are becoming increasingly important as pest control agents. They are superior to natural pyrethrins and cinerins in the fact that they combine high insecticidal activity, low mammalian toxicity with higher photostability. Prominent among these which are in commercial use are permethrin (III)⁴ and cypermethrin (IV)⁵. These insecticides are esters of 2,2-dimethyl, 3-vinyl substituted cyclopropane carboxylic acid esters with alcohols like *m*-phenoxy benzyl alcohol, 5-benzylfurylmethyl alcohol etc. For the synthesis of such insecticides, one of the key intermediates required is methyl, 1R cis, 3-formyl, 2,2-dimethyl cyclopropane carboxylate (V). The preparation of insecticides is then achieved by the reaction of appropriate Wittig reagents on (V). The preparation of compound (V) from cis-caronic acid has already been described in literature^{6,7}. So we were interested in finding a suitable method for the preparation of cis-caronic acid and for this purpose, the cheap and abundantly



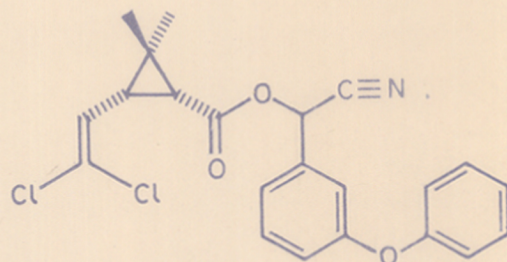
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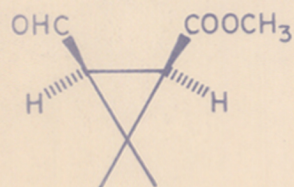
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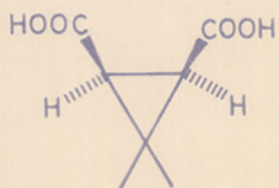
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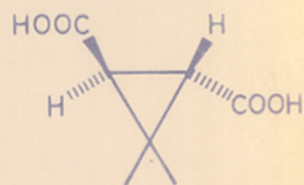
IV



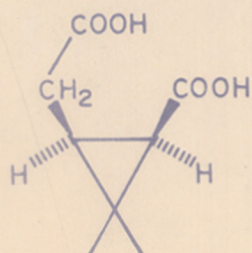
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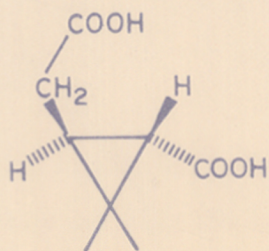
VI



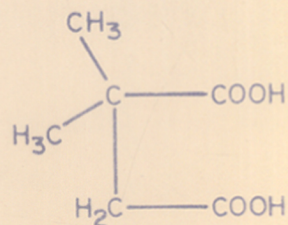
VII



VIII



IX

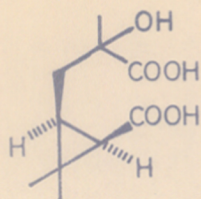


X

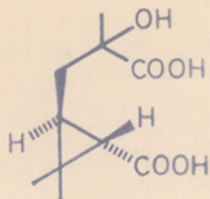
available (+) car-3-ene (I) was considered as the ideal starting material.

The oxidation of (+) car-3-ene (I) by potassium permanganate^{8,9} has been extensively studied. On oxidation in acetone the acid part was found to consist of a mixture of the following compounds which have been isolated and characterised through their ethyl esters, (a) cis and trans caronic acids, (VI and VII) (b) cis and trans homocaronic acids, (VIII and IX) (c) assy. dimethyl succinic acid, (X) (d) hydroxydicarboxylic acids (XI and XII). The hydroxy carboxylic acids on oxidation with potassium permanganate give the corresponding cis and trans keto carboxylic acids (XIII and XIV). Several methods have been described for the oxidation of (I) by potassium permanganate under different conditions^{10,11,12}. These methods, however, give rise to complex mixture of acids, as mentioned above and the isolation which involves fractionation of methyl or ethyl esters followed by saponification is rather tedious and in addition the yields are poor. Cis-caronic acid has also been obtained from car-3-ene 2,5-dione (XV)¹³ by oxidation with potassium permanganate. A survey of the literature indicated that compound (XV) itself has not been obtained in high yields from (I).

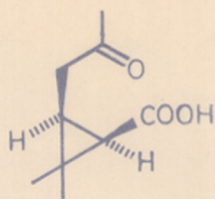
One of the methods by which (XV) can be obtained from (I) is by subjecting the latter to allylic oxidation.



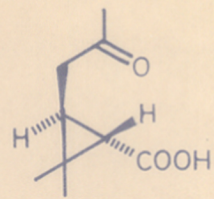
XI



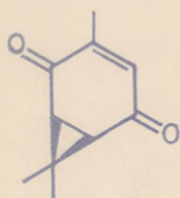
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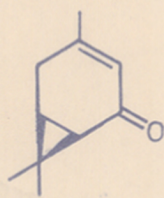
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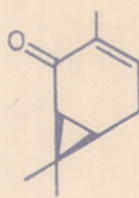
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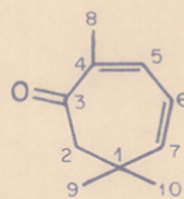
XV



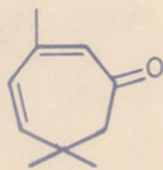
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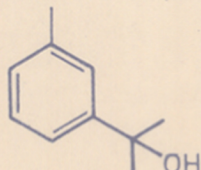
XVII



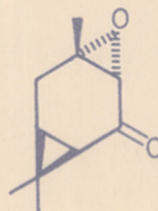
XVIII



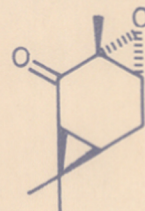
XIX



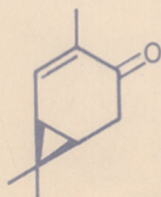
XX



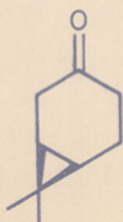
XXI



XXII



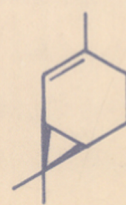
XXIII



XXIV



XXV



XXVI

During the oxidation of (I) by potassium permanganate¹⁴, the neutral portion left after the removal of acids, was found to consist of a complex mixture of compounds which have been separated by employing fractionation and column chromatography on silicic acid. Prominent among these are (-) car-3-ene-5-one (XVI), (75% of the neutral portion) and the remaining have been identified by spectral data and chemical reactions and are listed below (XVII to XXV). However, the diketone (XV) could not be isolated, though its presence along with XVI was detected by GLC analysis.

When the oxidation of (I) was carried out by chromic acid¹⁵ in acetone and pyridine although other allylic oxidation products were obtained, no trace of diketone (XV) could be detected.

In the methods described above the total neutral oxidation products could be obtained only in 10-15% yield, the major portion of (I) being recovered unchanged.

PRESENT WORK

The allylic oxidation by using sodiumdichromate and acetic acid with or without sodiumacetate has led to allylic oxidation products in fairly good yield in the case of many olefinic compounds^{16,17,18}. With a view to obtain the diketone (XV), (I) was subjected to allylic oxidation using sodium dichromate-acetic acid. Though normally, the oxidation by the above

reagents is carried out around 100°C or at refluxing temperature of acetic acid, taking into consideration the susceptibility of the cyclopropane ring system towards acid rearrangements, the reaction was carried out at room temperature for a prolonged time (48 hrs). The oxidation product after work up was found to consist of at least 5 major compounds. It was subjected to chromatography over alumina (Gr. II, 1:20 ratio) and eluted with solvents viz. pet.ether, pet.ether-benzene (1:1), benzene and chloroform. The earlier fractions eluted with pet.ether, which constituted about 70% of the total oxidation product was found to contain mostly unchanged (I) along with a minor proportion of rearranged hydrocarbons, according to IR spectra and comparative GLC and TLC.

The fraction eluted with pet.ether + benzene (1:1) showed the presence of five compounds according to TLC analysis. IR spectrum of some of these fractions indicated it to consist of acetates (bands at 1735, 1250 cm^{-1}). However even on repeated chromatography it was not possible to isolate them in pure state. In the earlier fractions of rechromatography, a small quantity of a hydrocarbon could be isolated in about 90% purity (GLC) and was identified as car-2-ene (XXVI) on the basis of spectral data and elemental analysis. Its NMR (CCl_4) spectrum shows signals at 9.25, 8.98 (3H, each s, cyclopropane methyls at C_7); centred at 9.37 (1H, m, C_6 -proton); 8.7

(1H, s, C₁-allylic proton); 8.42 (3H, s, vinyl methyl at C₃) and at 4.8 (1H, br, s, olefinic proton at C₂).

Some of the fractions eluted with benzene gave a liquid, TLC pure ketone (95% by GLC) which was further purified by distillation and identified as the 1,1,4-trimethyl, cyclohepta 2,4-diene-6-one (XIX) C₁₀H₁₄O, M⁺ 150, as per spectral data given below; IR bands at 1650 (>C=O, completely conjugated dienone); 1610 (conj. >CH = CH< and >C = CH-) 1380, 1365 (gem dimethyl); NMR (CCl₄) signals at: 8.92 (6H, s, gem dimethyl at C₁); 8.0 (3H, s, C₄ - vinyl CH₃); 7.5 (2H, s, C₇-CH₂ adjacent to >C=O); 3.95 (1H, d, J = 13.2 Hz, C₂-olefinic proton); 4.32 (1H, d, J = 13.2 Hz, C₃-olefinic proton) and 4.10 (1H, s, olefinic proton at C₅); UV $\lambda_{\max}$ 298 m μ , $\epsilon_{\max}$ 5500. It gives a 2:4 DNP derivative, C₁₆H₁₈O₄N₄, m.p. 173-175° [Lit.¹⁵ records 175-6° for 2:4 DNP of (XIX)]. This compound as reported earlier might have been formed by the acid rearrangement of ketone (XVI), expected to be formed from (I) by allylic oxidation in the same reaction. This compound has also been reported to have been formed during oxidation of (I) by chromium trioxide¹⁵ in acetone and pyridine and also by potassium permanganate¹⁴.

The tail fractions eluted with benzene (TLC single spot) on crystallisation from ethanol gave a solid C₁₀H₁₂O₂, m.p. 95-96° identified as the car-3-ene, 2,5-dione (XV) by spectral data described below; IR bands at 1650 (conj. >C = O)

1613, 858 (conj. $-\text{CH} = \text{C} <$); NMR (CCl_4) signals at 8.63, 8.67 (3H each, s, gem dimethyl at C_7); 3.07 (3H, s, C_3 -vinyl CH_3); 7.8 (2H, s, cyclopropyl protons at C_1 and C_6) and at 3.6 (1H, br, s, olefinic proton at C_4); UV λ_{max} 227 m μ , ϵ_{max} 8800; 241 m μ , ϵ_{max} 10,420. It gives a dioxime $\text{C}_{10}\text{H}_{14}\text{O}_2\text{N}_2$, m.p. 182-183° (Lit.¹³ for dioxime of XV m.p. 185°). The physical constants and spectral properties are thus in agreement for (XV) as reported in literature. However, the yield of the diketone works out to only 2.5% on the basis of oxidised material, though it could be isolated by a single chromatography on alumina.

The fractions eluted with chloroform were further purified by rechromatography over alumina, to get a liquid alcohol $\text{C}_{10}\text{H}_{14}\text{O}$, b.p. 75-76°/0.5 mm, M^+ 150 which was identified as 8-hydroxy m-cymene (XX) by spectral data; IR bands at 3400 (OH), 4 bands at 1961, 1887, 1792, 1754 (all weak), (m , disubstituted benzene¹⁹), 1605, 1587 (aromatic); NMR(CCl_4) signals at 8.5 (6H, s, methyls of hydroxy isopropyl); 7.65 (3H, s, CH_3 aromatic); 7.27 (1H, s, exchangeable with D_2O , OH proton); 3.04 and 2.67 (4H, m, aromatic protons).

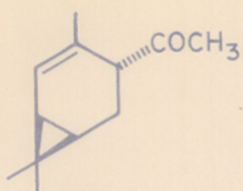
As observed earlier in the case of oxidation of (I) by chromium trioxide in acetone and pyridine, 8-hydroxy-m-cymene (XX) happens to be the predominant oxidation product in this oxidation also.

While this work was in progress, a convenient method for obtaining gig-caronic acid²⁰ from (+) car-3-ene was developed in our laboratory. The method consists in converting (+) car-3-ene (I) into 4- α -acetyl-car-2-ene (XXVII) by a Friedle-Crafts' type acylation, which is then subjected to oxidation by potassium permanganate either in acetone or aqueous medium. By this procedure, gig-caronic acid could be obtained in yields ranging from 35% to 45% on the basis of converted (XXVII).

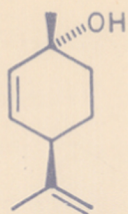
In this connection, we felt it desirable to examine some more transformations of 4- α -acetyl-car-2-ene (XXVII) which can be conveniently prepared from (I) in good yields.

Car-2-ene (XXVI) when treated with peracetic acid and sodium acetate in methylene dichloride is reported²¹ to give a mixture of compounds (XXVIII) and (XXIX) which are formed as a result of the rearrangement of the initially formed epoxide (XXX) under acidic conditions (Scheme I). With a view to ascertain the effect of the nature of the substituent at C₄-position on the cleavage of the C₂-C₃ epoxide of car-2-ene derivatives, the epoxidations of some 4-substituted car-2-ene derivatives were undertaken.

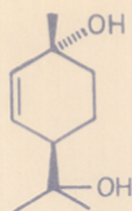
Car-3-ene (I) when treated with acetic anhydride in presence of Lewis acids like anhydrous zinc chloride gives in fairly good yield²², the 4- α -acetyl-car-2-ene (XXVII), which can be obtained in 95% purity (GLC) by fractionation. This compound, containing



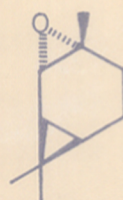
XXVII



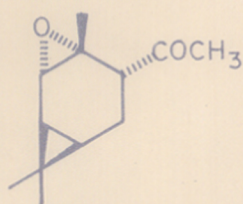
XXVIII



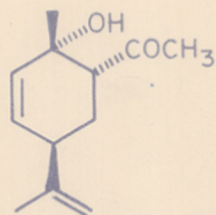
XXIX



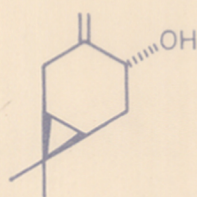
XXX



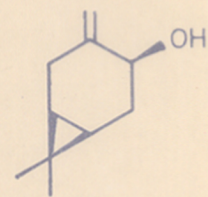
XXXI



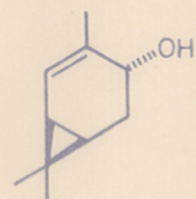
XXXII



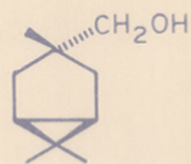
XXXIII a



XXXIII b



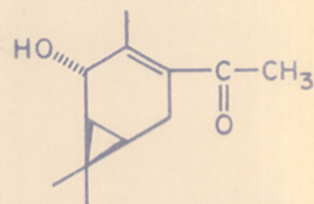
XXXIV



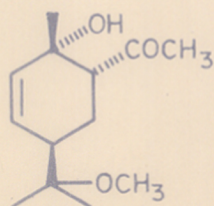
XXXV



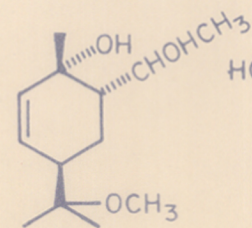
XXXVI



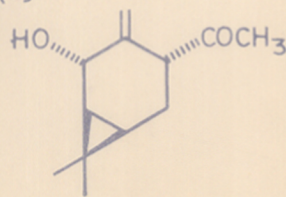
XXXVII



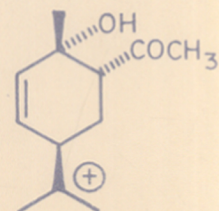
XXXVIII



XXXIX



XL



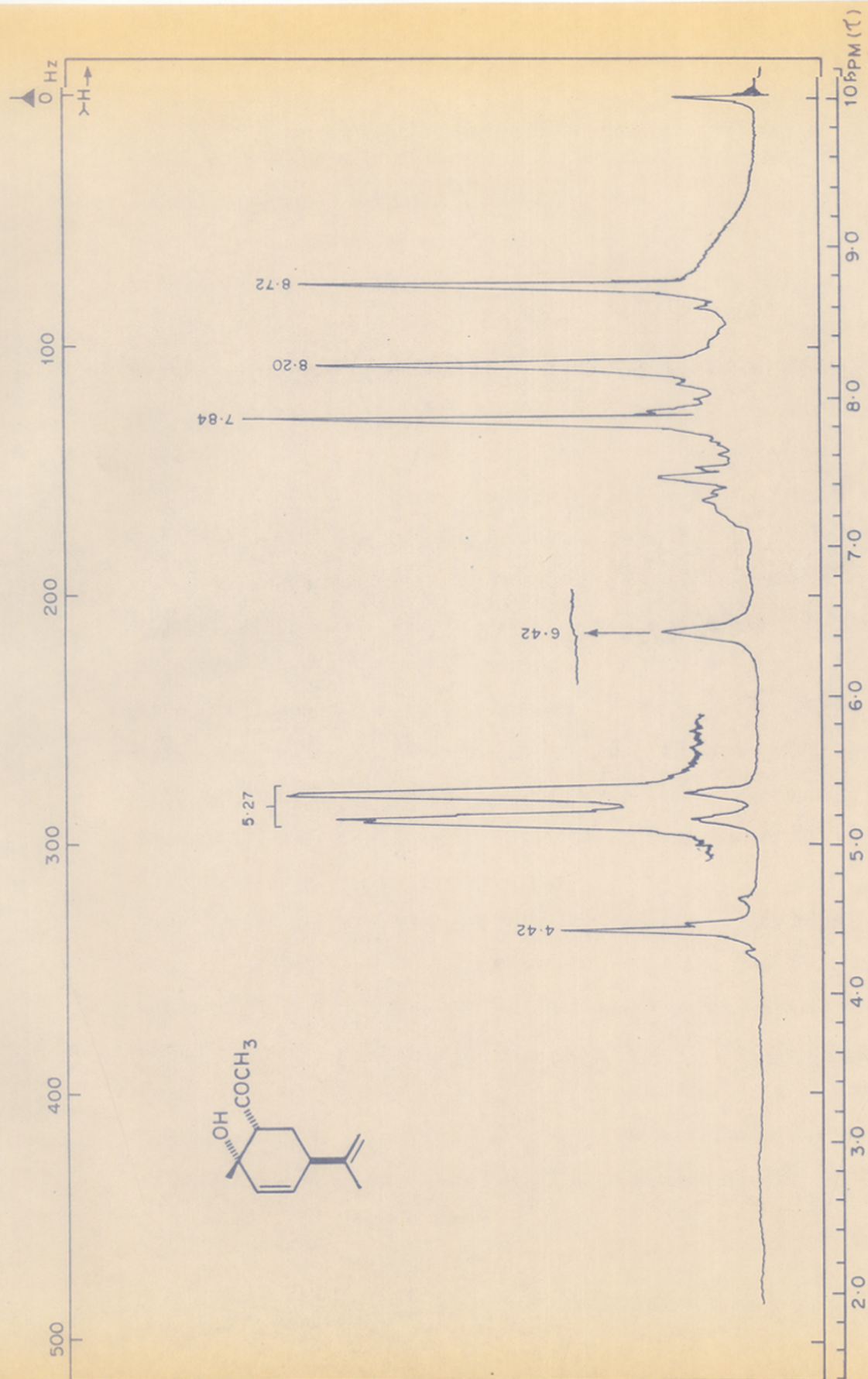
XLI

the acetyl group at the C₄-position was selected for our epoxidation studies. Treatment of (XXVII) with either *m*-chloroperbenzoic acid in chloroform at 10° or perbenzoic acid at 0°C, gives a mixture of two compounds (TLC), which could be separated by chromatography over silicic acid. The less polar compound, the major product of the reaction obtained as a liquid (TLC pure) has been identified as the epoxide²² (XXXI) C₁₂H₁₈O₂ by elemental analysis and spectral data; M⁺ 194; IR bands at: 1709 (C=O); 1153 (epoxide); NMR(CCl₄) signals at: 8.92, 9.02 (3H, each, s, methyls on cyclopropane at C₇); 9.17 (2H, br, m, cyclopropane protons at C₁ and C₆); 8.62 (3H, s, methyl at C₃-bearing oxygen); 7.90 (3H, s, -COCH₃ at C₄); 7.25 (1H, s, C₂-proton of epoxide) and centred at 7.54 (1H, m, C₄-proton). The epoxide (XXXI) is found to be sensitive to heat and unless a high vacuum of the order of 0.1 mm is employed, it could not be distilled without undergoing change. The spectral properties described above were in agreement with those reported in literature²².

The more polar compound, the minor product of the reaction (15%) has been obtained as a liquid (TLC pure). It is identified as (XXXII), C₁₂H₁₈O₂ by spectral data and elemental analysis. M⁺ 194; IR bands at: 3650 (OH), 1124 (tertiary -OH);

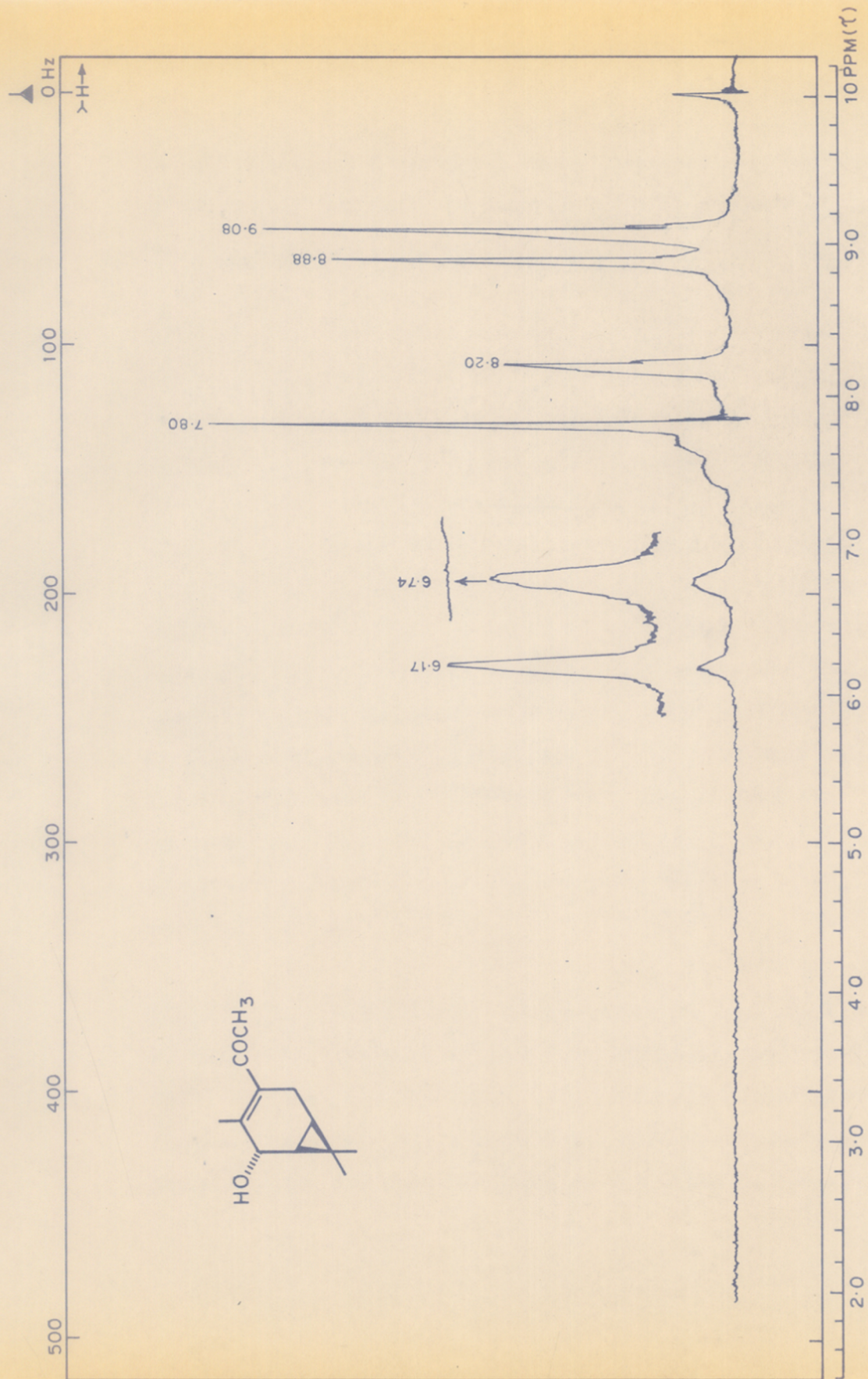
1709 ($>C=O$); 1658, 892 ($>C=CH_2$); 749 (cis- $CH=CH-$); NMR(CCl_4) (Fig. 1); signals at: 8.72 (3H, s, methyl at C_1); 8.2 (3H, s, vinyl methyl at C_8); 5.27 (2H, m, $>C=CH_2$, protons at C_{10}); 7.84 (3H, s, $-COCH_3$ at C_2) and at 4.42 (2H, br, s, olefinic protons at C_5 and C_6), 6.42 (1H, hydroxy proton exchangeable with D_2O). Compound (XXXII) might have been formed by the rearrangement of the epoxide (XXXI) under the acidic conditions of the reaction, as already observed in the case of car-2-ene epoxide (XXX). The amount of the rearranged compound (XXXII) could be considerably reduced, when perbenzoic acid carefully washed with ice-cold water is employed for epoxidation.

The rearrangement of oxiranes on alumina solid matrix to allylic alcohols and other rearranged products has been extensively studied. For example, when carane 3,4- α -epoxide (XXV) is rearranged on alumina solid matrix, a mixture of allylic alcohols (XXXIII and XXXIV) results²³ in addition to the rearranged alcohol (XXXV), while the corresponding β -epoxide (XXXVI) under similar conditions gives a mixture of three compounds in which the allylic alcohol (XXXIII) is also present. When an attempt was made to purify the crude epoxide (XXXI) on alumina (Gr. II, 1:20 ratio), it was observed that a major portion of the epoxide was rearranging to a more polar compound. This was confirmed by adsorbing the pure epoxide (XXXI) on a column of alumina (Gr. II, 1:20 ratio) for 24 hrs followed by elution, when an allylic alcohol assigned structure (XXXVII)

FIG. 1. NMR SPECTRUM OF COMPOUND (XXXII) (CCl_4)

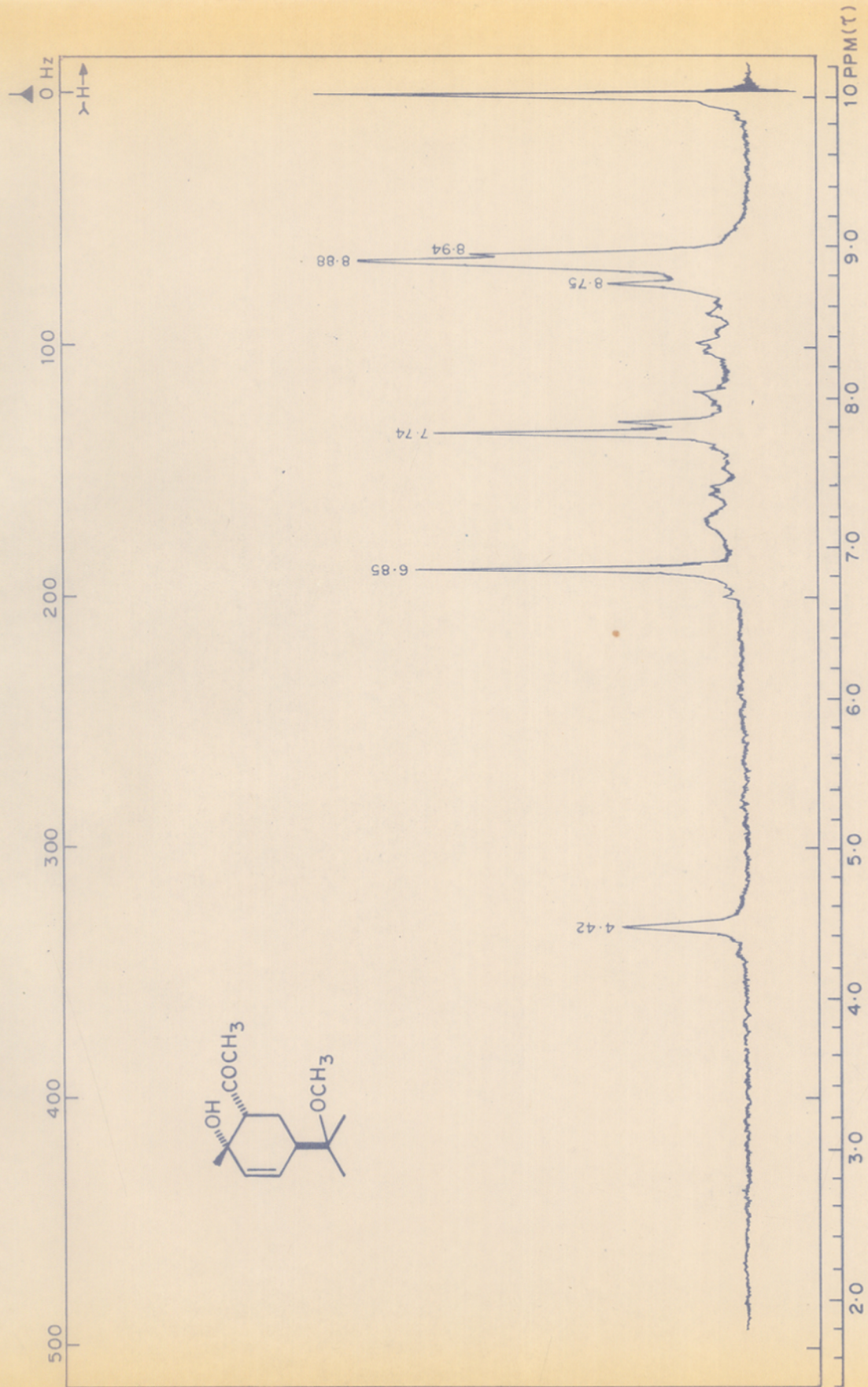
could be eluted with chloroform in the tail fractions of chromatography. Compound (XXXVII) a liquid, (attempts to purify the compound by distillation resulted in its rearrangement to other compounds as indicated by TLC analysis of the distillate) $C_{12}H_{18}O_2$, $(\alpha)_D - 24^\circ$ (c, 3), $M^+ 194$, shows IR bands at: 3478 (OH), 1695 (conj. $>C=O$), 1639 (conj. $>C=C<$); NMR (CCl_4) (Fig. 2); signals at: 8.88 (3H, s, methyl on cyclopropane); 9.08 (5H, br, s, CH_3 on cyclopropane and cyclopropane protons at C_1 and C_6); 8.2 (3H, s, vinyl methyl at C_3); 7.8 (3H, s, $-COCH_3$ at C_4); 6.74 (1H, s, exchangeable with D_2O , OH proton) and 6.17 (1H, s, proton at C_2); UV λ_{max} 246 m μ , ϵ_{max} 4000. The compound was found to be sensitive to heat and acids and even during the course of acetylation, (AC_2O/Py), in addition to the acetate other rearranged products were formed (TLC). Compound (XXXVII) is also obtained as the major product when the epoxide (XXXI) is treated with catalytic quantity of sodium hydroxide in methanol at room temperature.

It is already known that carane 2,3- α -epoxide (XXX) on chromatography over silicic acid rearranges to the diol (XXIX)²¹ while with metatitanic acid it rearranges to the monol (XXVIII)²¹. With a view to ascertain whether the epoxide (XXXI) which possesses the acetyl group at C_4 also rearranges in the same manner as (XXX) or differently, the epoxide (XXXI) was treated with dil. hydrochloric acid (1N)²⁴ in methanol at 10° for 24 hrs.

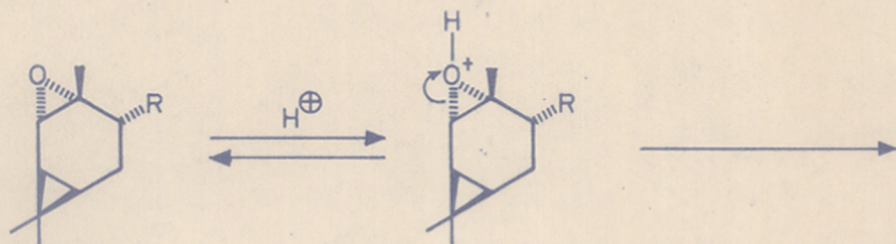
FIG. 2. NMR SPECTRUM OF ALLYLIC ALCOHOL (XXXVII) (CCl_4)

By this treatment a mixture of four compounds was obtained of which only two could be isolated pure, by chromatography. The less polar of the two was identified as (XXXII) according to comparative TLC analysis and spectral data (as given earlier). The more polar compound, a liquid (sensitive to heat, so could not be distilled without rearrangement), $C_{13}H_{22}O_3$ $M^+ 226$, $(\alpha)_D + 18^\circ$ (c, 2.5) has been identified as (XXXVIII) as per spectral data described below; IR bands at: 3636 (OH); 1704 ($>C=O$), 740 (gig-disubstituted double bond; NMR (CCl_4) (Fig. 3) signals at: 8.75, 8.88, 8.94 (9H, methyls at C_1 and C_8); 7.74 (3H, s, $COCH_3$ at C_2); 6.85 (3H, OCH_3 at C_3) and 4.42 (2H, br, s, olefinic protons at C_5 and C_6). The compound (XXXVIII) does not give an acetate under normal conditions (Ac_2O/Py at room temperature) indicating the tertiary nature of the hydroxyl group. These data suggest structure (XXXVIII) for the compound. The formation of (XXXVIII) from the epoxide (XXXI) by acid which follows the same course as in the rearrangement of carane 2,3 epoxide is shown in the scheme (I).

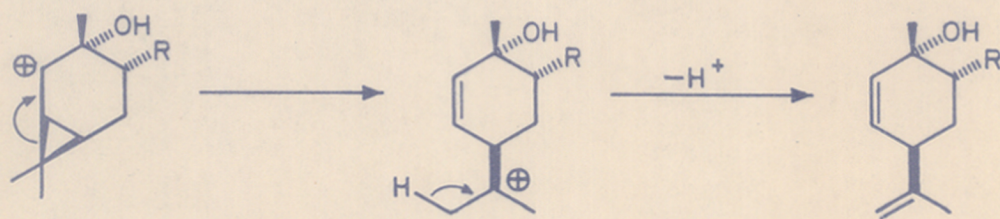
Sodiumborohydride reduction of (XXXVIII) gave the diol (XXXIX) as a liquid (probably epimers at C_{11}), $C_{13}H_{24}O_3$, $M^+ 228$, $(\alpha)_D + 77^\circ$ (c, 5) possessing the following spectral data; IR bands at: 3300 (OH), 740, 1640 (gig-disubstituted double bond); NMR (CCl_4) signals at: 8.85, 8.87, 8.94 (9H, methyls at C_1 and C_8); 8.74 (3H, d, $J = 6$ Hz, $C_{11}-CH_3$);

FIG. 3. NMR SPECTRUM OF COMPOUND (XXXVIII) (CCl_4)

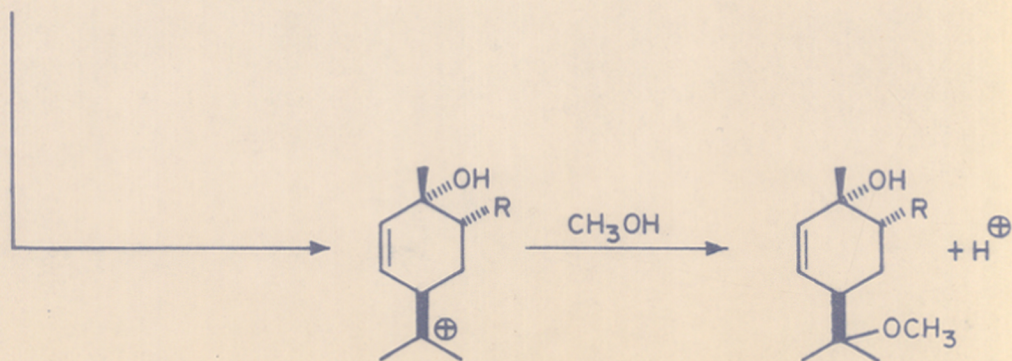
SCHEME - 1.



XXXI $R = -COCH_3$



XXXII $R = -COCH_3$



XXXVIII $R = -COCH_3$

6.85 (3H, s, OCH_3 at C_8); 6.08 (2H, br, s, exchangeable with D_2O , OH protons at C_1 and C_{11}) and at 4.4 (2H, br, s, olefinic protons at C_5 and C_6).

In conclusion it can be said that epoxide (XXXI) under mild alkaline conditions gives mostly the conjugated keto-alcohol (XXXVII), the acetyl group at C_4 , thus influencing its formation. The ^{non}conjugated double bond isomer (XL), however could not be detected in the reaction products. In presence of acid, the epoxide (XXXI) after initial protonation, rearranges to compounds (XXXII) and (XXXVIII) via the intermediate carbonium ion of structure (XLI). In this respect it resembles carene, 2,3 α -epoxide.

EXPERIMENTAL1. Oxidation of (+) car-3-ene by sodiumdichromate and acetic acid

(+) Car-3-ene (I, 55 g; approx. 0.4 mole) and acetic acid (200 ml) were placed in a 2 litre 3-necked flask, fitted with a mechanical stirrer. The mixture was stirred at room temperature and powdered sodium dichromate ($\text{Na}_2\text{Cr}_2\text{O}_7$; 148 g approx. 0.55 mole) was added in small portions during a period of 6 hr. The stirring was continued at the room temperature for 42 hrs. It was then diluted with water (1 litre) and extracted thrice with chloroform (3 x 250 ml). The chloroform layer was washed with aqueous sodiumcarbonate solution (5%), followed by water and dried. Distillation of chloroform gave the crude oxidation product (48 g). It was chromatographed over alumina (Gr. II, 960 g; 1:20 ratio) and eluted as shown below.

Chromatogram

<u>Fr.No.</u>	<u>Solvent</u>	<u>Volume in ml</u>	<u>Wt in g</u>	<u>TLC pattern</u>
1.	pet.ether	1250 (5 fractions of 250 ml)	18.75	Unreacted (+)car-3-ene (one spot)
2.	"	250	0.5	^a Minly one spot
3.	pet.ether + benzene (1:1)	1500 (6 fractions of 250 ml)	9.2	5 spots
4.	benzene	500	0.689	"
5.	"	250	1.43	one spot
6.	"	250	1.16	almost single spot
7.	benzene + chloro- form (1:1)	250	1.44	"
8.	Chloroform	500	7.87	one spot

Fraction 1 mostly contained unreacted (+) car-3-ene identified by IR spectrum and comparative GLC with an authentic sample.

Fraction 2 on rechromatography over alumina (Gr. I, 10g; 1:20 ratio) gave a hydrocarbon (GLC pure) in the pet.ether eluted fraction which was identified as car-2-ene (XXVI) by spectral data. M^+ 136.

Analysis

Found: C, 37.95; H, 11.68

$C_{10}H_{16}$ requires C, 38.16; H, 11.84%.

Fractions 3 and 4; both showing 5 spots in TLC analysis, showed in their IR spectra, bands at 1735 and 1250 cm^{-1} due to acetate. Attempts to separate the components of the mixture by chromatography were however, unsuccessful.

Fraction 5 (TLC single spot) was purified by distillation; b.p. 140-45° (bath)/3.5 mm (95% pure by GLC). M^+ 150 identified as (XIX) by spectral data.

IR bands: 1650, 1610, 1443, 1380, 1365, 1333, 1312, 1282, 1236, 1198, 1150, 1130, 1105, 1020, 976, 948, 913, 781, 749 cm^{-1} .

Analysis

Found: C, 80.12; H, 9.61

$C_{10}H_{14}O$ requires : C, 79.95; H, 9.39%.

It gave a 2:4 DNP derivative $C_{16}H_{18}O_4N_4$, m.p. 173-175° [Lit.¹⁵ records, m.p. 175-6° for 2:4 DNP of (XIX)].

Analysis

Found: C, 57.65; H, 5.64; N, 16.47

$C_{16}H_{18}O_4N_4$ requires C, 58.17; H, 5.49 and N, 16.96%.

Fraction 6 and 7 showing identical pattern were mixed together and crystallised from ethanol to give a pale yellow crystalline solid m.p. 96-97° identified by spectral data as (XV, 1.3 g).

IR bands: 1650, 1613, 1439, 1370, 1342, 1325, 1282, 1230, 1179, 1133, 1115, 1057, 1030, 1003, 986, 964, 926, 908, 858, 833, 790, 740, 700 cm^{-1} .

Analysis

Found: C, 72.86; H, 7.57

$C_{10}H_{12}O_2$ requires C, 73.14; H, 7.37%.

It gave a dioxime $C_{10}H_{14}O_2N_2$, m.p. 182-83° [Lit.¹³ records m.p. 185° for the dioxime of (XV)].

Analysis

Found: C, 61.51; H, 7.41; N, 14.01

$C_{10}H_{14}O_2N_2$ requires C, 61.83; H, 7.27; N, 14.42%

Fraction 8 (TLC almost pure) was further purified by rechromatography over alumina (Gr. II, 120 g, 1:15 ratio) and the fraction eluted with benzene + chloroform (8:1) gave TLC pure alcohol, identified by spectral data as 8-hydroxy m-cymene (XX), b.p. 75-76°/0.5 mm (GLC, 98% pure), M^+ 150. IR bands at: 3400, 1961, 1887, 1792, 1754, 1605, 1587, 1481, 1439, 1351, 1256, 1156, 1072, 1026, 1010, 990, 945, 894, 855, 811, 779 cm^{-1} .

Analysis

Found: C, 80.17; H, 9.16

$C_{10}H_{14}O$ requires C, 79.95; H, 9.39%.

2. Preparation of 4- α -acetyl car-2-ene (XXVII)

A mixture of acetic anhydride (220 ml; 2.33 moles), (+) car-3-ene (I, 136 g, 1 mole) was heated to 50° with stirring in an atmosphere of nitrogen. Anhydrous zinc chloride (24 g; 0.13 mole) was then added portionwise over a period of two hours. The mixture was stirred at 50° for additional 3 hr. and then poured into water (300 ml). The organic layer was extracted thrice with ether, washed with aqueous sodium bicarbonate solution (5%), water and dried. Evaporation of ether furnished the crude product, which was fractionated and the fraction boiling at 54-60°(vapour)/10 mm was collected. This fraction consisted of chiefly compound (XXVII, 97 g; GLC 98%).

3. Epoxidation of 4- α -acetyl car-2-ene

To a cooled solution of (XXVII, 24.3 g in 100 ml chloroform) a chloroform solution of perbenzoic acid (75 ml, 2.2N; 1.2 moles) was added and the homogeneous solution was allowed to stand at 0° for 48 hr. After dilution with chloroform (200 ml), it was extracted with aqueous sodiumbicarbonate solution (5%) to remove benzoic acid formed, washed with water and dried. Distillation of chloroform gave the crude epoxide (24 g), which consisted of three products according to TLC analysis. It was chromatographed on silicic acid (480 g; 1:20 ratio) and eluted as follows:

Chromatogram

<u>Fr.No.</u>	<u>Solvent</u>	<u>Volume in ml</u>	<u>Wt. in g</u>	<u>TLC pattern</u>
1.	Pet.ether	2000	-	-
2.	Pet.ether +benzene (2:1)	2000	5.2	one spot
3.	Pet.ether +benzene (1:1)	400	1.4	one spot
4.	"	600	0.5	2 spots
5.	"	1000	2.95	one spot
6.	Benzene	1000	3.2	2 spots
7.	Chloroform	200	1.7	2 spots
8.	Chloroform	800	1.54	2 spots
9.	Ethyl acetate	400	0.2	4 spots

Fractions 2 and 3 showed single spot in TLC analysis corresponding to that of (XXVII). IR superimposable with that of (XXVII). Fraction 4 being in small quantity was not examined further.

From fraction 5 the last traces of solvent were removed under high vacuum. The product thus obtained was identified as the epoxide (XXXI, 2.95 g) by spectral data M^+ 194.

IR bands at 1709, 1437, 1404, 1368, 1344, 1300, 1258, 1219, 1183, 1153, 1014, 928, 877, 865, 837 cm^{-1} .

Analysis

Found: C, 73.98; H, 9.12

$\text{C}_{12}\text{H}_{18}\text{O}_2$ requires C, 74.19; H, 9.34%.

Fractions 6 and 7 were mixed together and rechromatographed on silicic acid (50 g, 1:10 ratio) to get more of the epoxide (XXXI; 2.0 g). IR superimposable with that of epoxide (XXXI) obtained in Fraction 5.

Fraction 8 rich in the alcohol was purified by rechromatography over silicic acid to give in the tail fractions the pure alcohol (XXXII, 0.6 g) identified by spectral data.

M^+ 194.

IR bands at: 3650, 1702, 1652, 1449, 1344, 1212, 1167, 1124, 1000, 892, 846, 742 cm^{-1} .

Analysis

Found: C, 74.06; H, 9.46

$\text{C}_{12}\text{H}_{18}\text{O}_2$ requires C, 74.19; H, 9.34%.

4. Rearrangement of the epoxide (XXXI) over alumina to the allylic alcohol (XXXVII)

A column of alumina (Gr. II, 80 g) was developed with pet.ether as the eluent and a solution of (XXXI) in pet.ether (4 g in 15 ml) was adsorbed in the column. The adsorbed material was allowed to stand in contact with alumina for 24 hrs. It was then eluted with pet.ether, pet.ether + benzene (1:1), benzene, chloroform and ethylacetate. The fraction eluted with chloroform gave the allylic alcohol (XXXVII, 1.4 g); in the pure state (TLC).

$(\alpha)_D^{28} -24^\circ$ (c, 3), M^+ 194. It was identified by spectral data.

IR bands 3478, 1695, 1632, 1418, 1370, 1350, 1224, 1200, 1130, 1050, 1000, 978, 862 cm^{-1} .

Analysis

Found: C, 74.34; H, 9.60.

$\text{C}_{12}\text{H}_{18}\text{O}_2$ requires C, 74.19; H, 9.34%.

5. Rearrangement of the epoxide (XXXI) to the allylic alcohol (XXXVII) by treatment with alkali

To a solution of epoxide (XXXI, 0.5 g) in methanol (50 ml), a solution of potassium hydroxide (0.1 g in methanol 10 ml) was added. The reaction mixture was kept at room temperature for 24 hr. It was then diluted with water (150 ml) and extracted thrice with chloroform. The chloroform layer was washed with dil. HCl (5%), followed by water and dried. Removal of the chloroform gave a liquid (0.4 g) which was chromatographed over alumina (Gr. II, 10 g) as indicated in (4). The chloroform eluted fraction gave a liquid (0.2 g, TLC pure) identified as (XXXVII) by spectral data and rotation.

6. Rearrangement of epoxide (XXXI) by hydrochloric acid in methanol to compounds (XXXII) and (XXXVIII)

To an ice cooled solution of epoxide (XXXI, 7.8 g) in methanol (100 ml), hydrochloric acid (1N, 1 ml) was added. The reaction mixture was kept at 10° for 24 hr. It was then diluted with water (500 ml) and extracted thrice with chloroform (3 x 100 ml). The chloroform layer was washed with aqueous

sodium bicarbonate solution (5%), followed by water and dried. Removal of chloroform gave a product (7.5 g) which consisted of four compounds (TLC, 4 spots). It was chromatographed over alumina (Gr. II, 220 g, 1:30 ratio) and eluted as follows.

Chromatogram

<u>Fr.No.</u>	<u>Solvent</u>	<u>Volume in ml</u>	<u>Wt. in g</u>	<u>TLC</u>
1.	Pet.ether	500	0.7	2 spots
2.	Pet.ether + benzene (1:1)	500	0.35	3 spots
3.	Benzene	200	0.20	spots
4.	"	200	0.3	Mainly one spot
5.	Benzene + chloroform (2:1)	210	1.25	one spot
6.	Chloroform	200	0.4	3 spots
7.	"	300	3.1	Mainly one spot
8.	Ethylacetate	200	0.7	"

Fractions 1 to 3 containing 2 to 3 compounds being in small quantity were not investigated further.

Fractions 4 and 5 containing the less polar compound in major proportion, were mixed together and rechromatographed over silicic acid (22.5 g, 1:15 ratio) and the fraction eluted with a mixture of benzene and chloroform (7:1) furnished the alcohol (XXXII) in pure form, identified by comparative TLC, IR and NMR spectra. M^+ 194.

Analysis

Found: C, 74.48; H, 9.56.

$C_{12}H_{18}O_2$ requires C, 74.19; H, 9.34%.

Fractions 6 and 7 which were rich in the more polar compound were mixed together (3.8 g) and rechromatographed over silicic acid (57 g; 1:15 ratio) and eluted with benzene, benzene + chloroform mixtures, chloroform and ethylacetate. The fractions eluted with chloroform and ethylacetate gave the alcohol (XXXVIII) in the pure state (TLC). It was identified by spectral data and elemental analysis.

$(\alpha)_D^{28} + 18^\circ$ (c, 2.5); M^+ 226.

IR bands: 3636, 1704, 1632, 1460, 1376, 1359, 1135, 1094, 1069, 996, 919, 881, 823, 740 cm^{-1} .

Analysis

Found: C, 68.58; H, 10.06

$C_{13}H_{22}O_3$ requires C, 68.99; H, 9.80%.

7. Sodium borohydride reduction of alcohol (XXXVIII) to the diol (XXXIX)

To a solution of alcohol (XXXVIII, 1 g) in methanol (30 ml), sodiumborohydride (0.15 g) was added in small portions during half an hour under stirring and the reaction mixture stirred at room temperature for 24 hr. It was then diluted with water (200 ml), acidified with dil. HCl and extracted thrice with chloroform (3 x 50 ml). The chloroform layer was washed with water and dried. Removal of chloroform furnished a thick

liquid (0.85 g) which was purified by chromatography over silicic acid (10 g). The fraction eluted with chloroform (TLC pure) gave (XXXIX) (0.5 g).

$$(\alpha)_D^{27} + 77^\circ (c, 5); M^+ 228.$$

IR spectra: 3300, 1640, 1460, 1378, 1362, 1321, 1240, 1150, 1078, 1000, 935, 900, 879, 740 cm^{-1} .

Analysis

Found: C, 68.12; H, 10.30

$\text{C}_{13}\text{H}_{24}\text{O}_3$ requires C, 68.38; H, 10.59%.

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CHAPTER - IV

REVISED STRUCTURE OF NARDOSTACHONE
A SESQUITERPENIC KETONE ISOLATED FROM
NARDOSTACHYS JATAMANSI D.C.

S U M M A R Y

Nardostachone, a sesquiterpenic ketone isolated from Nardostachys latamansi D.C. which was initially assigned structure (VIII), has now been given revised structure (X), on the basis of spectral data (IR, UV, NMR, M^+ ion) and its identity shown with 1,8,9,10-tetrahydroaristolane-2-one, previously isolated from Nardostycone chinensis by Rücker.

Indian spikenard, Nardostachys jatamansi^{1,2} D.C.

(Valerianaceae) is a perennial herb growing at high altitudes upto 17,000 ft. in the Himalayan regions and Nepal. The rhizomes which are profusely covered with fibres are used in the indigenous system of medicine for treatment of hysteria, epilepsy and other nervous disorders, as an antidote to poisons, as a blood purifier, cure for infections of the throat and respiratory organs, leprosy and tubercular adinitis.

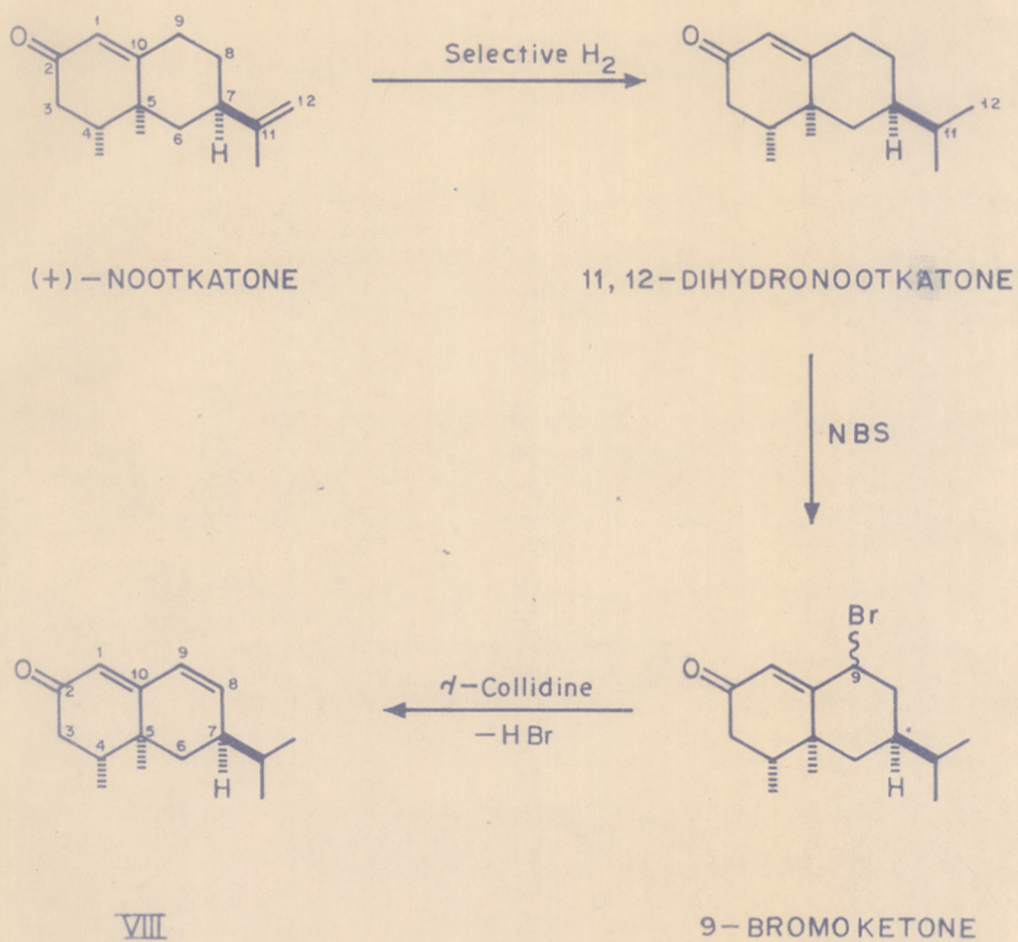
From the Indian variety of the plant, Choudhury et al. isolated jatamansic acid³ (I) and Govindachari et al. isolated jatamansone⁴ (valeranone) (II). From Nardostachys jatamansi (Roxl) D.C., compounds β -maaliene^{6,9} (III) and β -gurjunene⁵ (calarene)⁶ (IV) were isolated. Later Sastry et al. carried out the systematic chemical examination⁷ of Nardostachys jatamansi D.C. (black brown variety) in this laboratory. By employing low temperature solvent extraction procedure using 40-60° pet.ether, they obtained a concentrate from the roots of the plant. From the neutral portion of the concentrate they isolated the following compounds viz., nardol⁸ (V), β -gurjunene^{5,6} (calarene) (IV), aristolene⁹ (VI), valeranone¹⁰ (II) and calarenol¹¹ (VII). Subsequently, from the neutral portion, the same group (Sastry et al.) isolated a sesquiterpenic ketone, C₁₅H₂₂O, b.p. 130-35° (bath)/0.09 mm, (α)_D²⁷ + 209.3° (c, 3), which they named nardostachone and assigned the structure¹² (VIII) to it on the basis of spectral data and

chemical reactions. The spectral data given by them was as follows. IR bands at 1669, 1621, 1592 ($-\text{CO}-\text{CH}=\text{C}-\text{CH}=\text{CH}-$), 1370 ($-\text{CH}-\begin{smallmatrix} \text{Me} \\ \diagup \\ \text{Me} \end{smallmatrix}$), 1422 cm^{-1} ($-\text{COCH}_2$); UV $\lambda_{\text{max}}^{\text{EtOH}}$ 298 m μ ,

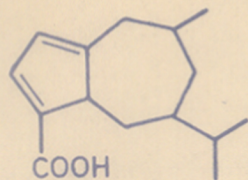
ϵ_{max} 15,200 (principle peak) and λ_{max} 235 m μ , ϵ_{max} 7478 (secondary peak), due to $-\text{CO}-$ group with extended conjugation. NMR signals at 3.64 (1H, quadruplet, $J_{\text{vic}} = 10\text{ Hz}$, $J_{\text{allylic}} = 5\text{ Hz}$) and 3.9 (1H, d, $J_{\text{vic}} = 10\text{ Hz}$) due to $-\text{CH}=\text{CH}-$ at C_8-C_9 , 4.42 (1H, s) due to $-\text{CH}=\text{C}-$ at C_1 , 7.77 (3H) due to $-\text{COCH}_2$ and one allylic proton at C_7 , 8.79 (3H, s) of quaternary methyl at C_5 , centred at 8.89 (6H, quadruplet, $J = 5.5\text{ Hz}$) due to $-\text{CH}-\begin{smallmatrix} \text{Me} \\ \diagup \\ \text{Me} \end{smallmatrix}$ and at 9.1 (3H, d, $J = 5.5\text{ Hz}$),

due to $-\text{CHMe}$ at C_4 . However, Pinder synthesized¹³ the ketone (VIII) as shown in Chart I and demonstrated that the synthetic ketone (VIII) is not identical with the natural ketone, on the basis of UV and NMR spectral data. Pinder pointed out that the reported UV absorption maxima ($\lambda_{\text{max}}^{\text{EtOH}}$ 298 m μ , ϵ_{max} 15,200) of nardostachone is not in agreement with structure (VIII) for which the calculated value is 280 m μ on the basis of Woodward's rule¹⁴. The reported⁷ UV maxima (317 m μ) for the semicarbazone of (VIII) is also not consistent with the structure assigned for the ketone. The synthetic ketone of Pinder had UV $\lambda_{\text{max}}^{\text{EtOH}}$ 283 m μ , ϵ_{max} 18,500. Its NMR spectrum in the olefinic region was very simple. In this region, only two signals appeared, at 3.8 (s, 2H, C_8-H and C_9-H in VIII) and

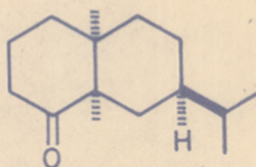
CHART I



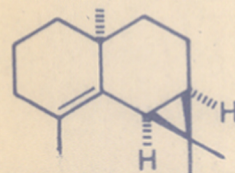
4.3 (s, slight evidence of splitting into a doublet, 1H, C_1 -H). A pair of doublets (6H and 3H) and a singlet (3H) (methyl groups in VIII) were also observed in the 9.0 τ region. The absence of coupling between the C_8 and C_9 protons and the allylic proton at C_7 in structure VIII, could be explained on the basis of the dihedral angle between them, which is about 90°¹³. The spectrum was very different in the low field region from that of nardostachone^{7,12} and it was apparent that the natural ketone could not be represented by structure (VIII). Support for this view was provided by the fact that the semicarbazone of synthetic nardostachone and that of (VIII), m.p. 230-32° (decomp.) and 213° (decomp.) respectively, were different, showing a large m.p. depression on admixture. On the basis of his new evidence Pinder confirmed that nardostachone could not be represented by (VIII), and suggested an alternative structure (IX), $C_{15}H_{20}O$, possessing cross-conjugated trienone system, on the basis of spectral evidence^{7,12}, already published by Sastry et al. However, according to Pinder, the structure (IX) could not explain some of the NMR signals, e.g. 9.10 τ (3H, d) assigned to $-CH-CH_3$ group and also the integration (1H) of the signal 4.42 τ which would have to be (2H) assuming that the hydrogens at C_1 and C_3 in (IX) have the same chemical shift.



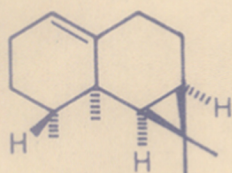
I



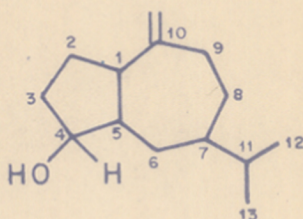
II



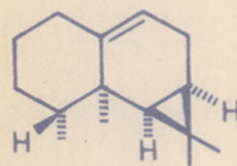
III



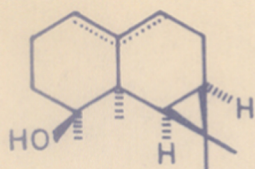
IV



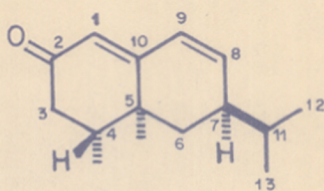
V



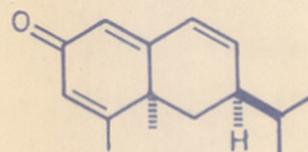
VI



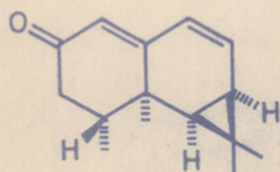
VII



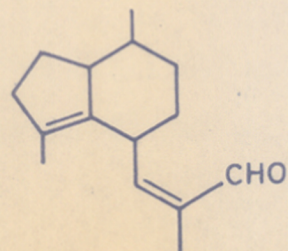
VIII



IX



X



XI

PRESENT WORK

This ambiguity of the NMR spectral data, pointed out by Pinder, prompted us to reinvestigate the structure of nardostachone and for this purpose, it was isolated again from the natural source, as per the procedure described⁷ and after careful purification by chromatography, a TLC pure sample of the ketone was obtained. The physical and spectral properties of the ketone are described below, on the basis of which the structure (X) is assigned to the ketone. The ketone has b.p. 132-35°(bath)/0.1 mm, n_D^{25} 1.5570, $(\alpha)_D^{28} + 215^\circ$ (c, 3). The high resolution mass spectrum of the ketone gives the molecular ion as 216. Accordingly and on the basis of elemental analysis the molecular formula is fixed as $C_{15}H_{20}O$. The IR spectrum (Fig. 1) shows bands at 1024, 879 (cyclopropane), 1658 (s, conj. $>C=O$), 1618, 1577 (conj. $-CH = C<$ and $-CH = CH-$ of the conjugated dienone system), and 1412 cm^{-1} ($-COCH_2$). The UV spectrum in ethanol shows λ_{max} 311 m μ , ϵ_{max} 12,940, as the main peak, instead of 298 m μ as reported by Sastry et al. The NMR (CCl_4) spectrum (Fig. 2) shows signals at: 9.14, 8.98 (3H, each, s, gemdimethyl on cyclopropane); 8.96 (3H, d, $J = 6\text{ Hz}$, C_4-CH_3); 8.86 (3H, s, quarternary methyl at C_5); 8.60 (1H, m, allylic cyclopropane proton at C_7); 7.76 (2H, m, $-CH_2$ at C_3 adjacent to $>C=O$); 4.40 (1H, s, conjugated olefinic proton at C_1) and at 3.90, 3.62

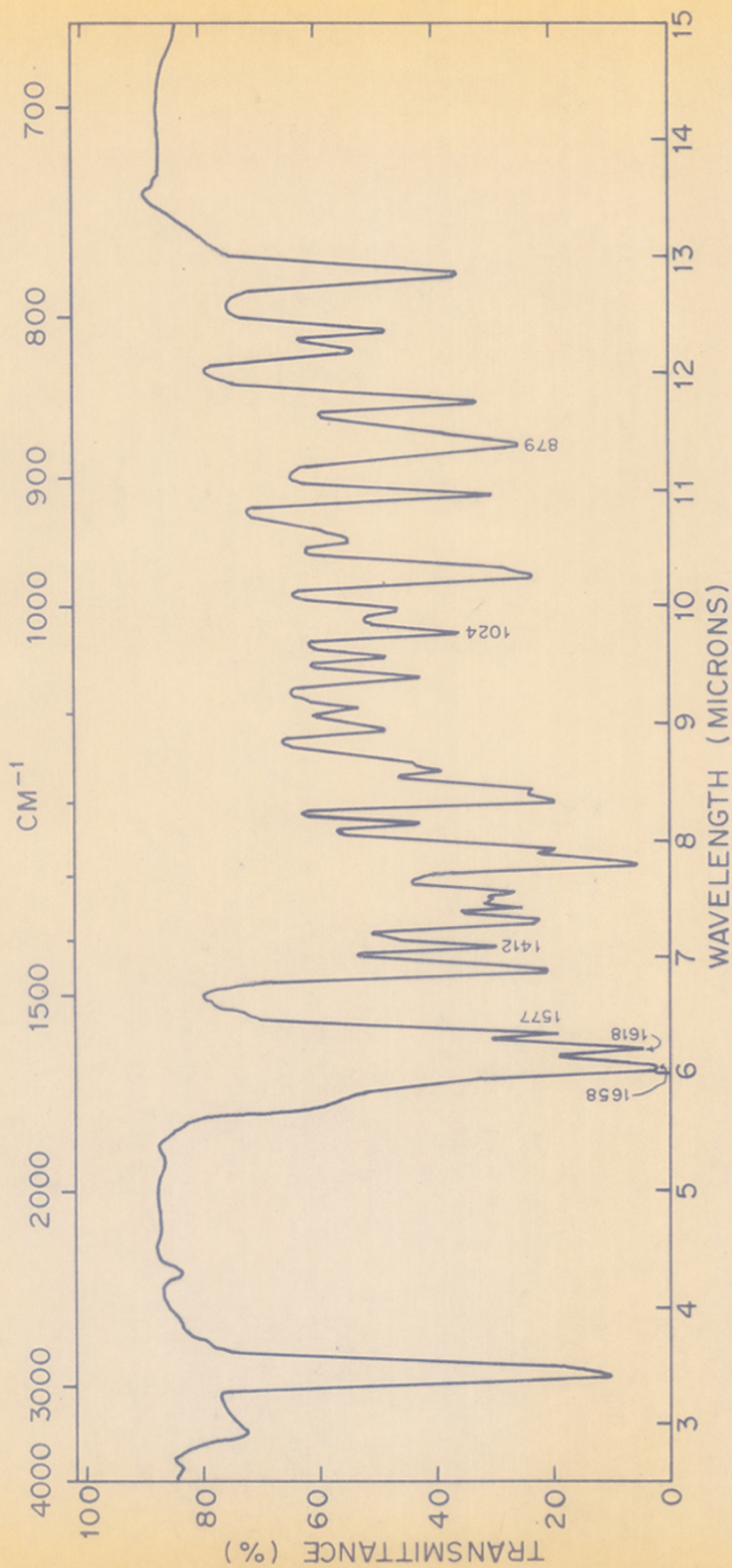
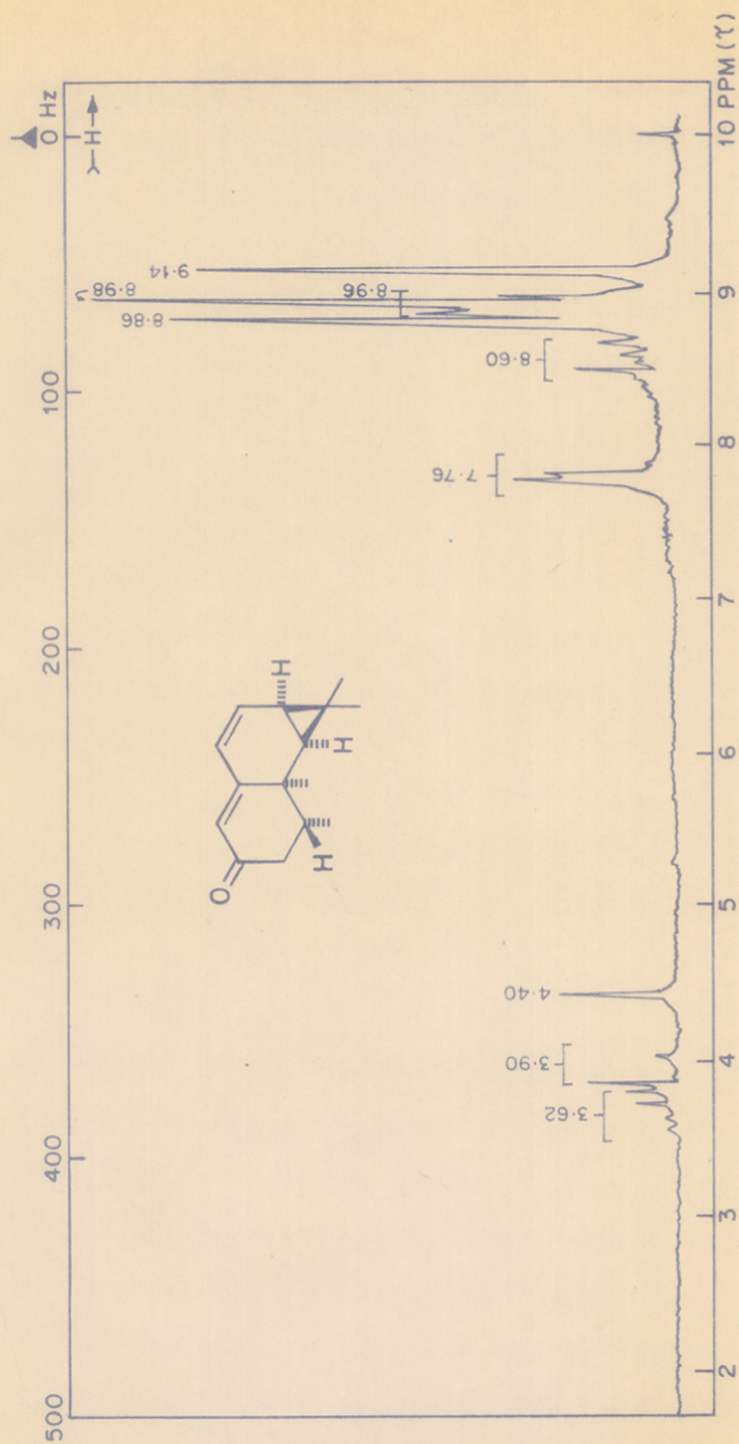


FIG.1. IR SPECTRUM OF NARDOSTACHONE (LIQ. FILM)

FIG. 2. NMR SPECTRUM OF NARDOSTACHONE (CCl_4)

(2H, m, $J_{vic} = 9.6$ Hz, C_8 and C_9 olefinic protons). It is known that in (+) car-3-ene and α -pinene, the methyl groups on the cyclopropane and cyclobutane rings are having different chemical shifts, which has been attributed to the shielding of one of the methyl groups by the π -electrons of the double bond. In this case also the methyls on cyclopropane which are similarly situated, differ in their chemical shifts. The protons at C_9, C_8 and C_7 form an ABX system with $J_{AB} = 9.6$ Hz. The spectral data described above are in complete agreement with the tricyclic structure (X) for nardostachone. This structure explains, more satisfactorily the spectral characteristics than the alternative structure (IX) proposed by Pinder¹³.

Indirect support for the structure (X) is available from its co-occurrence with β -gurjunene^{5,6} (IV), calareanol¹¹ (VII) and β -maaliene^{6,9} (III), all of which contain a cyclopropane ring.

Structure (X) was assigned¹⁵ previously by Rücker to the sesquiterpene ketone, $C_{15}H_{20}O$, from Nardostycone chinensis. Comparison of the physical and spectral data shows close similarity between the two ketones except for the magnitude of optical rotation. [Dr. G. Rücker has pointed out to us that the optical rotation reported in his paper for 1,8,9,10-tetra-dehydre aristolane-2-one and the dihydroproduct were in error. The correct values are $+230^\circ$ and $+160^\circ$ (in chloroform)]

respectively⁷. The identity of nardostachone with 1,8,9,10-tetrahydroaristolane-2-one is further confirmed by a comparison of IR, UV, NMR and mass spectra of the latter (kindly supplied by PROF. Rücker) with those of (X). This constitutes a proof that nardostachone shares the same carbon skeleton as that of β -gurjunene^{5,9} (IV).

We could isolate valeranone (II), valerenal (XI), nardol (V), n-hexacosanol, calarenol (VII) and β -sitosterol along with nardostachone (X) from the benzene eluted fraction of the main chromatography and thus confirmed the isolation of the previously reported compounds. However, we did not investigate fractions, other than benzene eluted fraction, obtained in the chromatography of the concentrate.

EXPERIMENTAL

1. Extraction of the root powder of *Nardostachys* *latamansi*, D.C.

A mixture of finely powdered roots (1 kg), pet.ether (40-60°, 3-L) was stirred for two hours, in a 10 L round bottom flask fitted with a mechanical stirrer. After keeping for fifteen minutes, the extract was filtered off. The operation was repeated twice with 2-L of solvent each time. From the combined filtrate the solvent was removed at 40° under reduced pressure. The concentrate obtained was dissolved in ethanol (350 ml) and cooled to 0° for 48 hr. The separated wax was filtered off and the alcohol removed from the filtrate at 60° under reduced pressure to yield 43 g of the concentrate (4.3%).

The concentrate (43 g) in ethereal solution was shaken with sodiumbicarbonate solution (10%) and processed in the usual fashion to yield acidic portion (0.7 g) and the neutral portion (42 g, 97%).

2. Chromatography of the neutral fraction

The neutral fraction (42 g) was chromatographed on alumina (Gr. II, 1050 g, 1:25 ratio) and eluted as follows:

Chromatogram (Table 1)

Fr.No.	Solvent	Volume in ml	Wt. in g	TLC pattern
1.	Pet.ether	4000	15.8	5 spots
2.	Benzene	4000	8.2	6 spots
3.	Ether	2000	3.4	3 spots
4.	Ethanol	1000	0.2	6 spots

For the isolation of the nardostachone, only the benzene fraction was investigated further.

The benzene fraction (8.2 g) was rechromatographed over alumina (Gr. II, 240 g, 1:30 ratio) and several fractions eluted with pet.ether, benzene and ether were collected.

The earlier fractions eluted with pet.ether on extensive rechromatography furnished valeranone (II) which was further purified through its semicarbazone to obtain pure (TLC) valeranone (II), b.p.125-32°(bath)/0.3 mm; (α)_D - 46.2° (c, 2); semicarbazone m.p. 206-08°. Its IR spectrum was superimposable on that of an authentic sample, [Lit.⁷ records for (II), b.p.120-30(bath)/0.2 mm, (α)_D²⁷ - 46.5° semicarbazone m.p.206-08°]

Analysis

Found: C, 81.38; H, 12.08

C₁₅H₂₆O requires C, 81.02; H, 11.79%.

Analysis of semicarbazone

Found: C, 69.05; H, 10.12; N, 14.76

C₁₆H₂₉ON₃ requires C, 68.77; H, 10.46; N, 15.04%.

The later pet.ether eluted fractions on repeated chromatography furnished valeranal (XI). The aldehyde was further purified through its semicarbazone to furnish pure (TLC) valeranal (XI), b.p.130-132(bath)/0.3 mm; $(\alpha)_D - 119^\circ$ (c, 3); semicarbazone m.p.170-72°. Its IR spectrum was superimposable on that of an authentic sample, [Lit.⁷ records for (XI), b.p.125-30°(bath)/0.2 mm; $(\alpha)_D - 120^\circ$ (c, 3.1); semicarbazone m.p.171-73°].

Analysis

Found: C, 82.02; H, 10.38,

$C_{15}H_{22}O$ Requires C, 82.51; H, 10.16%.

Analysis of semicarbazone

Found: C, 69.95; H, 8.94; N, 15.31

$C_{16}H_{25}ON_3$ requires C, 69.78; H, 9.15; N, 15.26%.

Rechromatography of the earlier benzene eluted fractions furnished nardol (V) b.p.121-26°(bath)/0.5 mm; $(\alpha)_D - 9.8^\circ$ (c, 3.0). Its IR spectrum was superimposable on that of an authentic sample [Lit.⁷ records for (V), b.p.120-25°(bath)/0.5 mm; $(\alpha)_D^{27} - 10.17^\circ$].

Analysis

Found: C, 80.71; H, 11.61

$C_{15}H_{26}O$ requires C, 81.02; H, 11.79%

Later benzene eluted fractions yielded n-hexacosanol m.p. and m.m.p. 79-80° [Lit.⁷ records for n-hexacosanol m.p.79-80°].

Rechromatography of some of the fractions eluted with ether furnished two pure (TLC) compounds (1) calarenol (VII) and (2) nardostachone (X). Nardostachone was further purified through its semicarbazone. The properties of these two compounds are given below.

Calarenol (VII), b.p. 123-27° (bath)/0.3 mm (α)_D²⁰ + 46.8° (c, 2.5). Its IR spectrum was superimposable with that of an authentic sample. [Lit.⁷ records for (VII), b.p. 120-25° (bath)/0.1 mm; (α)_D²⁰ + 47.9°].

Analysis

Found: C, 81.96; H, 10.75

C₁₅H₂₄O requires C, 81.76; H, 10.98%.

Nardostachone (X), b.p. 132-35° (bath)/0.1 mm; (α)_D²⁸ + 215° (c, 3); semicarbazone m.p. 230-32° (d). [Lit.⁷ records for (X) b.p. 130-35° (bath)/0.09 mm; (α)_D²⁷ + 209.3°; semicarbazone m.p. 230-32° (d)].

IR bands at 1658, 1618, 1577, 1462, 1412, 1376, 1208, 1024 980, 916, 879, 853, 825, 810 and 780 cm⁻¹.

Analysis

Found: C, 82.92; H, 9.65

C₁₅H₂₀O requires C, 83.28; H, 9.32%

Analysis of semicarbazone

Found: C, 70.08; H, 8.61; N, 14.92.

C₁₆H₂₃ON₃ requires C, 70.29; H, 8.48; N, 15.37%.

The last fractions eluted with ether solidified. The solid separated was crystallised from ethyl acetate to yield pure (TLC) β -sitosterol, m.p. and m.m.p. $134-35^{\circ}$ (Lit.⁷ records for β -sitosterol m.p. $134-35^{\circ}$). Its IR spectrum was superimposable on that of an authentic sample.

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