Studies in the Chemistry of Natural Products

A Thesis

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by

Hans M. Wagner.

The Royal Technical College,

Glasgow, C.1.

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ProQuest LLC.
789 East Eisenhower Parkway
P.O. Box 1346
Ann Arbor, MI 48106 – 1346

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Studies in the Chemistry of Natural Products.

Part I

The non-basic constituents of *Ephedra vulgaris*

Part II

The triterpenes of *Alstonia verticillosa* and experiments related to the structure of β-amyrin.

SUMMARY

Part I

The non-basic constituents of the plant Ephedra vulgaris were examined by chromatographic methods. An extract of the plant from which the alkaloids had been removed was saponified and the non-saponifiable matter was isolated. The latter was dissolved in ethanol and on chilling part of the material was precipitated. In this way an alcohol soluble portion and an alcohol insoluble portion was separated.

Chromatography of the latter yielded a mixture of long chain paraffins, a secondary alcohol, a primary alcohol mixture and a small quantity of a phytosterol as well as some recalcitrant gums. The paraffins were shown to be probably a mixture of nonacosane and hentriacontane, the secondary alcohol was identified as (+)-n-nonacosan-10-ol, the primary alcohol was shown to be a mixture of long chain aliphatic alcohols with a mean chain length of 28 carbon atoms and the phytosterol proved to be β-sitosterol.

Chromatography of the alcohol soluble fraction yielded an unidentified oil analysis of which gave the formula $C_{22}H_{40}O$ and which appeared to have a conjugated diene system. A second primary alcohol mixture was also

isolated with a mean chain length of 22 carbon atoms. Finally a large phytosterol fraction was isolated from the chromatogram. This was shown to consist of β -sitosterol only, for the absence of sitostanol was proved by the method of Anderson and Nabenhauer (J.A.C.S., 1924, 46, 1958) and the absence of stigmasterol was shown by the method of Windaus and Hauth (Ber., 1906, 39, 4378). The negative Tortelli-Jaffé test showed the absence of α -sitosterol and ergosterol. Furthermore oxidation of the sterol from Ephedra by the method of Barton and Jones (J., 1943, 599) led to the isolation of sitost-4-en-3-one only.

The residual gums from the chromatogram were examined for the presence of ketones by Girard's reagent but no ketonic material was isolated.

Part II

Three crystalline substances, which gave a positive Liebermann-Burchard test and which were obtained from a petroleum extract of the bark of the tree Alstonia verticillosa were examined chromatographically. The first material was shown to consist of a mixture of the triterpenes α - and β -amyrin and lupeol. The second fraction was hydrolysed and benzoylated and chromatography of the benzoates afforded α -amyrin-benzoate and lupeol-benzoate. Similar treatment of the third fraction yielded

α -amyrin, β -amyrin-benzoate and lupeol-benzoate.

A further quantity of β -amyrin was isolated from Manila Elemi resin. A new β -amyrene was prepared which can be used as a starting point in the study of the stereochemistry of rings A and B of β -amyrin. — The latter was oxidised as acetate with hydrogen peroxide to the saturated ketone β -amyranonyl-acetate. Reduction of this by the Kischner-Wolff technique yielded the saturated alcohol β -amyranol. Dehydration of the latter yielded a new hydrocarbon, β -amyrene, VII. Hydrogenation of this unsaturated compound gave a saturated hydrocarbon which was not identical with β -amyran, the saturated hydrocarbon previously obtained from β -amyrin by Ruzicka and Jeger (Helv.Chim.Acta, 1941, 24, 1179). It was therefore concluded that the dehydration probably took place by a retrapinacolone mechanism with a change in the structure of ring A of β -amyrin.

Contents

PART I

The Chemistry of the Non-Alkaloidal Constituents of *Ephedra Vulgaris*

Introduction

Introduction

The various species of Ephedra belong to the plant order Gnetaceae and occur in the Mediterranean region, the Himalaya and Altai mountains, the Andes in America, as well as in Texas and California. In Europe they can be found as far North as South Tyrol. The Ephedra are gymnospermous related to the pines and firs.

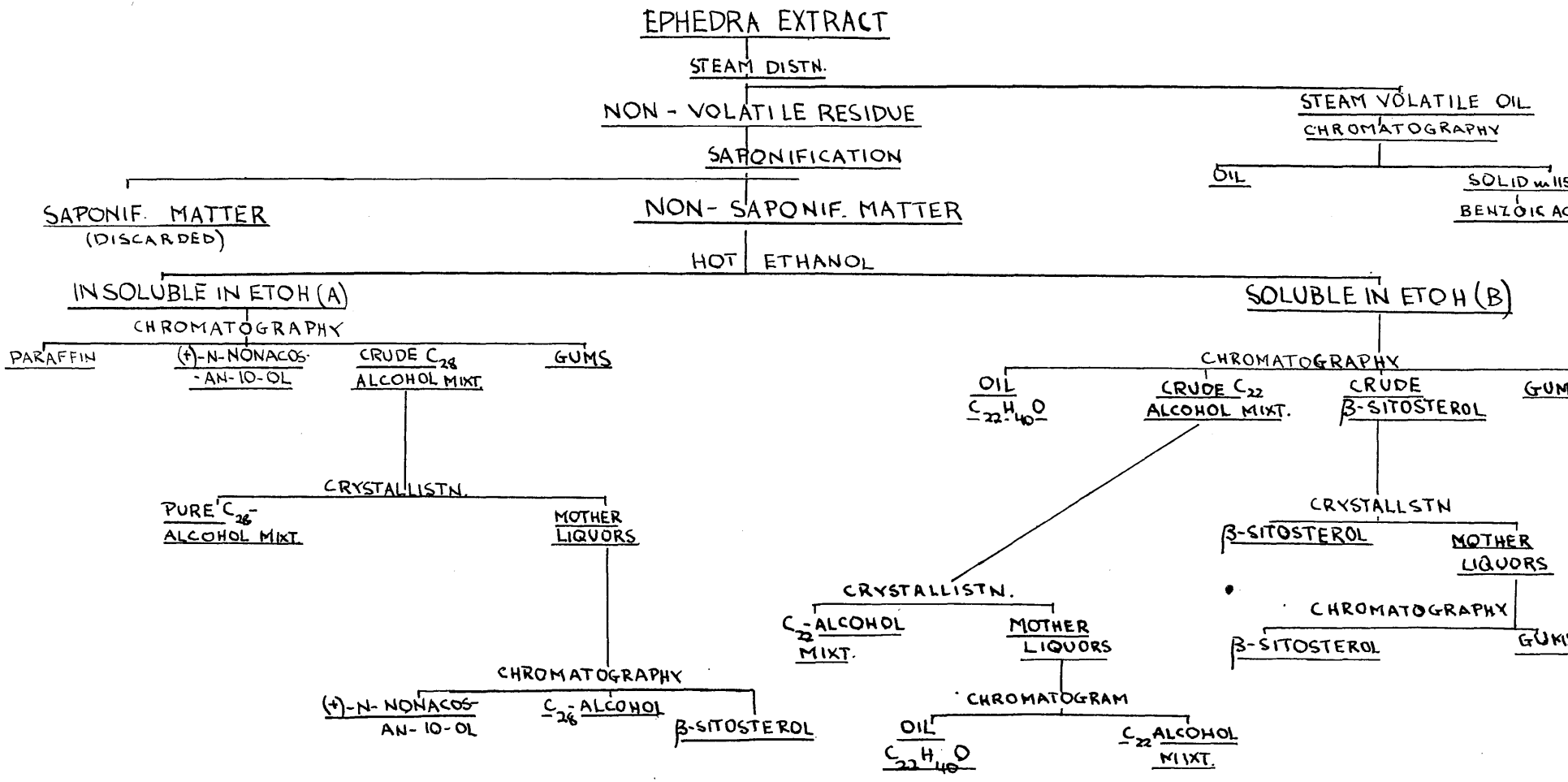
The Chinese herb Ma Huang (yellow astringent) was employed by native physicians for some 5000 years. The Chinese dispensatory (Pentao Kang Mu) written in 1569 by Chi-Cheng-Li mentions that the drug is valuable as an antipyretic, diaphoretic, circulatory stimulant and a sedative for coughs. An active principle was first isolated by Yamanashi in 1885 and two years later Nagai obtained the main alkaloid, ephedrine, for the first time in a pure form. Interest in Western medicine was created by the investigation of Chen and Schmidt which begun in 1923 as a result of a Chinese druggist's assurance that Ma Huang was really a potent drug. Today the alkaloid ephedrine produced largely synthetically, enjoys considerable popularity as a sympathomimetic and as a relief in the treatment of asthma. An excellent review of the properties of ephedrine and related substances can be found in the

work by Goodman and Gilman (1) and in the paper by Chen and Schmidt (2). Other alkaloids isolated from Ephedra include ψ ephedrine, l-norephedrine, nor-d- ψ -ephedrine, N-methylephedrine and d-N-methyl-ψ -ephedrine (3).

The non-alkaloidal constituents of the plant have however received only very scanty attention. Thus Klein's "Handbuch der Pflanzenanalyse" (Vienna, 1933) only briefly mentions the occurrence of catechins, mannose and cutin in various types of Ephedra. Kono (4) investigated the inorganic constituents of E.sinica and E.equisetina. A small quantity of a volatile oil and a tanin were obtained by Terry (5) from E.nevadensis. Hayden and Jordan (6) report the isolation of a catechol tanin, a saponin and a crystalline substance having glycosidal properties, in a study of the Chinese drug Ma Huang. A species of Ephedra from Tatshikistan, E.procera yielded 0.02% of a yellow brown oil on steam distillation in experiments by Isaev (7). Steam distillation was also employed by Chen and Mei (8) who obtained a neutral principle of formula $C_{10}H_{20}O_2H_2O$ and m.p. 120°. The anhydrous compound had m.p. 105°. They also isolated a volatile oil, b.p. 197° from Ma Huang. This was optically inactive and analysed to $C_8H_{12}O$.

Theoretical

ANALYSIS OF EPHEDRA



Theoretical

The ephedrine alkaloids are obtained by extracting the leaves and stems of the plant with benzene. The extract is then treated with mineral acid which removes the bases. These are then isolated from the aqueous acid solution by the addition of alkali. The solvent is recovered from the organic extract by distillation leaving the benzene soluble, non-basic components of the plant. A quantity of such a residue from Ephedra vulgaris was supplied by Messrs. T. & H. Smith, Ltd., Edinburgh, and the work reported below is concerned with this material.

The extract consisted of a dark green waxy solid with a strong penetrating odour. Steam distillation afforded a small amount of a pleasant smelling oil. Chromatography of this oil did not produce any definite compound but one fraction, after standing for several months partially crystallized. The resulting solid, $C_7H_6O_2$ (m.p. 122-123°) was shown to be benzoic acid (m.p. 122°) by the mixed m.p. which showed no depression. Confirmation of this identification was provided by the ultra-violet absorption spectra. The benzoic acid may have been formed by atmospheric oxidation of a small amount of benzaldehyde present in the steam volatile oil. No other solid materials were isolated. The small amount of material

available precluded the separation of the liquid components by distillation.

The non-saponifiable portion of the residue after steam distillation was obtained after hydrolysis of the extract with alcoholic alkali as a red-brown semi-solid amounting to 21% of the original extract. This was completely soluble in hot ethanol but on chilling, the solution deposited a yellow-brown waxy solid (A). After filtration and evaporation, the mother liquors afforded a dark brown gum (B).

Examination of the alcohol-insoluble material (A)

A solution of the waxy solid in a warm mixture of benzene and light petroleum deposited a crystalline solid on standing (see below). The mother-liquors were then examined by the liquid chromatogram method which was used throughout this work (15). The chromatogram was washed with solvents or mixtures of solvent of gradually increasing elution power and the eluate was collected in small fractions which were later combined according to the nature of the residue obtained on removal of the solvent. The main fractions obtained are described below in order of ease of elution.

The Paraffin fraction

From a consideration of the melting point, the ease with which the material passed through the chromatogram, the analytical data and the physical appearance, it was deduced that the first fraction consisted of a mixture of higher paraffins. Such mixtures are a common constituent of plants (16) and cannot be separated into pure compounds by chemical methods. In addition the mixtures are invariably composed of paraffins with an odd number of carbon atoms (21). By a comparison of X-ray data and melting points of various synthetic mixtures with naturally occurring paraffins, Chibnall (16) was able to determine the approximate

constitution of the natural products. Table I shows the melting points of a number of such synthetic mixtures of paraffins.

TABLE I (17)

PERCENTAGE COMPOSITION		m.p. °C
$C_{29}H_{60}$	$C_{31}H_{64}$	
100	0	63.4-63.6°
70	30	64.6-64.7°
60	40	64.8-65°
50	50	65.3-65.5°
40	60	65.8-66°
30	70	66.2-66.4°
0	100	67.6-67.8°

The paraffin from the Ephedra extract, analysis of which approximated to that required for $C_{30}H_{62}$ and which was probably a mixture of nonacosane and hentriacontane, since the naturally occurring paraffins are mixtures of compounds with an odd number of carbon atoms (21), had m.p. 65.3-65.6°. This corresponds to a mixture of 50% $C_{29}H_{60}$ and 50% $C_{31}H_{64}$ (Table I). However, since it was not possible to carry out an X-ray analysis of the substance, this could not be confirmed.

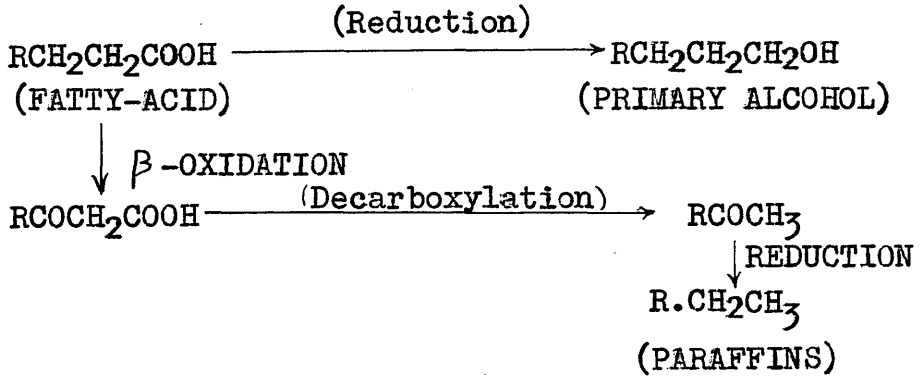
(+)-n-Nonacosan-10-ol

The second fraction was found to be identical with the solid which had separated earlier from the benzene light petroleum solution. The substance contained one acylable hydroxyl group and an acetate, a formate and a benzoate were obtained. Chromic acid oxidation afforded a ketone, which was characterised by the formation of an oxime. The analytical figures for the free alcohol and the acyl derivatives indicated the formula $C_{29}H_{59}OH$. The substance appeared to have the properties of the secondary alcohol (+)-n-nonacosan-10-ol, previously isolated by Chibnall, Piper, Pollard, Smith and Williams (18) from apple cuticle wax. Chibnall designated the alcohol as d-n-nonacosan-10-ol but modern convention favours (+)-n-nonacosan-10-ol since the compound has not been correlated to glyceraldehyde. A comparison of the new specimen of the alcohol, the derived ketone and oxime with authentic specimens of (+)-n-nonacosan-10-ol, nonacosan-10-one and nonacosan-10-one oxime, kindly supplied by Professor A.C. Chibnall, showed that the materials were in fact identical. (No depression of the mixed melting points was obtained). The optical rotation of the alcohol was negligible ($[\alpha]_D^{20}$ .13± .1°), in agreement with the findings of Chibnall et. al. (loc.cit.). In addition the absence

of any depression in the mixed melting point of the nonacosan-10-ol from Ephedra and that from apple cuticle wax indicates that the former is also the (+)-isomer since the melting point of a (±) mixture shows a considerable depression. Thus synthetic (±) nonacosan-10-ol has a melting point of 75° whereas (+)-nonacosan-10-ol shows a melting point of 82.2° (19).

Chibnall and Piper (21) suggested a possible metabolism sequence for the long-chain compounds in plants based on the results of the examination of a large number of plant and animal waxes. This theory modified earlier suggestions made by Channon and Chibnall (20) and later elaborated by Chibnall et. al. (19). According to this final hypothesis (21) primary alcohols (see below) are formed by the reduction of the corresponding fatty acids and the paraffins and secondary alcohols by the indirect decarboxylation of the corresponding acid with one more carbon atom.

SUGGESTED FORMATION OF PRIMARY ALCOHOLS AND PARAFFINS (21)



The basic building units in the synthesis of saturated acids and hydroxy acids are unsaturated acids which are formed in the plant from materials of shorter chain length. Thus n-nonacosan-10-ol (III) is probably formed from Δ^{9}-triacontenoic-acid (II) as shown below. The Δ^{11}-triacontenoic-acid (II) is synthesised from the doubly unsaturated acid (I) by reduction. The acid (I) is produced in the general synthesis of unsaturated fatty acids in the plant.

Hypothetical synthesis of n-nonacosan-10-ol in the plant (21).

UNSATURATED ACIDS (SHORT UNITS) $\longrightarrow$ $CH_3(CH_2)_{13}CH{=}CH(CH_2)_2CH{=}CH(CH_2)_9COOH$ (I)

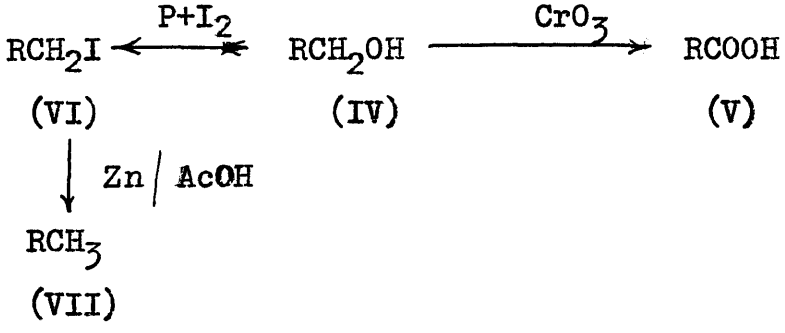
$\xrightarrow{\text{REDUCTION}}$ $CH_3(CH_2)_{17}CH{=}CH(CH_2)_9COOH$ (II) $\xrightarrow{\text{HYDRATION}}$

$CH_3(CH_2)_{18}CHOH(CH_2)_9COOH \xrightarrow{-CO_2} CH_3(CH_2)_{18}CHOH(CH_2)_8CH_3$ (III)

It should be noted however that (I) and (II) have not as yet been isolated from waxes. There are also other theories on the formation of long chain compounds in plants which are reviewed in a survey of the subject by Henshall and Smith (29).

The C_{28}-primary-alcohol mixture

The third fraction from the chromatogram gave a positive Liebermann-Burchard reaction but after repeated crystallization from ethyl-acetate a solid was obtained which did not give this colour test. The substance was shown to be an alcohol (IV) by the formation of an acetate and a 3:5-dinitrobenzoate. Chromic acid oxidation gave an acid (V) and reduction via the iodide (VI) afforded a paraffin (VII).



From these facts and the analytical data the material appears to be a mixture of several higher aliphatic primary alcohols. The analytical results correspond closely to an alcohol containing 28 carbon atoms and hence this alcohol is termed the "C_{28}-alcohol mixture" to distinguish it from a second, similar, mixture to be described later. The higher natural alcohols invariably occur as mixtures containing even number of carbon atoms (22; 16). The "C_{28} alcohol"

was therefore considered to be a mixture probably containing the C_{26}, C_{28} and C_{30} alcohols with a mean chain length of 28 carbon atoms. The analysis and equivalent of the derived C_{28}-acid mixture are in agreement with this as are the melting points of the C_{28}-paraffin mixture and the analyses and melting points of the alcohol acetate and 3:5-dinitrobenzoates. However, the analyses of the C_{28}-paraffin mixture suggested a somewhat lower chain length and the melting points of both the C_{28}-alcohol mixture (71°) and the C_{28}-acid mixture are somewhat lower than those recorded by Chibnall et. al. (16). It is suggested that these results may be due to traces of impurities such as paraffins or to the presence of more than three components in the mixture. In either case the m.p. will be depressed.

The following considerations have also been taken into account in the determination of the nature of the "C_{28}-alcohol mixture".

The alcohol corresponded in properties to the so called "ceryl alcohol" which has frequently been reported in the literature as a primary alcohol isolated from plant and animal waxes. Table II gives the melting points of a number of these alcohols and their derivatives.

TABLE II (22)

Ref.	Source	m.p. °C. Alcohol	Acetate	Acid	Paraffin
22.	Cocksfoot	79.7	59.5-59.6	87.7-87.9	56.4-56.6
23.	Chinese Wax	79	60	82	-
24.	Spinach Leaves	77-78	61.5	-	-
25.	Sunflower Oil	81.2	63.5	77.6	-
18.	Apple peel Wax	80.2-80.7	64.5-64.8	83-83.5	60-60.2
-	Ephedra Vulgaris	71	66	71	60-61

The "ceryl Alcohol" was considered to be a single compound by the early workers (24,25) containing 26 carbon atoms, but the melting points recorded were much lower than the extrapolated melting point of n-$C_{26}H_{54}O$ and it was suggested that this was due to branching in the carbon chain. However Francis, Piper and Malkin (23) fractionated a sample of "ceryl alcohol" from Chinese wax and concluded that their specimen consisted of a mixture of primary alcohols. This view was confirmed by Chibnall et.al (18) in their work on "ceryl alcohol" from apple cuticle and other waxes (22). Chibnall therefore suggested that all the "ceryl alcohols" quoted in the literature are mixtures of primary alcohols.

By methods similar to those used in the determination of the constituents of paraffins, namely, m.p. & X-ray measurements, Chibnall et.al.(16) deduced the composition of the primary alcohols in plants. Table III shows some of the results obtained.

TABLE III (16)

Source and Suggested Composition of Alcohol	m.p. °C	Derived Acid m.p. °C	Paraffin m.p. °C
Brussel sprouts	80.8	84.2	59.7
40%26* + 60%28	80.9	84.4	59.5
Cactus	82.4	85.7	63.7
50%28 + 50%30	82.6	87.3	63.7
White Mustard	85.0	87.8	66.4
20%28 + 40%30 + 40%32	85.6	87.9	66.5

*Number of carbon atoms in alcohol.

β-Sitosterol

The mother liquors from the crystallization of the C_{28} alcohol mixture on chromatographic treatment yielded (+)-n-nonacosan-10-ol, the C_{28} alcohol mixture and β-sitosterol. The latter accounted for the positive Liebermann-Burchard reaction observed in the mother liquors of the above alcohol fraction. The sterol fraction was examined more closely at a later stage. (see Page 17).

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The residual fractions from the chromatogram were recalcitrant gums from which no identifiable products could be obtained.

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Examination of the alcohol soluble material (B)

The reddish-brown gum was chromatographed in the usual manner. A large number of fractions were taken which were then combined into the following main fractions. As in the case of the alcohol insoluble portion (A) they are described in order of elution.

Compound $C_{22}H_{40}O$

The first fraction was a pale yellow oil, $C_{22}H_{40}O$ which appeared from its ultra-violet absorption spectrum and its colour with tetranitromethane to contain a conjugated diene system. No solid derivative could be obtained. (e.g. a maleic anhydride adduct). Catalytic hydrogenation experiments were inconclusive. The oil did not contain a carbonyl group reactive to 2:4-dinitrophenylhydrazine nor an active hydrogen atom (Zerewitinoff), nor a hydrolysable ether linkage (methoxyl determination). The molecular weight determination by the Rast method did not give consistent results. The small amount of the compound available precluded further study of this substance.

The C_{22}-primary alcohol mixture

The second fraction on crystallization from ethyl acetate yielded a colourless solid. Chromatography of the mother liquors afforded more of this material together with

more of the compound $C_{22}H_{40}O$ described above. The solid proved to be a second primary alcohol mixture and resembled the C_{28} alcohol mixture closely (e.g. specimens of the free alcohols did not show mixed melting point depressions). However the analytical results for the new mixture corresponded more closely to a mixture of alcohols with a mean chain length of 22 carbon atoms. This was confirmed by the data obtained for the derived acetate and 3:5-dinitrobenzoate and the acid produced by oxidation. This substance is therefore termed the "C_{22} alcohol mixture". The melting point of the mixture (67°) is, as would be expected, somewhat lower than the melting point (72°) of pure docosanol (26). The derived C_{22} acid mixture (m.p. 71°) appears to resemble the synthetic equimolar mixture of C_{21}, C_{22} and C_{23} acids (m.p. 73°) described by Francis, Piper and Malkin (23) although of course in the case of the natural C_{22} acid mixture no acid with an odd number of carbon atoms would be present. (see above, page 10).

From the above it will be seen that it was possible to achieve a rough separation of the higher primary alcohols from E. vulgaris into a C_{22} and C_{28} group because of their different solubilities in ethanol. In this connection it is of interest to note that Markley, Sando and Hendricks (27) separated the C_{24} and C_{28} groups of primary alcohols

(m.p. 73° and 79° respectively) from grape pomace by a long process of fractional crystallization from methanol, acetone and light petroleum. There appears to be no reference in the literature to the isolation of a C_{22} saturated alcohol from plant sources.

The sterol fraction

The third fraction from the chromatogram afforded a crystalline solid which gave a strong positive Liebermann-Burchard colour reaction. From the physical data, the substance appeared to be mainly β-sitosterol (IIX). Concentration of the mother liquors from the crystallization yielded more of the sterol which was purified by chromatography.

The total sterol fraction was then examined for the presence of other phytosterols since Jones, Wilkinson and Kerlogue (30) showed that a sample of β-sitosterol from wheatgerm oil, although having properties very similar to those quoted for pure β-sitosterol was far from homogeneous. Thus wheat-germ oil sitosterol was found to contain triacontane, sitostanol (XI) and an unidentified substance as well as β-sitosterol (30; 13).

The Tortelli-Jaffé colour reaction of the sterols from Ephedra was negative showing the absence of α-sitosterol (IX) and ergosterol (XII). (This reaction is given by sterols

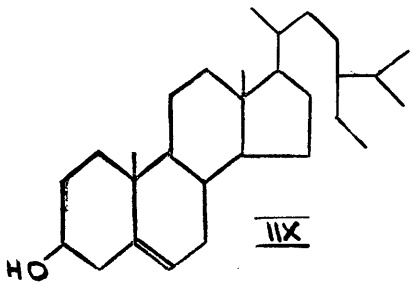
with a double bond between two tertiary carbon atoms or a double bond which easily isomerises to such a position) (31).

Bromination of the sterol acetate according to the method of Windaus and Hauth (33) gave no insoluble tetrabromo derivative indicating the absence of stigmasterol (X).

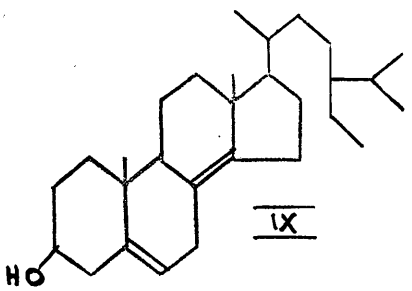
Saturated sterols e.g. sitostanol do not give the Liebermann-Burchard test. This fact was used by Anderson

TABLE IV

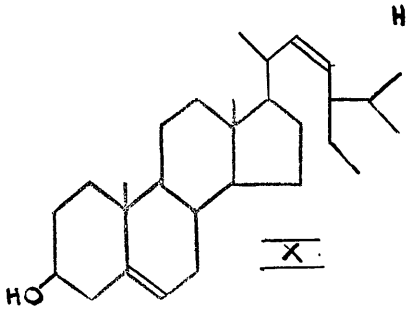
THE COMMON PHYTOSTEROLS



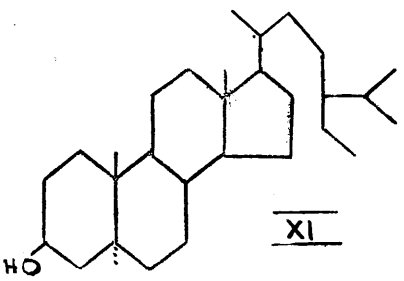
IIIX



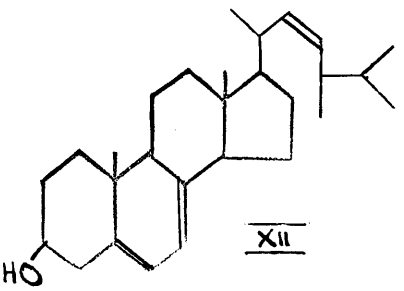
IX



X



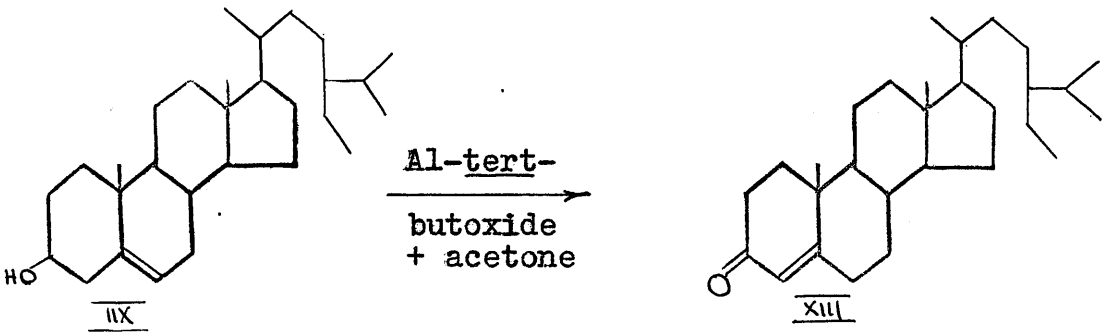
XI



XII

and Nabenhauer (12) to isolate sitostanol from a mixture containing unsaturated sterols. The sterols were dissolved in carbontetrachloride and treated with acetic anhydride and sulphuric-acid in the usual way. The The unsaturated sterols were thus destroyed. The addition of water gave an acid layer and a carbontetrachloride layer, the latter containing the saturated sterols. When this method was applied to the sterols from E.vulgaris, no material was isolated from the carbontetrachloride extract indicating the absence of sitostanol ((XI).

Oxidation of the sterol fraction by the Oppenauer method was finally used to prove that only β-sitosterol was present. Barton and Jones (13) found that by oxidation, followed by chromatography of the product an effective separation of plant sterols was possible. Thus it was shown (13) that the sterols from Tall-oil contained sitostanol and β-sitosterol, for the former was oxidised in the presence of aluminium-tertiary-butoxide and acetone, to sitostanone and the latter to sitost-4-en-3-one. The sitostanone was eluted more easily from the chromatogram than the sitost-4-en-3-one (XIII) and the two were thus readily separated. In the case under examination here sitost-4-en-3-one (XIII) was the only ketonic substance isolated.



XIII

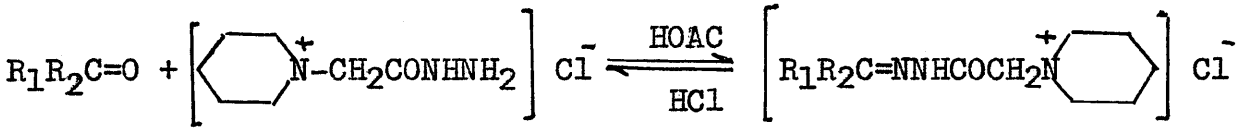
Thus Ephedra vulgaris appears to be a source of remarkably pure β-sitosterol. In this connection it may be noted that Wallis and Chakravorty (34) isolated pure β-sitosterol from cotton-seed oil.

The residual fractions

The final fractions from the chromatogram yielded only recalcitrant gums. Attempts to prepare crystalline acyl derivatives which could be separated and purified by chromatography proved fruitless.

No ketonic material had been isolated throughout the analysis of the Ephedra extract and the gum was therefore examined by the method evolved by Girard (28). The acetylated gum was treated with Girard's reagent P, (pyridine-acetohydrazide hydrochloride, XIV). This compound combines with ketonic substances to form hydrazone

derivatives (XV) having a polar group which makes the substances watersoluble. The ketones are then readily regenerated after removal of the non-ketonic matter, by hydrolysis with mineral acid.



(XIV) (XV)

However no ketonic material was isolated from the gum.

CONCLUSION

The following compounds have been isolated by chromatographic methods from an extract of Ephedra vulgaris: a mixture of higher paraffins, (+)-n-nonacosan-10-ol, two mixtures of higher aliphatic primary alcohols, β-sitosterol and an unidentified diene containing oxygen. In addition a small quantity of benzoic acid has been separated from the steam volatile portion.

It has been shown that chromatography provides an excellent means for the separation of waxes, primary and secondary higher aliphatic alcohols and is a convenient alternative to Chibnall's (18) phthalic anhydride procedure.

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EXPERIMENTAL

EXPERIMENTAL

"Light petroleum" refers to the fraction b.p. 60-80°. The alumina used in the chromatographic experiments was supplied by Savory and Moore, standardised according to Brockmann (Grade II). Melting points are uncorrected.

Steam distillation experiments

The total benzene extract of E.vulgaris was freed from alkaloids in the usual manner. Removal of the benzene from the alkaloid free extract gave a green coloured waxy solid. Steam distillation of this solid (2 kg.) afforded a pale yellow oil. On warming this under reduced pressure (50°/3 mm.) a small amount (20 mg.) of a colourless crystalline solid sublimed which after one crystallization from light petroleum had m.p. 113-117°. This was not examined further. A solution of the oil (4.7 g.) in light petroleum (50 c.c.) was adsorbed on a column (30 x 1.5 cm.) of alumina. Development and elution in the usual way gave the following fractions :

Fraction	c.c.	Eluant	Product after removal of Solvent
1.	60	Light petroleum	Colourless pleasant smelling oil (2 g.)
2.	35	benzene + 2% ethanol	Colourless oil which deposited crystalline material after standing for several months (1.2 g.)
3.	115	benzene + 4% ethanol	Colourless oil (1.0 g.)

Fraction 1. gave a yellow colour with a solution of tetranitromethane in chloroform, suggesting the presence of one or more unconjugated double bonds.

Fraction 2. The solid from this fraction crystallized from light petroleum (40-60°) as colourless hexagonal plates (30 mg.) m.p. 122-123°. Ultra-violet absorption spectrum in ethanol showed λ_{max} at 2250 Å and 2720 Å; E_{max} = 11000 and 700 respectively.

Found : C 68.72; H 5.17%

Calc.for: $C_7H_6O_2$; C 68.9; H 4.93%

A mixed m.p; with authentic benzoic acid (m.p. 122°) showed no depression. The ultra-violet absorption spectrum for benzoic acid in ethanol showed λ_{max} 2250 Å and 2720 Å; E_{max} = 11500 and 900 respectively.

Isolation and preliminary separation of the non-saponifiable components.

The non-volatile residue from the steam distillation (500 g.) was heated under reflux with methanolic potassium hydroxide solution (5%; 1500 c.c.) for two hours, allowed to cool and diluted with water (3.5 l). Continuous extraction of the saponification mixture with ether gave a brown waxy solid. This was entirely soluble in hot ethanol (800 c.c.) but on chilling a yellow-brown waxy solid separated which was collected and freed from any retained solvent by distillation with benzene (500 c.c.). After removal of the benzene there remained a light brown waxy solid (A; 49 g.). The alcoholic mother liquors on evaporation gave a gum which was freed from traces of solvent by distillation with benzene. The residue (55 g.) after removal of the benzene was a dark brown gum (B.)

The saponifiable portion of the Ephedra extract containing the hydrolysis product of chlorophyll and fatty acids was not examined further.

Examination of the alcohol-insoluble fraction

A warm solution of the waxy solid (A; 49 g.) in a mixture of benzene (630 c.c.) and light petroleum (270 c.c.) deposited a crystalline solid (11.4 g.), m.p. 78-79°, after

crystallization from benzene. The benzene-light petroleum solution was adsorbed on a column (40 x 5 cm.) of alumina which was then washed with solvents and developed and eluted as follows :-

Fraction	c.c.	Eluant	Product After Removal of Solvent
A1	3600	Benzene-light petrol. (7:3)	Colourless waxy solid m.p. 55-60° (4.82 g.)
A2	1500	do. + 1% ethanol.	Yellow gum (0.62 g.)
A3	500	do.	Colourless solid (1.91 g.)
A4	3100	do.	Red gummy solid (17.98 g.)
A5	2000	do.	Brown gum (1.06 g.)
A6	4400	Benzene-light petrol. (7:3) + 2% ethanol.	Brown gum (1.43 g.)
A7	2500	do. + 5% ethanol.	Brown gum (0.6 g.)
A8	3600	do. + 8% ethanol.	Brown gum (0.93 g.)
A9	2600	Benzene-ethanol (1:1)	Brown gum (1.37 g.)
A10	1100	Ethanol	Brown gum (0.14 g.)

Fraction A1, paraffin mixture. After repeated crystallization from ethyl-acetate a mixture of higher paraffins (3 g.) was obtained as small colourless plates, m.p. 65-65.5°.

Found : C, 85.0; H, 14.5%

Calc.for 50% $C_{29}H_{60}$ + 50% $C_{31}H_{64}$: C, 85.23: H, 14.8%

A mixture of 50% n-nonacosane (m.p. 63.4-63.6°) and 50% n-hentriacontane (m.p. 67.6-67.8°) has m.p. 65.3-65.6° (Chibnall et.al., 17).

Fraction A2: On crystallization from ethyl-acetate it gave a small amount (0.1 g.) of a colourless solid m.p. 66.69° which was probably grossly impure n-nonacosan-10-ol.

Fraction A3: On crystallization from ethyl-acetate a colourless powder (1.67 g.) m.p. 78-79° was obtained. No melting point depression could be observed in a mixed m.p. with the solid which separated prior to chromatography. Crystallization of the combined specimens from benzene and ethyl-acetate gave (+)-n-nonacosan-10-ol (10 g.) as hard colourless nodules, m.p. 81.5-82.5°, undepressed on admixture with a sample of the authentic alcohol (m.p.81.9-82.2°), $[\alpha]_D^{20}$ + 0.13° (± .1°), (c = 1.9 in chloroform)

Found : C, 81.90; H, 14.3%; M.W.(Rast) 417

Calc.for $C_{29}H_{60}O$: C, 82.07; H, 14.2%; M.W.424.8

(+)-n-Nonacosan-10-yl-acetate : Acetylation of (+)-n-nonacosan-10-ol (1 g.) by heating under reflux with acetic anhydride (30 c.c.) afforded (+)-n-nonacosan-10-yl-acetate which separated from a mixture of methanol and ethanol in small colourless rectangular plates, m.p.

44-45°, $[\alpha]_D^{20}$ + 0.54° (± 0.2°), (c = 1.8 in chloroform)

Found : C, 79.60; H, 13.18%; M.W.469

Calc.for : $C_{31}H_{62}O_2$: C, 79.75; H, 13.39%; M.W.466.8

Chibnall et.al. (18) record m.p. 44.5-45°.

(+)-n-Nonacosan-10-yl formate : A solution of (+)-n-nonacosan-10-ol (1 g.) in a mixture of formic acid (98/100%; 20 c.c.) and dioxan (15 c.c.) was heated under reflux for 8 hours. After distillation of the solvents under reduced pressure, the residual (+)-n- nonacosan-10-yl formate crystallized (charcoal) from a mixture of methanol and ethanol as colourless plates (0.7 g.) m.p. 57-58°, $[\alpha]_D^{20}$ + 0.32° (± 0.2°), (c = 0.8 in chloroform)

Found : C, 79.38; H, 13.75%

$C_{30}H_{60}O_2$ requires C, 79.58; H, 13.36%

(+)-n-Nonacosan-10-yl benzoate: A mixture of (+)-n-nonacosan-10-ol (0.5 g.) benzoyl chloride (7 g.) and pyridine (10 c.c.) was heated under reflux for 30 minutes and then kept overnight at room temperature. The solution was extracted with ether and the extract was washed with dilute hydrochloric acid, sodium hydroxide solution and finally water, dried (sodium sulphate) and the solvent was removed by distillation. The residual (+)-n-nonacosan-10-yl benzoate (0.55 g.) was obtained as colourless needles from a mixture of ethanol and methanol, m.p. 35-35.5°, $[\alpha]_D^{20}$ + 0.66° (± 0.2°),

(c = 1.5 in chloroform).

Found : C , 81.36; H , 12.56%

$C_{36}H_{64}O_2$ requires C , 81.77; H, 12.2%

n-Nonacosan-10-one : To a suspension of (+)-n-nonacosan-10-ol (2 g.) in glacial acetic acid (40 c.c.) was added a solution of chromium trioxide (8 g.) in water (8 c.c.) and acetic acid (8 c.c.) during 10 minutes with stirring. After warming for one hour at 100° the mixture was poured into water. The n-nonacosan-10-one which separated gave colourless, lozenge shaped plates (1.32 g.), m.p. 73.5-74.5°, on crystallization from a mixture of ethanol and methanol.

Found : C , 82.26; H , 13.51%

Calc.for $C_{29}H_{58}O$: C , 82.39; H , 13.82%

There was no depression of m.p. on admixture with an authentic specimen of the ketone m.p. 74.3-74.8°.

n-Nonacosan-10-one oxime : A mixture of n-nonacosan-10-one (0.5 g.), hydroxylamine hydrochloride (0.2 g.) and powdered potassium hydroxide (0.35 g.) in ethanol (40 c.c.) was heated under reflux for 8 hours. The solid which separated on pouring into water crystallized from ethanol giving the oxime as colourless crystals (0.37 g.) m.p. 48.5-49.5° which did not depress the m.p. (49.5-50°) of an authentic specimen.

Fraction A4 : One crystallization of this fraction from

ethyl acetate gave a mixture of higher aliphatic alcohols as an amorphous solid, m.p. 55-69°. After 8 crystallizations the material was obtained as colourless nodules (5 g.) m.p. 71°. It was not possible to raise the mp. by repeated crystallization.

Found : C, 81.80; H, 14.50%

Calc. for $C_{26}H_{54}O$: C, 81.59; H, 14.22%

Calc. for $C_{28}H_{58}O$: C, 81.95; H, 14.20%

Calc. for $C_{30}H_{62}O$: C, 82.27; H, 14.09%

For an equimolecular mixture of C_{26} (m.p. 79.5-79.8°), C_{28} (m.p. 83.2-83.4°) and C_{30} (m.p. 86.3-86.5°) alcohols, Chibnall et.al. (16) record m.p. 80.7°. The material obtained from the mother liquors from these crystallizations gave a positive Liebermann-Burchard reaction and were examined as described later (Page 33).

The C_{28}-alcohol-acetate : The C_{28}-alcohol (240 mg.) and acetic anhydride (4.5 c.c.) were heated under reflux for 90 minutes. The reaction mixture was worked up in the usual manner and after repeated crystallization from ethanol the C_{28}-alcohol-acetate was obtained as colourless nodules m.p. 66°.

Found : C, 80.52; H, 13.77%

Calc. for $C_{30}H_{60}O_2$: C, 79.64; H, 13.27%

Calc. for $C_{32}H_{64}O_2$: C, 80.01; H, 13.42%

Chibnall et.al. (16) record m.p. 64.4° for an equimolecular mixture of C_{26} (m.p. 60.0-60.1°), C_{28} (m.p. 64.6-64.8°) and C_{30} (m.p. 69.1-69.2°) alcohol acetates.

The C_{28}-alcohol-3:5-dinitrobenzoate : The C_{28} alcohol (0.5 g.), 3:5-dinitrobenzoylchloride (0.5 g.) and pyridine (6c.c.) were warmed on a steambath for two hours and then poured into 2N-sulphuric acid solution (30 c.c.). The mixture was extracted with ether (4 x 20 c.c.) and the extract was washed with dilute sodium-hydroxide solution and water, dried (sodium sulphate) filtered and the solvent was distilled off. On trituration with ethanol the residue yielded a solid which after repeated crystallization from ethanol gave the C_{28}-alcohol-3:5-dinitrobenzoate as small colourless plates (0.3 g.) m.p. 71-72°.

Found : N , 4.93%

$C_{35}H_{60}O_6N_2$ requires N , 4.78%

The C_{28}-acid : (cf. Chibnall et.al; 22) The C_{28}-alcohol was dissolved in warm glacial acetic acid (40 c.c.) and a solution of chromium trioxide (1 g.) in glacial acetic acid (12 c.c.) was added during 15 minutes with stirring. The solution was agitated for a further 40 minutes, diluted with water and extracted with warm benzene (6 x 20 c.c.). The benzene solution was dried (sodium sulphate), filtered and the solvent was distilled off. The residue was taken up

in ether (100 c.c.) and the sodium salt of the acid was precipitated by shaking with sodium ethoxide solution, prepared from sodium (3 g.) and ethanol (75 c.c.). The suspension was centrifuged for 20 minutes at 1500 r.p.m. and the supernatant liquors were decanted. The sodium salt was decomposed with dilute hydrochloric acid and extracted with ether. The extract was washed with water, dried (sodium sulphate), filtered and the solvent was distilled off. The residue was triturated with acetone when a solid separated. The C_{28}-acid mixture was obtained as a colourless crystalline powder, m.p. 70-71°, on crystallization from acetone.

Found : C, 79.52; H, 13.57%; equivalent by titration 416.9

Calc. for $C_{28}H_{56}O_2$: C, 79.24; H, 13.21%; equivalent 424.

For an equimolecular mixture of C_{26} (m.p. 87.8-88°), C_{28} (m.p. 90.8-91.1°) and C_{30} (m.p. 93.8-94°) acids, Chibnall *et.al.* (16) record m.p. 83.4°.

The C_{28}-paraffin : (*cf.* Levene, West and van der Scheer, 32) The C_{28}-alcohol (1.0 g.) was heated with iodine (0.4 g.), red phosphorus (0.1 g.) and benzene (10 c.c.) on a waterbath for six hours. The solution was diluted with ether, filtered and washed with dilute sodium thiosulphate solution to remove excess iodine. It was then washed with water,

dried (sodium sulphate) filtered and on evaporation of the solvent a red gum was obtained. To this was added glacial acetic acid (15 c.c.) and zinc dust (1 g.) and the solution was warmed on a waterbath for 48 hours. A stream of hydrogen chloride gas was passed through the solution for two minutes every 3 hours. The zinc had then dissolved and the solution was diluted with water when the product separated. It was collected and crystallized repeatedly from ethyl-acetate when the C_{28}-paraffin was obtained in small colourless plates m.p. 60-61°

Found : C , 84.7; H , 14.9%

Calc. for $C_{24}H_{50}$: C , 85.1; H , 14.9%

Calc. for $C_{28}H_{58}$: C , 85.7; H , 14.3%

For an equimolecular mixture of C_{26} (m.p. 56.4-56.6°), C_{28} (m.p. 61.3-61.5°) and C_{30} (m.p. 65.6-65.8°) paraffins, Chibnall et.al. (16) record m.p. 61.8°.

Fractions A5-A10: These fractions did not afford any crystalline material on attempted purification and no products could be identified.

Examination of the mother liquors from the crystallization of the C_{28} alcohol mixture.

The combined mother liquors from the crystallization of fraction A4 gave a gummy residue (12 g.) on evaporation

of the solvent. The gum gave a positive Liebermann-Burchard colour test. A solution of the material in light petroleum (200 c.c.) was adsorbed on a column (22 x 2.5 cm.) of alumina. The chromatogram was developed and eluted as follows :-

Fraction	c.c.	Eluant	Product after removal of Solvent
1	1400	Light petroleum	yellow gum (0.58 g.)
2	2100	light petroleum-benzene (4:1)	Yellow waxy solid (0.31 g.)
3	5100	light petroleum-benzene-ether (6.6:2.5:1)	Brown gum (3.4 g.)
4	1000	light petroleum-benzene (7:3) + 1/2% ethanol	Red gum (0.84 g.)
5	500	do + 1% ethanol	Reddish semi solid (3.97 g.)
6	1700	benzene-ethanol (1:1)	Red gum (0.91 g.)

Fractions 1, 4 and 6 did not afford any crystalline product. Fraction 2 after repeated crystallization from ethyl-acetate gave (+)-n-nonacosan-10-ol (0.1 g.) m.p. 81°. A mixed m.p. with an authentic specimen showed no depression.

Fraction 3 yielded the C_{28}-alcohol mixture m.p. 69-71° (1.5 g.) on crystallization from ethyl-acetate. A mixed m.p. with authentic C_{28}-alcohol showed no depression.

Fraction 5 gave a positive Liebermann-Burchard test and on crystallization from ethanol afforded β-sitosterol as colourless hexagonal plates (3.0 g.) m.p. 137°; $[\alpha]_D^{20}$ -35.2°(c = 2.30 in chloroform.)

Found : C, 82.2; H, 12.4%

Calc. for $C_{29}H_{50}O$; 1/2 C_2H_5OH : C, 82.4; H, 12.2%

β-Sitosteryl-acetate: β-Sitosterol (200 mg.) was converted into the acetate by heating under reflux with acetic anhydride (12 c.c.). Crystallization from ethanol yielded β-sitosteryl-acetate m.p. 125°, $[\alpha]_D^{20}$ -39.5° (c = 1.44 in chloroform). A mixed m.p. with an authentic specimen of β-sitosteryl-acetate from the alcohol soluble fraction showed no depression.

Examination of the alcohol-soluble fraction

A solution of the solid (B; 55 g.) in light petroleum (1 l.) and benzene (100 c.c.) was adsorbed on a column (40 x 5 cm.) of alumina and developed and eluted as follows :-

Fraction	c.c.	Eluant	Product After Removal of Solvent
B1	6000	light petroleum-benzene (4:1)	Pale yellow oil (5.73 g.)
B2	13700	light petrol-benzene (1:1)	Yellow gum (12.08 g.)
B3	2100	benzene	Red gum (1.72 g.)
B4	2100	do.	Red gum (1.10 g.)
B5	6800	light petrol-benzene (3:7) + 1/2% ethanol	Reddish semi solid (3.14 g.)
B6	4700	do + 1% ethanol	Red gum (15.2 g.)
B7	10700	do.+ 4% ethanol	Red gum (6.8 g.)
B8	6500	benzene-ethanol (1:1)	Red gum (5.0 g.)
B9	1920	ethanol	Red gum (0.11 g.)

Fraction B1 : This fraction was combined with the oil obtained in the purification of fraction B2, (5.58 g.). Distillation gave a yellow viscous oil b.p. 100-120°/ 1.5×10^{-4} mm. (The residue had an odour resembling that of heated rubber). A pure specimen was obtained by redistillation, the fraction b.p. 108°/1.47×10^{-4}mm; n_D^{19} = 1.4741 (3 g.) being collected.

Found : C , 82.47; H,12.78% M.W.(Rast) 229,259,275.

$C_{22}H_{40}O$ requires C , 82.43; H , 12.58% M.W. 320.

Ultra-violet absorption spectrum (in ethanol) =

λ_{max} = 2280 Å, E_{max} = 6000 (assuming M.W. = 300). The compound gave a yellow brown colour with tetranitromethane in chloroform and was optically inactive.

Attempted formation of a maleic anhydride adduct :

Attempts to form an adduct by heating the oil ($C_{22}H_{40}O$) with maleic anhydride under reflux in benzene solution resulted in the recovery of starting material. The following method was also applied (cf. Windaus and Luttringhaus, 9).

The compound (0.4 g.) was dissolved in dry xylene (6 c.c.) and was heated with maleic anhydride (0.3 g.) in an autoclave at 135° for 12 hours. The reaction mixture was allowed to cool and the xylene was distilled off under vacuum. The residue was warmed with potassium hydroxide (0.3 g.) and methanol (5 c.c.) for two hours on a steambath. The solution was diluted with water and extracted with light petroleum (3 x 20 c.c.; b.p. 60-80°). The extract was dried (sodium sulphate). The aqueous layer was acidified with dilute hydrochloric acid and extracted with ether (3 x 20 c.c.). After filtration both the ether and light petroleum solutions were evaporated. The latter left no residue while the former gave a brown oil which showed an ultra-violet absorption spectrum similar to the starting material and gave a yellow brown colour with tetranitromethane in chloroform. It was probably unchanged starting material.

Attempted hydrogenation of the oil : Platinum oxide catalyst (0.1 g.) was suspended in pure dry ethyl acetate and reduced by shaking with hydrogen. The oil (0.32 g.) in ethyl acetate (35 c.c.) was added and agitation with hydrogen was continued at room temperature and atmospheric pressure for 10 hours.

The volume of hydrogen absorbed was 14.2 c.c. (corrected to N.T.P.)

The volume of hydrogen required to saturate one double bond = 48.2 c.c. (assuming M.W. = 300).

The catalyst was filtered off and the solvent was distilled off under vacuum. The residue, a yellow oil, showed an ultra-violet absorption spectrum similar to the starting material, although the intensity was lower (E = 4000), and a strong yellow brown colour was obtained with tetranitromethane in chloroform, indicating that probably only partial hydrogenation had occurred. Lack of material prevented further work on the compound $C_{22}H_{40}O$.

Fractions B2 and B3 : The combined fractions were crystallized from ethyl acetate when they yielded the crude C_{22}-primary alcohol mixture as a colourless amorphous solid (6.4 g.) m.p. 62-64°. The mother liquors afforded a dark red gum (7.38 g.) on evaporation. A solution of this in

light petroleum (200 c.c.) was filtered through a column (18 x 1 cm.) of alumina which was then washed with light petroleum. After removal of the solvent from the eluate, a yellow oil (5.58 g.) was obtained which was added to fraction B1. Continued washing of the column with light petroleum-benzene mixture (7:3; 1100 c.c.) produced more of the crude C_{22}-alcohol. The combined alcohol fractions were repeatedly crystallized from ethyl acetate affording the C_{22}-primary alcohol mixture, (5 g.) as colourless nodules, m.p. 67°

Found : C , 80.98; H , 14.04%

Calc. for $C_{22}H_{46}O$: C , 80.9; H , 14.19%

The C_{22}-alcohol-3:5-dinitrobenzoate : 3:5-Dinitrobenzoylation by the method described for the C_{28}-alcohol mixture gave the C_{22}-alcohol mixture-3:5-dinitrobenzoate as small colourless plates, m.p. 71°, from ethanol.

Found : C , 66.63; H , 9.29; N , 5.4%

$C_{29}H_{48}O_6N_2$ requires : C , 66.89; H , 9.29; N , 5.39%

The C_{22}-acid : This was prepared from the C_{22}-alcohol by the method used in the preparation of the C_{28}-acid. Repeated crystallization of the product from acetone gave the C_{22}-acid mixture as colourless nodules, m.p. 70-71°

Found : C,78.1; H,12.98%; equivalent (by titration) 358

Calc. for $C_{22}H_{44}O_2$: C,77.6; H,13.02; equivalent 342.

Fractions B4 and B5 : These fractions gave small amounts of the C_{22}-alcohol mixture, m.p. 68°, on crystallization from ethyl acetate. A mixed m.p. with authentic C_{22}-alcohol showed no depression.

Fraction B6: This fraction gave a positive Liebermann-Burchard reaction. Repeated crystallization from ethanol afforded β-sitosterol (5.2 g.) as colourless hexagonal plates, m.p. 137° $[\alpha]_D^{20}$ -35.6° (c = 1.0 in chloroform).

Found: (after drying at 78°/0.01 mm. for 2 hours)

C, 81.05; H, 12.2%

Calc. for $C_{29}H_{50}O$; C_2H_5OH : C, 80.87; H, 12.2%

Found: (after drying at 110°/0.01 mm. for 4 hours

C, 81.68; H, 12.11%

Calc. for $C_{29}H_{50}O$; 1/2 C_2H_5OH : C, 82.04;, H 12.2%

Cook and Paige (10) record m.p. 137.5-138.5°, $[\alpha]_D$ -34°, Wallis and Chakravorty (34) record m.p. 136-137°, $[\alpha]_D$ -36.6°, and Simpson and Williams (35) record m.p. 135-135.5°, $[\alpha]_D$ -34.2°.

β-Sitosteryl-acetate : β-Sitosterol (3 g.) was heated under reflux for 75 minutes with acetic anhydride (35 c.c.). On cooling β-sitosteryl acetate precipitated and was obtained as colourless rods, m.p. 125.5-127°, $[\alpha]_D^{20}$ -40° (c = 1.30 in chloroform) after crystallization from ethanol.

Found: C, 81.58; H, 11.53%

Calc. for $C_{31}H_{52}O_2$: C, 81.49; H, 11.48%

Cook and Paige (loc.cit) record m.p. 126.5-127.5°, Wallis and Chakravorty (34) record m.p. 125-126°, $[\alpha]_D$ -41°.

β-Sitosteryl-benzoate: β-Sitosterol (0.5 g.) was heated on a steambath with benzoyl-chloride (1.8 c.c.) and pyridine (12 c.c.) for two hours. The product was worked up in the usual way and after crystallization β-sitosteryl-benzoate was obtained in colourless rectangular plates m.p. 144-145° $[\alpha]_D^{20}$ -11.2° (c = 2.0 in chloroform).

Found : C, 83.3; H, 10.35%

Calc. for $C_{36}H_{54}O_2$: C, 83.5; H, 10.4%

Cook and Paige (10) record m.p. 145.5-146.5°, Wallis and Chakravorty (34) record m.p. 146-147°, $[\alpha]_D$ -13.8°, and Simpson and Williams (35) record m.p. 145-146°, $[\alpha]_D$ -14.2°.

Examination for traces of other phytosterols in the sitosterol fraction.

The absence of α-sitosterol was deduced from the negative Tortelli-Taffé colour test.

Examination for stigmasterol: (cf. Windaus and Hauth, 33) β-Sitosteryl-acetate (1.8 g.) was dissolved in ether (20 c.c.) and a solution of bromine (0.5 c.c.) in glacial acetic acid (20 c.c.) was added. The solution was allowed

to stand at room temperature for 14 hours but no precipitate of tetrabromstigmasteryl-acetate was observed.

The solution was then debrominated (cf. Wallis and Fernholz, 11) by the addition of zinc dust (1.2 g.) to the solution which was then heated on the steambath for two hours. The residual zinc was filtered off and the solution was diluted with water (50 c.c.) and extracted with ether (3 x 30 c.c.). The ether solution was dried (sodium sulphate), filtered and the solvent was removed by distillation. The residue, β -sitosteryl-acetate, was crystallized from ethanol when it had m.p. 124°. A mixed m.p. with authentic β -sitosteryl-acetate showed no depression.

Examination for saturated sterols (sitostanol): (cf. Anderson and Nabenhauer, 12). The β -sitosteryl-acetate (2.g.) was dissolved in carbontetrachloride (33 c.c.) and the solution was placed in a separating funnel. Acetic anhydride (10 c.c.) was added with shaking followed by the dropwise addition of sulphuric acid (10 c.c.). The solution changed colour from red to violet, blue and finally green. After standing for a few minutes a little water was added and the deeply coloured acid formed a layer above the faintly green carbontetrachloride solution. The latter was separated and washed several times with water

until colourless, dried (sodium sulphate) and evaporated. No residue was obtained indicating the absence of sitostanol.

Oxidation of sterol fraction : (cf. Barton and Jones, 13) A solution of the sterol fraction (0.9 g.) and aluminium -t-butoxide (1.2 g.) in dry benzene (30 c.c.) and dry acetone (12 c.c.) were heated under reflux for 18 hours. The solution turned pale yellow and a little gelatinous material separated. The solution was washed with dilute sulphuric acid (3 x 15 c.c.) and water (2 x 15 c.c.), dried (sodium sulphate), filtered and the solvent was distilled off. The residue, a yellow gum, was dried in vacuo and then dissolved in light petroleum (30 c.c.) and adsorbed on a column (34 x 2 cm.) of alumina and developed and eluted as follows :

Fraction	c.c.	Eluant	Product after removal of Solvent
a.	70	benzene	Colourless, non-ketonic solid (0.05 g.)
b.	660	benzene and 2% ether	Colourless solid, m.p. 81-82°. (0.69 g.)
c.	240	benzene and 5% ethanol	Colourless crystals, m.p. 134° (0.15 g.)

Fraction a. The material did not give the Liebermann-Burchard test and was not further examined.

Fraction b. This fraction yielded sitost-4-en-3-one, on crystallization from ethanol as colourless plates, m.p. 84° $[\alpha]_D^{20}$ + 86° (c = 1.60 in chloroform). The ultra-violet absorption spectrum in ethanol shows λ_{max} 2420 Å and E_{max} 17870.

Found : C, 84.8; H, 11.7%

Calc. for $C_{29}H_{48}O$: C, 84.4; H, 11.7%

Barton and Jones (loc.cit.) record m.p. 88° $[\alpha]_D$ + 85.8°

Sitost-4-en-3-one-2:4-dinitrophenylhydrazone: This derivative was prepared by adding Brady's solution (2 c.c.; Brady, J. 1931, 756) to a solution of sitost-4-en-3-one (0.1 g.) in methanol. The bright red precipitate was crystallized from chloroform-ethanol mixture yielding sitost-4-en-3-one-2:4-dinitrophenylhydrazone, m.p. 253°. The ultra-violet absorption spectrum in chloroform showed λ_{max} 2860 Å and 3930 Å; E_{max} 11360 and 29900 respectively.

Found : C, 71.01; H, 8.67%

Calc. for $C_{35}H_{52}O_4N_4$: C, 70.91; H, 8.84%

Barton and Jones (13) record m.p. 253°.

Fraction c. The crystalline solid afforded unchanged β-sitosterol, m.p. 135°, from ethanol. A mixed m.p. with authentic β-sitosterol showed no depression.

Examination of the mother liquors of fraction B6

The combined mother liquors from the crystallization of the

β-sitosterol yielded a second crop of crystalline sitosterol (2.5 g.), m.p. 125-127°, $[\alpha]_D^{18}$ -28 (c = 1.42 in chloroform). The solid was dissolved in light petroleum (100 c.c.) and adsorbed on a column (14 x 2 cm.) of alumina and eluted as follows :

Fraction	c.c.	Eluant	Product after removal of Solvent
1	1500	light petroleum-benzene (7:3)	Yellow waxy solid, (0.1 g.) Liebermann-Burchard negative.
2	100	do. + 1% ethanol.	Yellow waxy solid, (0.102 g.)
3	500	do.	Crystalline solid (2.310 g.)

Fractions 1 and 2: were combined and crystallized from ethyl acetate to yield the C_{22}-alcohol mixture, m.p. 68°. No melting point depression was observed on admixture with an authentic C_{22}-alcohol.

Fraction 3 afforded β-sitosterol on crystallization from ethanol as colourless plates, m.p. 137°, $[\alpha]_D^{20}$ -35.2° (c = 1.3 in chloroform).

β-Sitosteryl-acetate was prepared as described above (page 40) and was obtained pure on crystallization from ethanol, m.p. 125°, $[\alpha]_D^{20}$ -37.9°, (c = 1.4 in chloroform).

The β-sitosteryl-acetate (2 g.) was examined for the presence of traces of stigmasterol by the method described above (page 41). No tetrabromstigmasteryl-acetate could

be isolated.

Acetylation and chromatography of the non-crystalline residue of fraction B 6.

After removal of the second crop of β-sitosterol described above, the solvent was removed from the mother liquors of fraction B6 by vacuum distillation, leaving a dark brown gum, (7 g.). This was acetylated by allowing the solution to stand at room-temperature with pyridine (40 c.c.) and acetic anhydride (15 c.c.) for 15 days. The solution was poured into dilute sulphuric acid (400 c.c.) and extracted with ether (4 x 100 c.c.). The extract was washed with water, dried, (sodium sulphate), filtered and the ether was removed by distillation leaving a dark gum (7 g.). This was dissolved in light petroleum (200 c.c.) and adsorbed on a column (24 x 3.5 cm.) of alumina and eluted as shown below :

Fraction	c.c.	Eluant	Product after removal of solvent
1	3000	light petroleum-benzene (1:1)	Yellow non-crystallizable gum (1.169 g.) Liebermann negative.
2	800	benzene-ether (3:1)	Yellow gum (0.363 g.)
3	2000	benzene + 1% ethanol.	Red gum (3.926 g.); a small amount of solid crystallized from ethanol.
4	1600	benzene-ethanol (9:1)	Red gum (1.050 g.)

Fractions 1, 2 and 4 could not be induced to crystallize and no product was identified.

Fraction 3: A colourless solid (0.2 g.) was obtained on crystallization from ethanol. It gave a positive Liebermann-Burchard test. Further crystallization afforded β-sitosteryl-acetate m.p. 124°, $[\alpha]_D^{20}$ -39.4° (c = 1.1 in chloroform). A mixed m.p. with authentic β-sitosteryl-acetate, m.p. 125.5-126° showed no depression.

Fractions B7 and B9 : The gums (11.9 g.) were combined and acetylated by heating under reflux with acetic anhydride (125 c.c.) for 90 minutes. The product was worked up in the usual way yielding the acetylated gum (12 g.)

Attempted separation of ketonic material by Girard's reagent P. cf. Reichstein and Van Euw (14). The acetylated gum was dissolved in methanol (200 c.c.) and glacial acetic acid (17 c.c.) and Girard's reagent P (12 g.) were added to the solution which was then heated under reflux for one hour. The solution was chilled overnight and was then neutralised with 10% w/v sodium carbonate solution until weakly alkaline to phenolphthalein, keeping the temperature below 10° (ice-salt bath). The methanol was removed by distillation under vacuum at 10°. The solution was then cooled to -10° (acetone-carbon dioxide bath) and extracted with ice-cold pure ethyl acetate (5 x 150 c.c.). The extract

was washed with dilute sodium hydroxide solution (150 c.c.) water (100 c.c.) dried (sodium sulphate) and after filtration the solvent was removed by vacuum distillation leaving the non-ketonic fraction as a dark gum (11.3 g.)

The aqueous solution was then carefully acidified by the addition of dilute hydrochloric-acid until the solution was acid to litmus. Extraction with pure ethyl acetate (3 x 100 c.c.) gave ketonic fraction I.

The dilute acid solution was then acidified more strongly by the addition of concentrated hydrochloric acid (60 c.c.). Extraction with ethyl acetate as above yielded ketonic fraction II.

On removal of the solvent from both fraction I and II only very small gummy residues were obtained from which no ketonic derivatives could be isolated with 2:4-dinitrophenyl-hydrazine.

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Part II

The Triterpenes of *Alstonia Verticillosa*

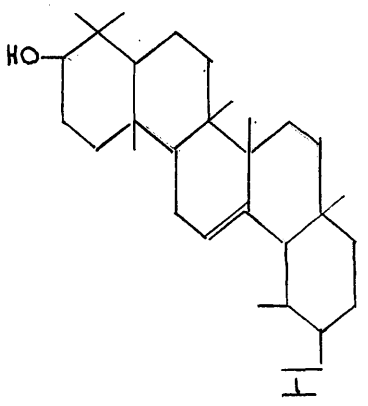
and Experiments on β-Amyrin.

Historical

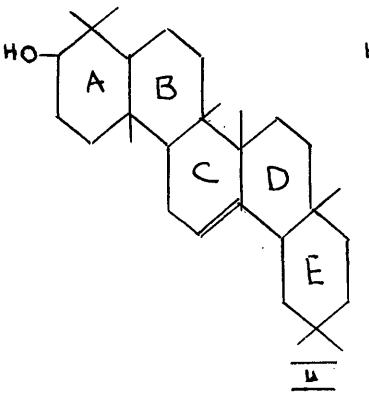
Introduction

The triterpenes are a group of naturally occurring compounds containing thirty carbon atoms which are considered to be arranged so that they can be divided theoretically into six isopentane units. On dehydrogenation with selenium they yield homologues of naphthalene (1). They are widely distributed among plants and occur free or as esters or combined with sugars forming glycosides. The triterpenes include monohydric alcohols such as the isomeric α - and β -amyrins which are found in many resins, diols such as betulin isolated from birch (2) and a number of hydroxy acids such as elemolic acid from Manila Elemi resin (3).

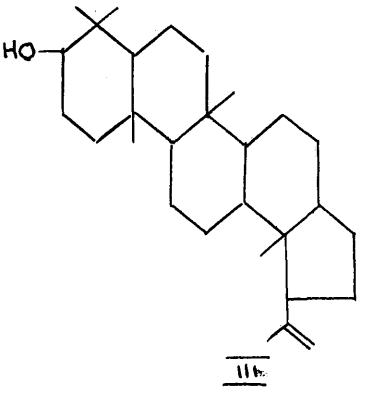
Extensive research (4,5,61) into the chemistry of the triterpenes has established that they are pentacyclic compounds, with the exception of the open chain compound squalene and a number of tetracyclic compounds. The pentacyclic substances can all be related to one or other of three parent alcohols, α -amyrin (I), β -amyrin (II) and lupeol (III).



I

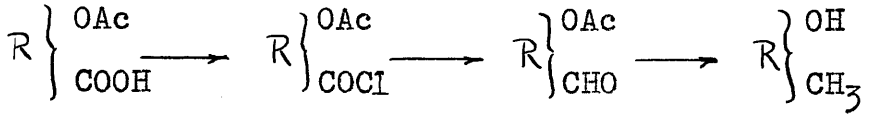


II



III

Thus the hydroxy acid oleanolic acid, with its hydroxyl group protected was converted into its acid chloride, Rosennmund reduction of which gave the corresponding aldehyde which on reduction by the Kischner-Wolff method afforded β-amyrin (37) according to the following scheme :-



Until recently it was not possible to convert one parent compound into another but Ames, Halsall and Jones (6) have been able to form common derivatives of β-amyrin (II) and lupeol (III) and have shown that β-amyrin and lupeol are identical in rings A, B, C and D, with the exception of the location of the double bond and possibly of some stereochemical differences.

The Structure of β-Amyrin

β-Amyrin ($C_{30}H_{50}O$) is a monohydric alcohol forming acyl derivatives. The yellow colour with tetranitromethane shows the presence of one double bond which is however resistant to catalytic hydrogenation. Nevertheless the presence of this has been confirmed by chromic acid oxidation of β-amyrin-benzoate (15,16) to β-amyrenonyl-benzoate in which the occurrence of an αβ unsaturated ketone has been demonstrated spectroscopically.

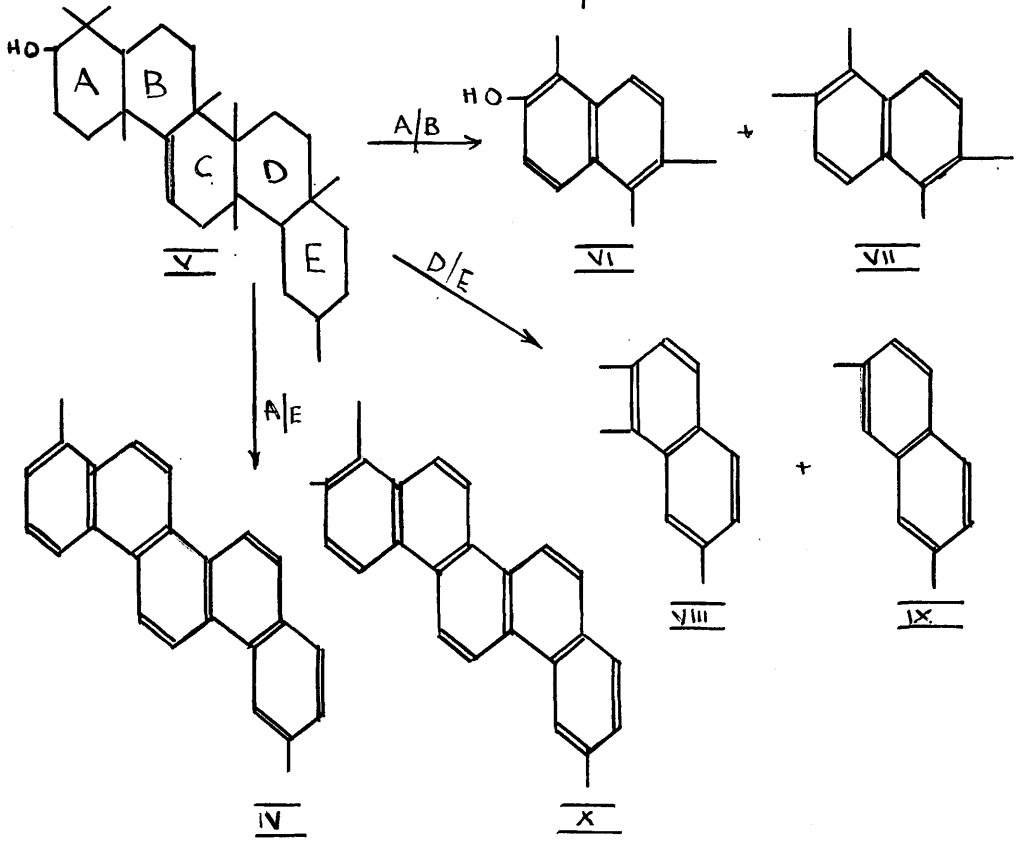
Reduction of this ketone with sodium and alcohol followed by dehydration yielded a dehydro compound in which the presence of a conjugated diene system has been shown spectroscopically. Furthermore oxidation of β-amyrin-acetate with hydrogen peroxide (17) gave β-amyranonyl-acetate in which it could be shown (light absorption and no colour with tetranitromethane) that a keto group had replaced one double bond. Reduction of this compound by the Kischner-Wolff technique afforded the saturated alcohol, β-amyranol, which by further oxidation and Kischner-Wolff reduction yielded the saturated hydrocarbon β-amyran (18).

From a comparison of the molecular formula of β-amyrin ($C_{30}H_{49}OH$) with the straight chain paraffin $C_{30}H_{62}$ it becomes evident that β-amyrin contains twelve hydrogens less than the saturated open chain compound. It is clear therefore since one double bond is present, that β-amyrin is pentacyclic.

Dehydrogenation. In 1929 Ruzicka (19) after vigorous heat treatment of the mixed amyrin isomers obtained a number of hydrocarbons which afforded naphthalene homologues on treatment with selenium or sulphur. Dehydrogenation experiments on a mixture of the isomeric amyrins yielded 1:2:7-trimethylnaphthalene (VIII, 20), 1:2:5:6-tetramethyl-

naphthalene (VII, 20), 1:2:3:4-tetramethylbenzene (20), 2:7-dimethylnaphthalene (IX, 20), 1:5:6-trimethyl-2-naphthol (VI, 21) and 1:8-dimethylpycene (IV, 22). In addition to all these β-amyrin afforded phenanthrene and 2-hydroxy-1:8-dimethylpycene (X, 19,20,21,22,23,24).

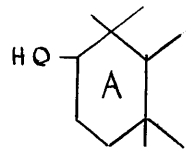
The isolation of 1:8-dimethylpycene (IV) indicated that the pentacyclic system of the triterpenes was a reduced pycene nucleus. Considering all the products of dehydrogenation and applying the "isoprene rule" Ruzicka postulated the formula (V) for β-amyrin in 1937 (25).



It should be noted that the formation of 1:2:5:6-tetramethylnaphthalene (VII) and 1:2:3:4-tetramethylbenzene was accounted for by the postulation of a retrapinacolone dehydration involving the 2-hydroxyl group, as a preliminary step to the dehydrogenation. This theory was confirmed by the dehydrogenation of β-amyrene (20).

The structure of β-amyrin was modified to formula (II) by Haworth (26), which accounts better for all the known data (see below) and which is in agreement with the most recent evidence (6,27,28,61).

Degradation experiments: By the stepwise oxidative degradation of ring A of hederagenin and oleanolic acid, both of which are members of the β-amyrin series of triterpenes, the structure of this ring (XI) was rigidly established (29,30).

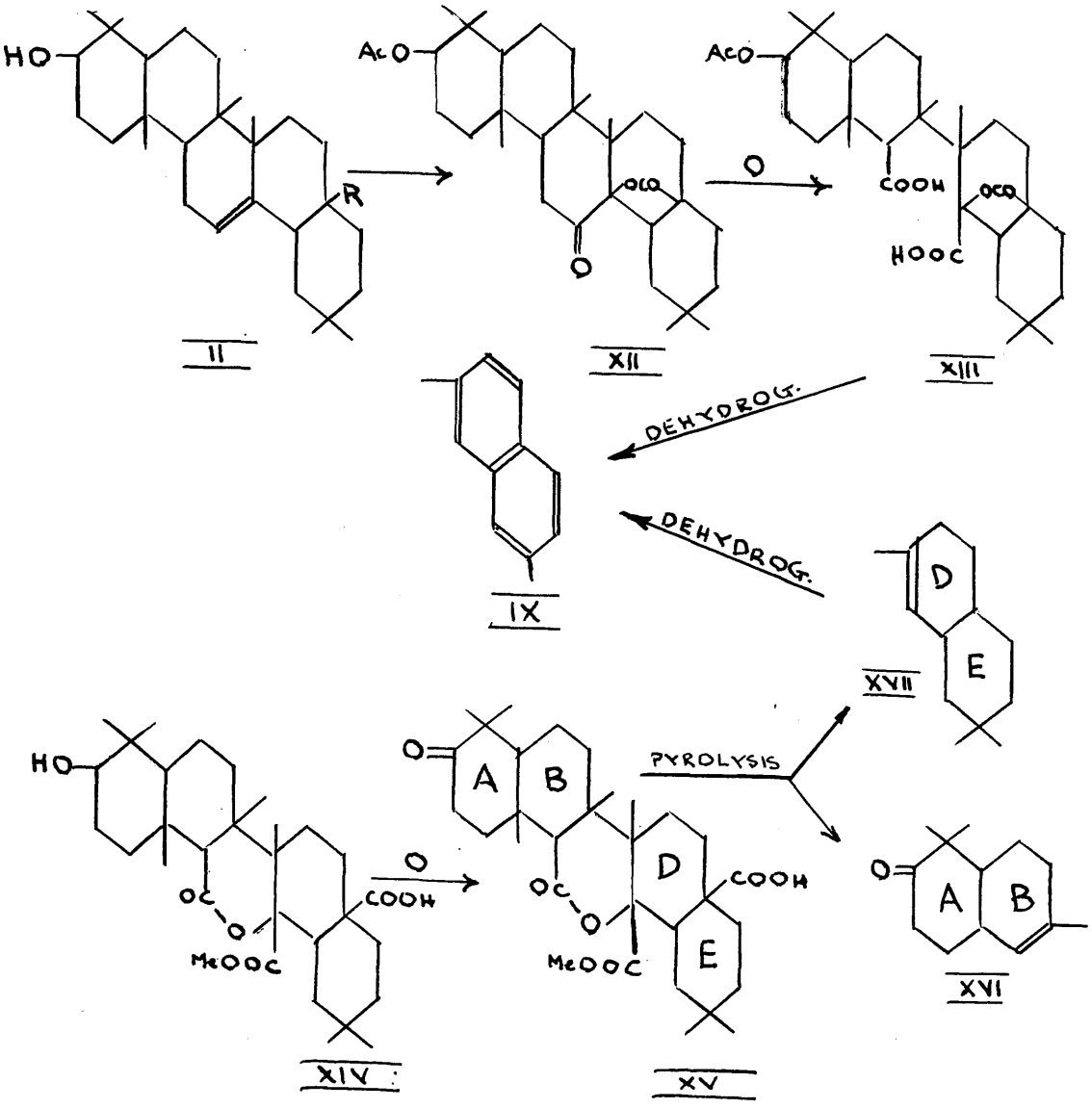


XI

The position of the double bond in Ring C of the hydropycene structure of β-amyrin was elucidated from experiments on oleanolic acid (p. 52).

Oxidation of ketoacetyloleanolic lactone [(XII) obtained

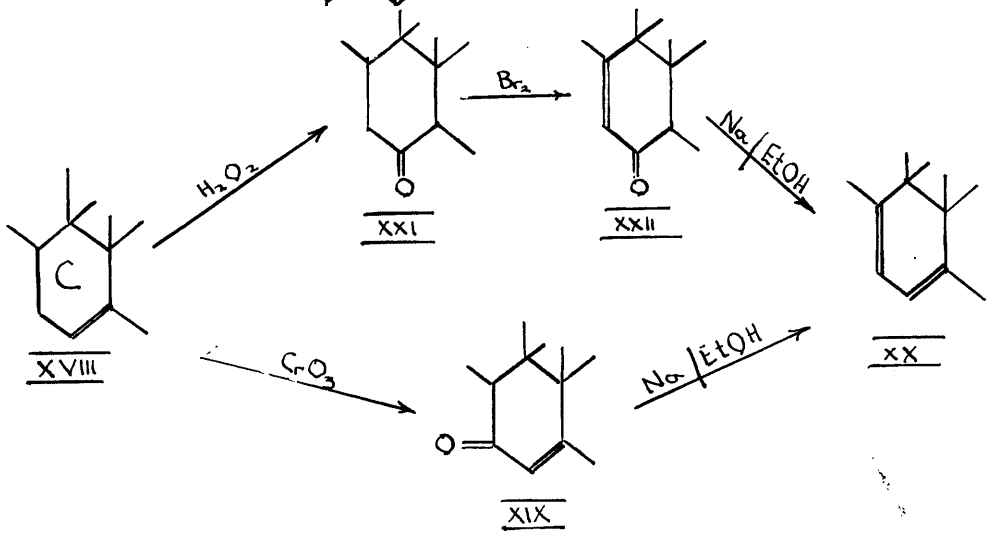
from (II, R=COOH for oleanolic acid) afforded acetyl oleanolic lactone dicarboxylic acid (XIII) which on dehydrogenation yielded 2:7-dimethylnaphthalene (IX,31). Proof that (IX) was derived from rings D and E was furnished by a series of reactions starting from the monomethyl ester of isooleanolic lactone dicarboxylic acid (XIV) which was obtained from the dimethyl-ester of (XIII) by alkaline hydrolysis. Oxidation of (XIV) yielded the ketone (XV) which on pyrolysis gave the ketone (XVI) and the hydrocarbon (XVII) in almost equal amounts. The latter on dehydrogenation yielded (IX), (32,33). The compound (XV) was thus split into two portions both of which could be degraded to naphthalene derivatives which confirmed the supposition (25) that the double bond was in ring C and that this ring was opened on the oxidation of (XII) to (XIII).



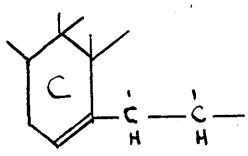
It has already been shown (p. 52) that β-amyrin benzoate yields an $\alpha\beta$ unsaturated ketone, β-amyrenonyl-benzoate, on oxidation with chromic acid. The ketone is formed by the oxidation of a methylene group next to an ethylenic linkage (15). Treatment of β-amyrenonol with sodium and alcohol, followed by esterification with acetic

anhydride gave a dienyl-acetate, β -amyradienyl-I-acetate which had presumably been formed from β -amyrenonol by the reduction of the carbonyl group to a secondary alcohol followed by the dehydration of the latter (34). Spectroscopy showed that the dienol contained a conjugated system of two double bonds in one ring. It follows that β -amyrin contains the system (XVIII) and β -amyrenonol and β -amyradienol-I can be represented by the partial formulae (XIX) and (XX) respectively.

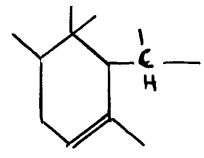
Furthermore, β -amyranonol, made from β -amyrin by oxidation with hydrogen peroxide (17) was shown to be a saturated keto-alcohol (35), which on bro[illegible]on as acetate, yielded an α β unsaturated ketone, iso-β-amyrenonyl-acetate which could be converted into β -amyradienol-I-acetate (XX) by sodium and alcohol reduction and treatment with acetic anhydride. If β -amyrin is represented by (XVIII), then β -amyranonol and iso-β -amyrenonol contain fragments (XXI) and (XXII) [illegible]tively.



Finally Picard and Spring (36) have established that partial dehydrogenation is brought about by the treatment of β -amyrenonyl esters with bromine giving the corresponding esters of β -amyradienonol. Light absorption data demonstrated that these esters contain the system -CO-C=C-C=C- which means that β -amyrin contains either fragment (XXIII) or (XXIV).



XXIII



XXIV

Formula (II) includes fragment (XXIII) and the reactions described above thus help to confirm the veracity of the Haworth formulation for β -amyrin.

Alstonia Verticillosa

α -And β -amyrin are frequently associated in nature with lupeol e.g. in Alstonia costulata (7). In the work to be described in the following pages, the triterpenes of another member of the Alstonia species, Alstonia verticillosa are examined.

The Alstonia species (family Apocynaceae) are forest

trees occurring in India, the Philippines, Java, Timor, Australia and tropical Africa (8). The barks were used in the tropics as anti-malarial drugs which resulted in their chamical examination by a number of workers. This led to the isolation of several alkaloids, e.g. alstonine and alstonidine, porphyrine and porphyrosine from A. constricta and echitamine from A. verticillosa, A. congensis, A. spathulata and A. Gilletii (9). Modern methods of investigation have however shown that these substances are not effective antimalarials (10).

Theoretical

Introduction

The work to be described on the following pages is divided into two parts :-

The first part deals with the analysis of the triterpene fraction of an extract from the bark of the Australian tree Alstonia verticillosa.

The second part is concerned with the chemistry of β -Amyrin, one of the triterpenes isolated from Alstonia and deals with the establishment of a route to a compound which would permit a study of some stereochemical aspects of the structure of β -Amyrin. The preparation of a new β -amyrene is described.

Part I.

The Triterpenes of Alstonia Verticillosa

In a study to find naturally occurring substitutes for quinine Sharp, (11,12) examined the barks of various types of the Alstonia species occurring in Australia. In the case of A. verticillosa (12) exhaustion of the powdered bark with light-petroleum afforded a rubbery extract which was partly soluble in ethanol. The alcohol solution yielded several crops of crystalline solids, of increasing solubility in ethanol, all of which gave characteristic colourations with the Liebermann-Burchard reagent. Sharp (loc.cit.) did not examine these products but considered them to be "sterols". One crop of the crystals did appear to be homogeneous and had a melting point close to that of the triterpene, lupeol, but a mixed melting point with authentic lupeol showed a depression of 40°.

The examination of these "sterols" forms the subject of the work reported below. The specimens were supplied by Dr. T.A. Henry of the Wellcome Research Laboratories. They consisted of three fractions, the first and least soluble of which melted over a range below 160°, the second had a melting point range between 160-180°, while the last melted between 180-190°. All the fractions gave a violet

colour with the Liebermann-Burchard reagent suggesting the presence of triterpenoids rather than steroids. The fact that the fractions melted over a considerable range showed that they were probably mixtures.

Fraction I

In an effort to separate the various constituents of the first fraction, the material was examined by the liquid chromatogram method (13). A large number of arbitrary fractions were collected which were combined according to their melting points into three main groups A, B and C.

It appeared however, that these fractions (A,B and C) were not homogeneous. (e.g. the melting points were not sharp even after crystallization). It was thought moreover, that some of the products isolated from the column might be present as esters (the ease of elution seemed to show that there were no functional groups present which would cause strong adsorption on the alumina). The three fractions (A,B and C) were therefore saponified and the hydrolysed products were acetylated to facilitate subsequent separation since the free triterpenols do not crystallize readily. Each of the fractions of acetates was then chromatographed separately.

Fraction A yielded a mixture of α- and β-amyrin-acetate

characterised by the optical rotation, melting point and analysis but even further chromatography and repeated crystallization did not permit a separation of the two isomers.

Fraction B afforded lupeol-acetate. It was possible to separate β-amyrin-acetate and lupeol-acetate from fraction C by fractional crystallization of the acetates obtained from the chromatogram. The amount of pure β-amyrin-acetate was very small and the bulk of fraction C consisted of lupeol-acetate. The latter was hydrolysed and converted into the benzoate which confirmed the identification.

Fraction II

The second fraction was then examined in the light of the results obtained with the first fraction. The material was saponified and the mixture of triterpene alcohols was benzoylated since mixtures of triterpene benzoates are more easily separated than the acetates by chromatography (14). The benzoates were adsorbed on a column of alumina and carefully eluted, and as in the case of fraction I it was possible to combine the large number of fractions into six larger groups (fractions D,E,F,G,H,I).

Fraction D appeared to consist of amyrin-benzoates (melting point and optical rotation) and fractional

crystallization afforded five crops of crystalline material. The 1st crop was shown to be lupeol-benzoate while the other four were characterised as α -amyrin-benzoate. The third crop was thought to contain some β -amyrin-benzoate (a slightly higher optical rotation than with the other crops was recorded). It was therefore extracted with ether, in which β -amyrin-benzoate is only slightly soluble. Only a very small amount of material remained undissolved and even this was shown to be α -amyrin-benzoate after further crystallization. The ether soluble portion consisted entirely of α -amyrin benzoate. The α -amyrin-benzoate from the combined crops of fraction D was hydrolysed and converted into its acetate, confirming the identification.

Fraction E, a fraction obtained from the mother liquors of fraction D, was also shown to be α -amyrin-benzoate.

Fractions F, G and H consisted of lupeol-benzoate but a small amount of α -amyrin-benzoate was isolated from the mother liquors of the crystallization of fraction F. The lupeol-benzoate was hydrolysed and the acetate was prepared in the same way as the α -amyrin-benzoate of fraction D.

Fraction I was not easily eluted from the chromatogram and did not crystallize readily. It was considered to consist of free triterpene alcohols and was not examined.

Fraction III

The third fraction from the Alstonia extract was examined by the same method as the second fraction. The material was hydrolysed and benzoylated and the benzoates were chromatographed. Here again the large number of fractions obtained were combined into three groups (fractions K,L,M) each of which was fractionally crystallized.

In fraction K it was possible to separate α - and β - amyrin-benzoate by crystallization in distinction to the amyrin acetates which could not be separated in this manner (see first fraction above).

Fraction L also consisted of a mixture of the two amyrin isomers and again they were readily separated by fractional crystallization. In both fractions K and L, the β -isomer was predominant.

Fraction M consisted entirely of lupeol-benzoate.

Part II

Studies in the Stereochemistry of β -Amyrin.

The Preparation of a new β -Amyrene.

In the introduction a brief outline was given of the steps which led to the elucidation of the structure of β -amyrin. Little, however, has been known until recently about the stereochemistry of β -amyrin and the pentacyclic triterpenes in general.

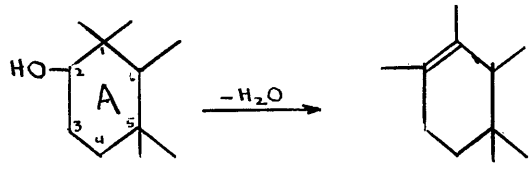
It has already been stated (see introduction) that β -amyrin and lupeol have the same structure in rings A, B, C and D with the exception of the double bond in ring C of β -amyrin (6). Meisel, Jeger and Ruzicka (46) demonstrated that α - and β -amyrin had the same configuration in rings A and B and possibly also in rings B and C. Furthermore Ruzicka, Gutmann, Jeger and Lederer (47) have shown that rings A and B of these triterpenes were trans-locked, for oleanolic acid was degraded to 2:5:5:9-tetramethyl-trans-decalin-1-carboxylic acid which had the same stereochemical configuration as abietic acid, for which it had already been proved that rings A and B were trans-locked (48). However it was possible that epimerisation may have occurred during the degradation of the oleanolic acid to the trans-decalin-carboxylic acid (49) and therefore a more rigorous study of the stereochemistry

of rings A and B is necessary.

Barton and Holness (51) have shown that the rings D and E of the β-amyrin group were cis-locked for it was possible to epimerise rings D and E of 11-ketooleanolic acid into the more stable trans-form.

To study the stereochemistry of rings A and B of β-amyrin it was desired to prepare a compound having a double bond between C_1 and C_2 which would make it possible to introduce functional groups in rings A and B. It is with the preparation of such a compound that the work described below is concerned.

In the introduction (p. 55) it was stated that Ruzicka showed that a retrapinacolone dehydration took place during the dehydrogenation of β-amyrin with selenium thus:



XXV XXVI

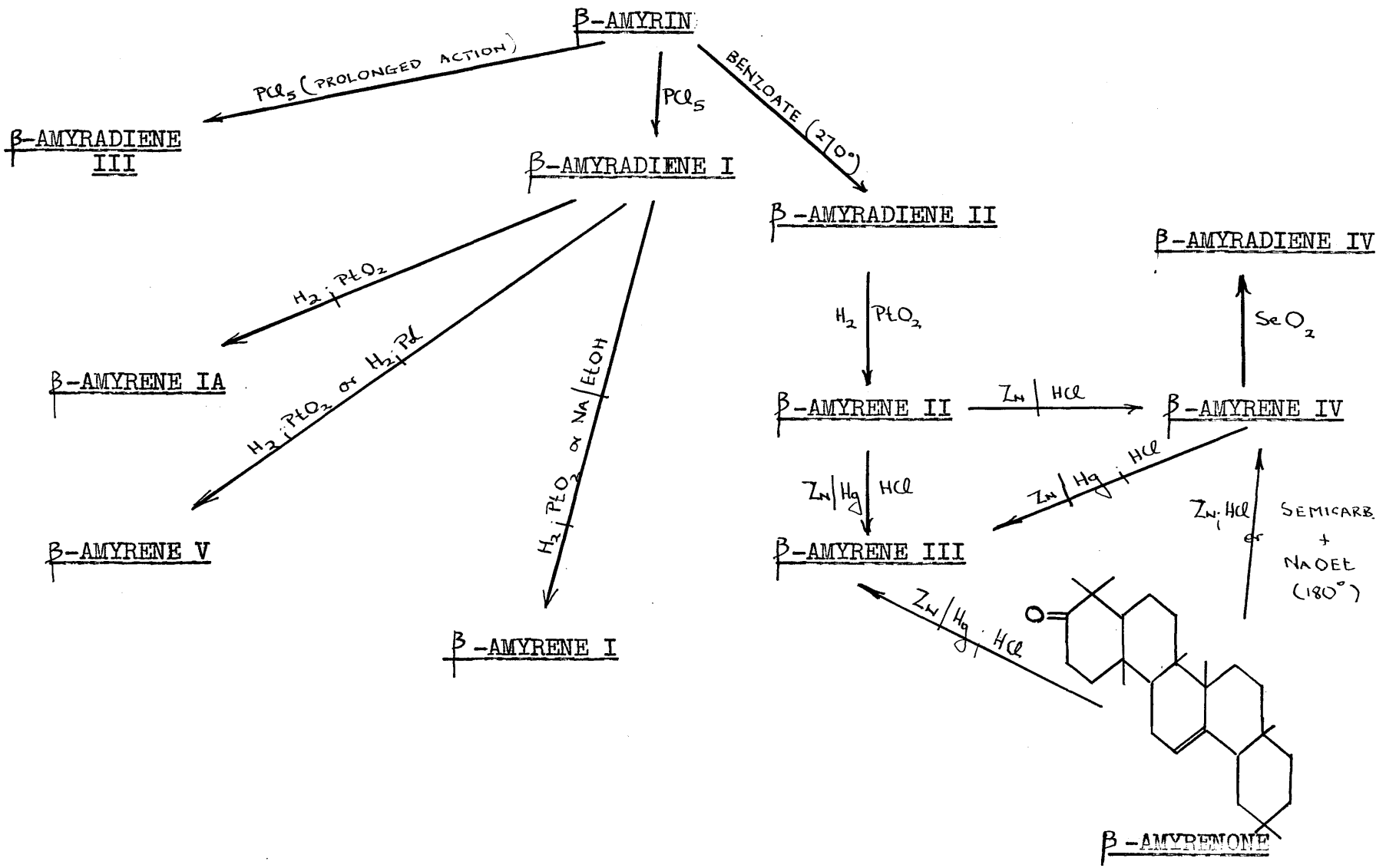
β-amyrin itself has been dehydrated in the past and depending on the dehydrating agent employed, a number of β-amyradienes have been isolated.

In 1887 Vesterberg (52) treated β-amyrin with phosphorus pentachloride. A doubly unsaturated hydrocarbon,

β -amyradiene I (m.p. 170-175°) was isolated which has since been prepared by Ruzicka (53) and Winterstein and Stein (54). Catalytic hydrogenation of this compound yielded β -amyrene I (m.p. 92-93°) which still contained one double bond which was however resistant to hydrogenation (53,54,21). Further hydrogenation experiments on β -amyradiene I led to the separation of two isomeric β -amyrenes; the β -amyrene I described above and β -amyrene V (m.p. 84-5°; 21). The latter was also obtained by Spring (17) by the hydrogenation of β -amyradiene I with palladium catalyst and the same author obtained β -amyrene I by the reduction of the diene with sodium and ethanol. Winterstein and Stein (54) isolated yet another monoene, β -amyrene IA (m.p. 209°) by the fractional crystallization from acetone of the product of hydrogenation of β -amyradiene I.

The pyrolysis of β -amyrin-benzoate at 270-300° (54) or heating the p -toluenesulphonylchloride of β -amyrin dissolved in pyridine at 100° (56) yielded β -amyradiene II (m.p. 148-150°) which on hydrogenation gave β -amyrene II (m.p. 162-165°; 54). Clemmensen reduction of this brought about an isomerisation to β -amyrene III (m.p. 187-189.5°; 54). The latter was also formed when β -amyrenone was reduced by the Clemmensen technique. β -Amyrene II on treatment

S C H E M E II


β-AMYRIN
PCl5 (PROLONGED ACTION)
PCl5
BENZOATE (270°)
β-AMYRADIENE III
β-AMYRADIENE I
β-AMYRADIENE II
β-AMYRADIENE IV
H2 | PtO2
H2 | PtO2 or H2 | Pd
H2 | PtO2 or Na | EtOH
SeO2
β-AMYRENE IA
β-AMYRENE II
Zn | HCl
β-AMYRENE IV
Zn | Hg | HCl
Zn | Hg | HCl
Zn | Hg | HCl
Zn, HCl or SEMICARB. + NaOEt (180°)
β-AMYRENE V
β-AMYRENE III
β-AMYRENE I
β-AMYRENONE

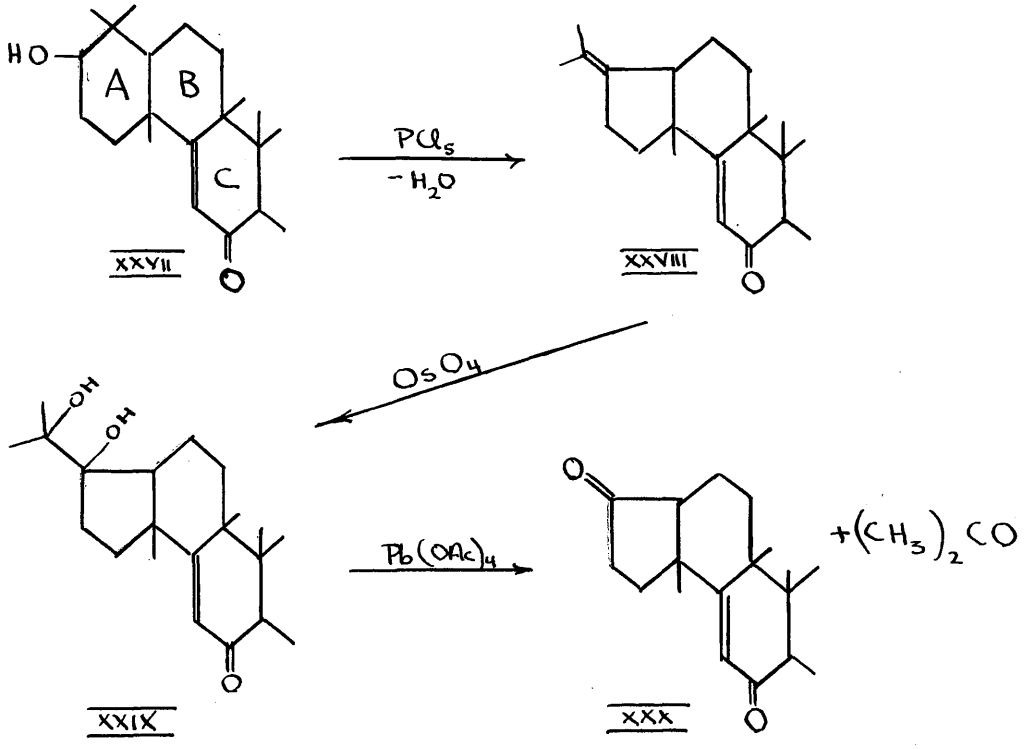
with zinc and hydrochloric acid isomersied to β-amyrene IV (m.p. 162-163°) which could also be obtained from β-amyrenone by reduction with zinc and hydrochloric acid (54) or by the Kischner-Wolff reduction of the semicarbazone of β-amyrenone (57). β-Amyrene IV could be converted into β-amyrene III by treatment with zinc amalgam and hydrochloric acid (54). Oxidation of β-amyrene IV with selenium dioxide yielded a further diene, β-amyradiene IV (m.p. 218°; 57).

Finally β-amyradiene III (m.p. 103°) was produced by the prolonged action of phosphorus pentachloride on β-amyrin. Scheme II shows the reactions described above.

In none of the dienes or β-amyrenes was the position of the unsaturated centers established.

Recently however Ruzicka threw some light on the mechanism of the dehydration of the hydroxyl group at C_2 of the pentacyclic triterpenes in a study of the dehydration products of α-amyrin (58). α-Amyrenonol [(XXVII), obtained from α-amyrin-acetate by chromic acid oxidation, followed by hydrolysis] was dehydrated with phosphorus pentachloride yielding α-amyradienone I (XXVIII) which on oxidation with osmium-tetroxide gave a diol $C_{30}H_{48}O_3$ (XXIX). The latter was oxidised with lead-tetraacetate yielding the diketone (XXX) and acetone, which was isolated in a 70% yield (58).

Ruzicka et.al. concluded that a retrapinacolone dehydration had taken place with the formation of an isopropylene side chain and a consequent reduction of ring A from a 6- to a 5-membered ring.



These results were in agreement with the spectroscopic evidence (58).

Ruzicka also showed that the dehydration of lupeol took place by a similar mechanism (59). In the case of the β-amyrin group of triterpenes Ruzicka, Rudowski,

Norymbersky and Jeger (60) demonstrated that the dehydration proceeded in the same manner in degradation experiments on oleanolic acid.

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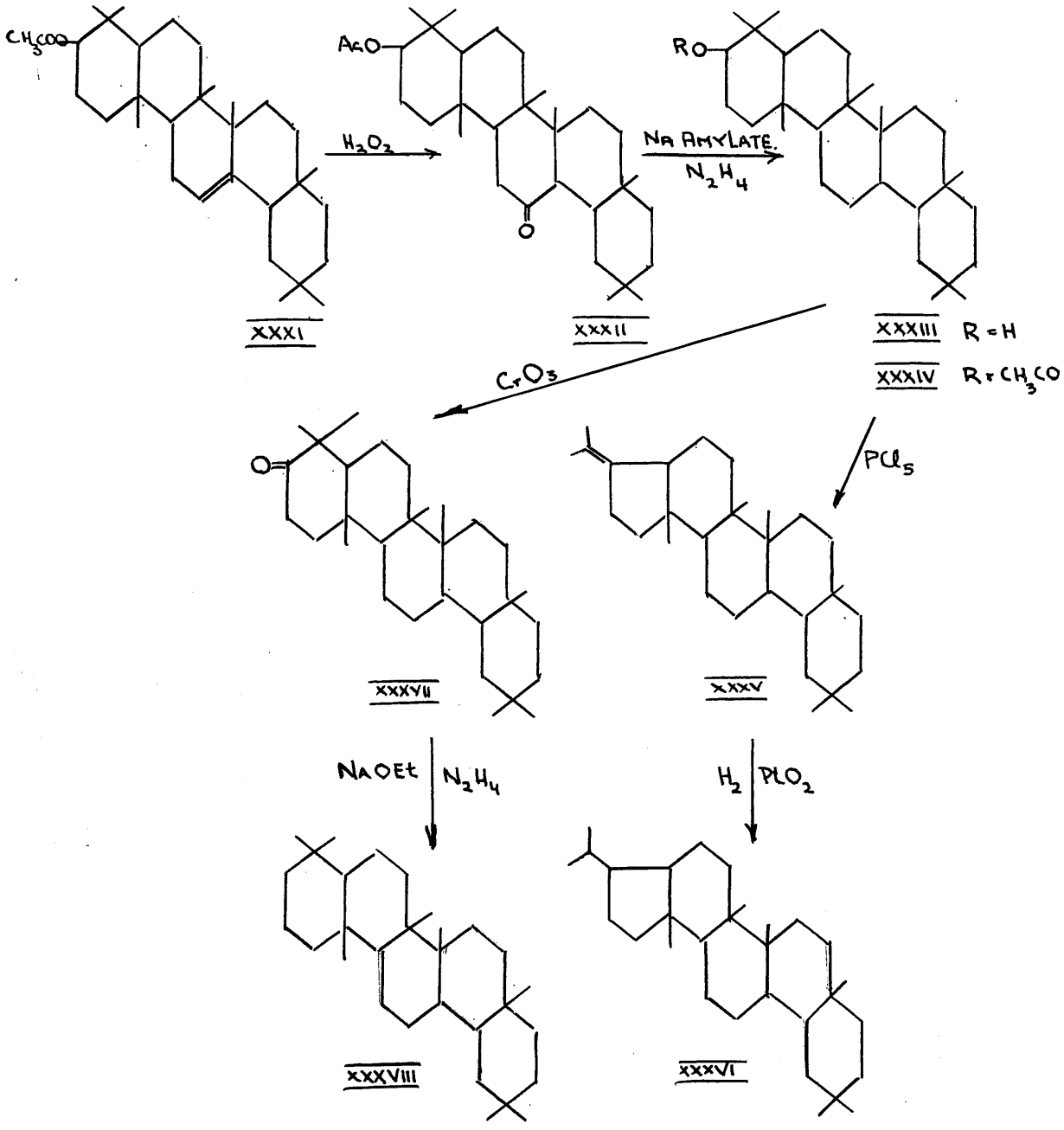
The yields of β-amyrin from the extract of A.verticillosa were very small and a larger quantity of the triterpene was therefore isolated from Manila Elemi resin as β-amyrin-benzoate. This was hydrolysed to the free alcohol and acetylated to yield β-amyrin-acetate (XXXI).

It was desirable that there should be no functional groups in the molecule apart from the hydroxyl group in ring A and therefore the double bond in ring C between C_{12} and C_{13} had to be removed. It has already been stated (see introduction p. 52) that the double bond is resistant to catalytic hydrogenation and consequently it was removed as follows :

β-Amyrin-acetate (XXXI) was oxidised with hydrogen-peroxide to the saturated ketone, β-amyranonyl-acetate (XXXII; 17) which on reduction by the Kischner-Wolff technique yielded the saturated alcohol β-amyranol (XXXIII; 18). The latter was purified through its acetate (XXXIV).

Dehydration of the β-amyranol (XXXIII) with phosphorus pentachloride in light petroleum solution afforded a new β-amyrene, termed β-amyrene VI (XXXV). This compound


CH3COO
AcO
RO
H2O2
Na AMYLATE.
N2H4
CrO3
PCl5
NaOEt
N2H4
H2
PtO2
XXXI
XXXII
XXXIII R=H
XXXIV R=CH3CO
XXXVII
XXXV
XXXVIII
XXXVI

was hydrogenated catalytically. One molecule of hydrogen was taken up and a saturated β-amyran, termed β-amyran II (XXXVI) was obtained. The compound did not give a yellow colour with tetranitromethane. This β-amyran II differed considerably in its physical properties from the β-amyran (m.p. 172-173°) isolated by Ruzicka (18) who obtained the saturated hydrocarbon by the oxidation of β-amyranol (XXXIII) to β-amyranone (XXXVII), Kischner-Wolff reduction of which gave β-amyran (XXXVIII; 18). The mode of formation of the latter precluded any changes in ring A of the molecule.

It was therefore concluded that the dehydration took place by a retrapinacolone mechanism as postulated by Ruzicka et.al. (58,59,60).

Since the yields at each stage of the experiments were very small, it was not possible to confirm the structure of β-amyrene VI (XXXV).

CONCLUSION

Part I

The so-called "sterol" fractions isolated by Sharp (12) from Alstonia verticillosa were examined by chromatographic methods and fractional crystallization of the acetates and benzoates of the substances. No steroid material was isolated and the material consisted entirely of lupeol, α-amyrin and β-amyrin in that order of abundance.

It is possible that the triterpeneols are present in the bark as esters but no definite evidence was obtained.

Part II

β-Amyrin-acetate was oxidised to β-amyranonyl-acetate and the latter was reduced to β-amyranol. This compound on dehydration yielded a new β-amyrene, β-amyrene VI. It was suggested that the dehydration took place with a retrapinacolone mechanism as postulated by Ruzicka and his co-workers (58,59,60), since the hydrogenation product of β-amyrene VI was not identical with the saturated β-amyran obtained by Ruzicka (18).

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Experimental

Experimental

All melting points are uncorrected. The alumina used for the chromatograms was supplied by Savory and Moore and standardised according to Brockmann (Grade II). Light petroleum refers to the fraction boiling between 40-60°.

Alstonia verticillosa : Fraction I

Chromatography

The powdered solid (10 g.) was dissolved in light petroleum (500 c.c.) and was adsorbed on a column (45 x 5 cm.) of alumina and developed and eluted as shown below. The volume of each fraction was 100 c.c.

			After Removal of Solvent	
Fraction	Eluant	Weight	Product	m.p.
1-16	light petroleum	0.0 g.		
17-69	light petroleum 98; benzene 2	0.0 g.		
70-74	do.	0.122 g.	colourless solid	172-190°
75-77	light petroleum 90; benzene 10	0.165 g.	do.	164-194°
78-79	do.	0.162 g.	do.	172-188°
80-82	do.	0.304 g.	do.	172-186°
83-85	do.	0.208 g.	do.	168-186°
86-89	do.	0.047 g.	do.	168-185°
90-92	do.	0.130 g.	do.	176-194°
93-95	do.	0.139 g.	do.	186-194°

		After Removal of Solvent		
Fraction	Eluant	Weight	Product	m.p.
96-98	light petroleum 90; benzene 10	0.155 g.	colourless solid	170-186°
99-103	do.	0.177 g.	do.	165-180°
104-107	do.	0.244 g.	do.	135-170°
108-109	do.	0.111 g.	do.	158-180°
110-111	do.	0.078 g.	do.	162-200°
112-115	do.	0.100 g.	do.	164-188°
116-120	do.	0.133 g.	do.	140-162°
121-123	do.	0.150 g.	do.	154-180°
124	do.	0.136 g.	do.	164-180°
125-128	do.	0.082 g.	do.	162-182°
129-132	do.	0.147 g.	do.	160-180°
133-134	do.	0.109 g.	do.	154-184°
135-136	do.	0.071 g.	do.	166-190°
137-138	do.	0.086 g.	do.	178-200°
139-141	do.	0.110 g.	do.	170-190°
142-144	do.	0.105 g.	do.	152-172°
145-148	do.	0.083 g.	do.	168-192°
149-150	do.	0.063 g.	do.	164-182°
151-152	do.	0.034 g.	do.	166-180°
153-156	do.	0.122 g.	do.	154-180°
157-158	do.	0.148 g.	do.	174-200°
159-162	do.	0.052 g.	do.	186-200°

		After Removal of Solvent		
Fraction	Eluant	Weight	Product	m.p.
163-166	light petroleum 90; benzene 10	0.068 g.	colourless solid	164-180°
167-170	do.	0.149 g.	do.	176-192°
171-174	do.	0.054 g.	do.	182-196°
175-177	do.	0.049 g.	do.	166-183°
178-181	do.	0.146 g.	do.	172-192°
182-185	light petrol 85; benzene 15	0.051 g.	do.	162-180°
186-189	do.	0.058 g.	do.	164-188°
190-192	do.	0.039 g.	do.	162-180°
193-198	do.	0.102 g.	do.	162-180°
199-202	do.	0.098 g.	do.	168-200°
203-205	light petrol 80; benzene 20	0.076 g.	do.	172-200°
206-208	light petrol 70; benzene 30	0.029 g.	do.	182-196°
209-213	light petrol 60; benzene 40	0.113 g.	do.	170-186°
214-220	light petrol 50; benzene 50	0.048 g.	non crystallisable gum.	
221-223	do.	0.063 g.	do.	
224-230	benzene	0.157 g.	do.	
231-234	benzene 90; ether 10	0.129 g.	colourless solid.	126-136°
235-239	benzene 98; ethanol 2	0.059 g.	do.	134-144°

Fraction	Eluant	After Removal of Solvent: Weight	Product	m.p.
240-245	benzene 96; ethanol 5	0.686 g.	colourless solid	138-148°
246	do.	1.157 g.	do.	136-148°
247	do.	2.596 g.	do.	134-146°
248	benzene 90; ethanol 10	0.361 g.	do.	132-142°
249-254	ethanol	0.00g.		

All the solid fractions gave a pink colour with the Liebermann-Burchard reagent.

The following mixed melting points between the fractions shown below were obtained :-

Fractions	m.p.
(70-74) and (83-85)	164-200°
(86-89) and (96-98)	166-186°
(99-103) and (110-111)	164-184°
(116-120) and (129-132)	152-160°
(133-134) and (145-148)	158-180°
(153-156) and (159-162)	158-180°
(70-74) and (159-162)	148-182° *
(104-107) and (129-132)	130-150° *
(112-115) and (137-138)	156-174° *

Fraction	m.p.
(108-109) and 124	154-178° *
124 and (137-138)	152-176° *
(175-177) and (182-185)	164-180°
(186-189) and (199-202)	162-184°
(137-138) and (145-148)	168-186°

* denotes m.p. depression.

Based on the m.p. and mixed m.p. the fractions were combined as follows :-

fractions 70-111 to form fraction A

fractions 137-200 to form fraction B

fractions 235-248 to form fraction C

Fraction A.

After repeated crystallization from acetone and ethanol the colourless needles had m.p. 181-200°.

Hydrolysis of fraction A: The fraction A (0.75 g.) was heated under reflux with ethanolic potassium hydroxide solution (4%; 40 c.c.) on a steambath for 2 hours. The solution was allowed to cool and poured into water (300 c.c.) and then extracted with ether (4 x 50 c.c.). The extract was washed with water (2 x 50 c.c.), dried (sodium sulphate), filtered and the ether was distilled off. The gummy residue (0.7 g.) was dried under vacuum.

Acetylation of fraction A: The gum obtained on hydrolysis

(0.7 g.) was treated with pyridine (9 c.c.) and acetic anhydride (15 c.c.) for 2 hours on a steambath. After cooling the solution was poured into dilute sulphuric acid (100 c.c.) and extracted with ether (4 x 30 c.c.). The extract was washed with dilute sodium hydroxide solution (40 c.c.) and water (50 c.c.). The ether layer was dried (sodium sulphate), filtered and the solvent was distilled off. The residue (0.7 g.) of fraction A acetates was crystallized from ethanol yielding prisms, m.p. 182-196°.

The acetates (0.7 g.) were dissolved in light petroleum (30 c.c.) and adsorbed on a column (52 x 1.5 cm.) of alumina and developed and eluted with light petroleum (600 c.c.), light petroleum-benzene mixture (75:25; 900 c.c.) and benzene-ethanol mixture (95:5; 600 c.c.). Three fractions were obtained. The first (0.476 g.) after repeated crystallization from chloroform-methanol yielded fine needles m.p. 195-197°; $[\alpha]_D^{18}$ + 80.1° (c = 1.695 in chloroform.) A mixed m.p. with authentic α-amyrin-acetate (m.p. 226°) gave m.p. 200-218°.

Found :- C, 81.61; H, 11.22%

Calc. for $C_{32}H_{52}O_2$:- C, 81.99; H, 11.11%

The second fraction (0.043 g.) was a non crystallisable gum and was discarded. The third fraction (0.201 g.) crystallized from ethanol in felted needles (m.p. 176-183°).

On acetylation by the same method as used above it yielded fine needles from chloroform-methanol, m.p. 196-197°, which showed no depression in a mixed m.p. with the first fraction.

Re-chromatography of fraction A acetates. The fraction A acetates (0.282 g.) were dissolved in light petroleum (20 c.c.) and adsorbed on a column (14 x 1 cm.) of alumina and developed and eluted with light petroleum (100 c.c.), and light petroleum-benzene mixture (300 c.c.). One fraction (0.278 g.), a colourless solid, was obtained. After repeated crystallization from ethanol and methanol-chloroform fine needles of a mixture of α - and β -amyrin acetate m.p. 198-200°, $[\alpha]_D^{16}$ + 78.6° (c = .686 in chloroform) were obtained. A mixed m.p. with a 1:1 mixture of authentic α - and β -amyrin-acetate (m.p. 195-200°) showed no depression.

Found :- C, 81.7; H, 11.2%

Calc. for $C_{32}H_{52}O_2$:- C, 81.99; H, 11.11%

It was not possible to separate the acetates by further crystallization from chloroform-methanol mixture.

Fraction B

Hydrolysis and acetylation : Fraction B was hydrolysed and acetylated by the method used for fraction A. The mixed fraction B acetates (0.7 g.; m.p. 186-195°) were dissolved in light petroleum (25 c.c.) and adsorbed on a column

(40 x 1.5 cm.) of alumina. The chromatogram was developed and eluted as shown in the table below. The volume of each fraction was 100 c.c.

			After Removal of Solvent	
Fraction	Eluant	Weight	Product	m.p.
1-5	light petroleum	0.0 g.		
6-10	light petroleum 75; benzene 25	0.0 g.		
11-14	light petrol 50; benzene 50	0.212 g.	colourless solid	198-200°
15-17	do.	0.062 g.	do.	197-199°
18-24	benzene	0.0 g.		
25-36	benzene 90; ether 10	0.0 g.		
37-42	benzene 99; ethanol 1	0.379 g.	colourless solid	178-195°
43-50	benzene 50; ethanol 50	0.0 g.		

Fractions 11-17 were combined and crystallized repeatedly from ethanol and methanol-chloroform mixture giving needles of lupeol-acetate, m.p. 214°, $[\alpha]_D^{18}$ + 47.5° (c = 0.689 in chloroform). Mixed melting points with authentic lupeol-acetate (m.p. 212-213°) and with a specimen of lupeol-acetate from fraction C (see below) showed no depression.

Found :- C , 81.69; H ,11.06%

Calc. for $C_{32}H_{52}O_2$:- C , 81.99; H, 11.11%

Fractions 37-42 were acetylated in the usual way with pyridine (4 c.c.) and acetic anhydride (10 c.c.) yielding needles of lupeol-acetate, m.p. 210-212°, $[\alpha]_D^{18}$ + 47.2° (c = .732 in chloroform) from methanol-chloroform mixture. A mixed m.p. with authentic lupeol-acetate showed no depression.

Found :- C, 81.5; H, 11.0%

Calc. for $C_{32}H_{52}O_2$:- C, 81.99; H, 11.1%

Fraction C

Hydrolysis and acetylation: Fraction C (4 g.) was hydrolysed and acetylated in the same way as fraction A. On crystallization from ethanol the acetates (4.1 g.) were obtained as needles m.p. 196-198°, $[\alpha]_D^{18}$ + 47.75° (c = 1.63 in chloroform).

Further crystallization did not increase the m.p. The acetates (3.4 g.) were dissolved in light petroleum (60 c.c.) and adsorbed on a column (20 x 2.5 cm.) of alumina. The column was developed and eluted as shown below. The volume of each fraction was 100 c.c.

Fraction	Eluant	After Removal of Solvent Weight	Product	m.p.
1-4	light petrol 75; benzene 25	0.0 g.		
5-12	do.	1.751 g.	colourless solid	175-180°
13-18	light petrol 50; benzene 50	0.599 g.	do.	182-196°
19-25	do.	0.0 g.		
26-36	benzene 99; ethanol 1	0.451 g.	needles crystallizing with difficulty from ethanol	
37-41	ethanol	0.0 g.		

Fractions 7-18 were combined and fractionally crystallized from ethanol until three crops of crystalline material were obtained.

Crop I. After repeated crystallization from chloroform-methanol mixture, β-amyrin-acetate (0.025 g.) m.p. 240°, $[\alpha]_D^{18}$ + 82.3° (c = 0.546 in chloroform), was obtained. A mixed m.p. with authentic β-amyrin-acetate showed no depression.

Found :- C, 81.97; H, 11.02%

Calc. for $C_{32}H_{52}O_2$:- C, 81.99; H, 11.11%

Crops II and III had m.p. 206-208° and 204-207° and

$[\alpha]_D^{18}$ + 45.9° (c = 0.71 in chloroform) and $[\alpha]_D^{18}$ + 46.7°, (c = 0.832 in chloroform) respectively. They were combined and after repeated crystallization from chloroform-methanol mixture yielded lupeol-acetate (0.75 g.) m.p. 213°, $[\alpha]_D^{18}$ + 46.3 (c = .639 in chloroform). A mixed m.p. with authentic lupeol-acetate showed no depression.

Found :- C , 81.83; H , 11.28%

Calc. for $C_{32}H_{52}O_2$:- C , 81.99; H, 11.11%

Hydrolysis of lupeol-acetate : The lupeol-acetate(0.75 g.) was heated under reflux with methanolic potassium hydroxide solution (5%; 70 c.c.) on a steambath for four hours and the solution was worked up in the usual way. The product (0.7 g.), lupeol, was obtained as a gummy solid.

Lupeol-benzoate : The lupeol (0.7 g.) obtained in the previous experiment was dissolved in pyridine (10 c.c.) and heated on a steambath with benzoyl-chloride (3 c.c.) for three hours. The solution was diluted with benzene (80 c.c.) and washed with dilute sulphuric acid (3 x 60 c.c.) and water (2 x 50 c.c.). The benzene solution was dried (sodium sulphate), filtered and the solvent was distilled off under vacuum. The residue (0.7 g.) on repeated crystallization from ethanol-benzene mixture yielded lupeol-benzoate, m.p. 262°, $[\alpha]_D^{18}$ + 62.9° (c = .636 in chloroform) as shiny plates. A mixed m.p. with authentic lupeol benzoate

showed no depression.

Found :- C , 83.47; H , 10.32%

Calc. for $C_{37}H_{54}O_2$:- C , 83.73; H , 10.25%

Fractions 26-36 These fractions were acetylated by the method of acetylation used above (Fraction A). The product after several crystallizations from chloroform-methanol mixture gave lupeol-acetate, m.p. 210°, $[\alpha]_D^{18}$ + 46.8° (c = 0.790 in chloroform). A mixed m.p. with authentic lupeol-acetate showed no depression.

Alstonia verticillosa : Fraction II

Hydrolysis : Fraction II (20 g.) was dissolved in boiling benzene (120 c.c.) and heated under reflux on a steambath for 20 hours with methanolic potassium hydroxide solution (5%; 700 c.c.). The solution was concentrated until solid began to appear and was then poured into water (1600 c.c.). The mixture was extracted with ether (5 x 200 c.c.) and the ethereal layer was washed with water (200 c.c.), dried, (sodium sulphate), filtered and finally the solvent was distilled off leaving a colourless amorphous mass (20 g.)

Benzoylation of the hydrolysed product :- The dried solid (20 g.) was dissolved in pyridine (40 c.c.) and the solution was heated on a steambath for 6 hours with benzoyl-chloride (18 c.c.). The dark red solution was diluted with benzene (300 c.c.) and washed with dilute hydrochloric acid (2 x 200 c.c.)

dilute sodium-hydroxide solution (2 x 200 c.c.) and finally with 5% brine solution (2 x 200 c.c.). The benzene layer was dried (sodium sulphate), filtered and the solvent was distilled off under vacuum, leaving a light-brown crystalline solid (23 g.) which was dissolved in light petroleum (700 c.c.) and adsorbed on a column (35 x 5 cm.) of alumina and developed and eluted as shown below. The volume of each fraction was 200 c.c.

		After Removal of Solvent		
Fraction	Eluant	Weight.	Product	m.p.
1-8	light petroleum	0.0 g.		
9-10	light petrol 90; benzene 10	0.565 g.	solid crystallizing from ethanol-benzene	192-194°
11-14	do.	0.549 g.	do.	192-195°
15-16	do.	4.001 g.	do.	191-195°
17	do.	2.860 g.	do.	192-196°
18	do.	1.951 g.	do.	212-222°
19	do.	1.081 g.	do.	232-238°
20-21	light petrol 80; benzene 20	.812 g.	do.	236-244°
22-24	do.	1.632 g.	do.	232-236°
25	do.	1.472 g.	solid crystallizing from acetone-benzene	232-238°

Fraction	Eluant	After Removal of Solvent: Weight	Product	m.p.
26-28	light petrol 80; benzene 20	1.047 g.	solid crystallized from acetone-benzene	239-250°
29-32	do.	.795 g.	do.	246-252°
33-38	do.	.712 g.	do.	236-244°
39-43	do.	.607 g.	do.	236-244°
44-56	light petrol 70; benzene 30	.539 g.	do.	244-250°
57-77	do.	.810 g.	do.	236-248°
78-85	light petrol 70; benzene 29, ethanol 1.	1.669 g.	yellow gum	
86-92	do.	.476	do.	
93-97	benzene 50; ethanol 50.	.135	do.	

All the fractions gave a pink colour with the Liebermann-Burchard reagent.

Based on the melting points the fractions were combined as follows after one crystallization from an acetone-benzene mixture :-

Fractions from Chromatogram	Combined to form Fraction	Weight	m.p. °C	$[\alpha]_D^{18}$ in chloroform
9-17	D	5.9 g.	190-192°	+84.7°(c=2.52)
mother liquors of 9-17	E	0.190 g.	170-175°	-
18-21	F	1.64 g.	232-236°	+68.6°(c=1.175)
22-25	G	1.49 g.	248-254°	+63.8°(c=1.153)
26-77	H	1.12 g.	248-256°	+64.9°(c=1.020)
78-97	I	2.01 g.	-	-

Fraction D

Fractional crystallization from ethanol-benzene mixture gave five crops of material :

Crop	I	II	III	IV	V
Weight	0.2 g.	0.302 g.	3.84 g.	0.215 g.	0.320 g.
m.p.	262-263°	198°	196°	196-197°	197°
$[\alpha]_D^{18}$ in chloroform.	+64.2° c= .876	+93.6° c=1.440	+94.4° c=1.228	+94.1° c=1.04	+91.9° c= .664

Crop I The lupeol-benzoate crystallized in colourless plates and a mixed m.p. with authentic lupeol-benzoate (m.p. 263°) showed no depression.

Crop II The material was further crystallized combined with the crystals from the mother liquors of fraction G (see below) from ethanol-benzene mixture yielding sturdy prisms of α-amyrin-benzoate, m.p. 198° $[\alpha]_D^{18}$ + 93.8°, (c = 1.325 in chloroform. A mixed m.p. with authentic α-amyrin-benzoate (m.p. 197°) showed no depression.

Found : C , 83.8; H , 10.6%

Calc. for $C_{37}H_{54}O_2$:- C , 83.73; H , 10.25%

Crop III The microcrystalline solid was shaken vigorously with ether (10 c.c.) for 3 minutes. Only very little material remained undissolved (0.2 g.). The suspension was filtered and the ether was distilled off. After repeated crystallization from ethanol-benzene mixture, the ether soluble fraction afforded large prisms of α-amyrin-benzoate m.p. 198°, $[\alpha]_D^{18}$ + 92.4° (c = 2.37 in chloroform). A mixed m.p. with an authentic specimen of α-amyrin-benzoate showed no depression.

Found :- C ,83.42; H ,10.30%

Calc. for $C_{37}H_{54}O_2$:- C , 83.73; H ,10.25%

The ether insoluble fraction after three crystallizations from ethanol-benzene mixture also yielded prisms of α-amyrin-benzoate, m.p. 198°, $[\alpha]_D^{18}$ + 94.6° (c = 1.46 in chloroform). A mixed m.p. with the α-amyrin-benzoate from the ether-soluble fraction showed no depression.

Crops IV and V: Both these crops after further crystallization from ethanol-benzene yielded α-amyrin-benzoate, m.p. 198° and 197°, $[\alpha]_D^{18}$ + 92.8° (c = 1.362 in chloroform) and + 93.8° (c = 1.273 in chloroform) respectively.

Hydrolysis of α-amyrin-benzoate: The α-amyrin-benzoate (1.9 g.) was hydrolysed by the method described for the hydrolysis of the crude fraction II of Alstonia (see above) using benzene (15 c.c.) and 5% methanolic potassium-hydroxide solution (100 c.c.). This afforded crude α-amyrin (1.4 g.) which was not purified.

Acetylation of α-amyrin : The crude α-amyrin (1.4 g.) was acetylated by the method used for fraction A (see above p. 81) using pyridine (10 c.c.) and acetic anhydride (15 c.c.). The product after repeated crystallization from methanol-chloroform mixture yielded α-amyrin-acetate, m.p. 225° $[\alpha]_D^{18}$ + 74.9° (c = .82 in chloroform). A mixed m.p. with an authentic specimen of α-amyrin-acetate, (m.p. 226°) showed no depression.

Found :- C , 82.13; H, 10.96%

Calc. for $C_{32}H_{52}O_2$:- C , 81.99; H ,11.11%

Fraction E

The solid after crystallization from ethanol benzene mixture afforded α-amyrin-benzoate (0.05 g.), m.p. 197°, $[\alpha]_D^{18}$ + 93.1° (c = 1.621 in chloroform). A mixed m.p. with

an authentic specimen of α-amyrin-benzoate showed no depression.

Fraction F

The material, after fractional crystallization from acetone-benzene yielded shiny platelets of lupeol-benzoate m.p. 262-263°, $[\alpha]_D^{18}$ + 63.5° (c = .944 in chloroform). A mixed m.p. with an authentic specimen of lupeol-benzoate showed no depression.

Found :- C, 83.99; H, 10.36%

Calc. for $C_{37}H_{54}O_2$:- C, 83.73; H, 10.25%

Fractions G and H

The fractions were combined and fractionally crystallized from acetone-benzene mixture. The top crops yielded plates of lupeol-benzoate, m.p. 262°, $[\alpha]_D^{18}$ + 63° (c = 1.365 in chloroform). A mixed m.p. with authentic lupeol-benzoate showed no depression.

Found :- C, 83.42; H, 9.86%

Calc. for $C_{37}H_{54}O_2$:- C, 83.73; H, 10.25%

The mother liquors from the crystallization gave large prisms of α-amyrin-benzoate (0.2 g.), m.p. 198°, $[\alpha]_D^{18}$ + 93.0° (c = 1.794 in chloroform) which were worked up with the α-amyrin-benzoate of crop II, fraction D (see above p. 91).

Hydrolysis of lupeol-benzoate and acetylation of lupeol :

The lupeol-benzoate of fractions F,G and H (0.9 g.) was

hydrolysed and then acetylated by the method used for α-amyrin-benzoate (see above p. 93). The product was repeatedly crystallized from methanol-chloroform mixture affording lupeol acetate, m.p. 217-218°, $[\alpha]_D^{18}$ + 46.8°, (c = .876 in chloroform). A mixed m.p. with an authentic specimen of lupeol-acetate showed no depression.

Found :- C ,81.87; H ,11.08%

Calc. for $C_{32}H_{52}O_2$:- C ,81.99; H ,11.11%

Fraction I

The material crystallized only with difficulty from ethanol in long needles. It was considered to be a mixture of triterpene alcohols and was not further examined.

Alstonia verticillosa fraction III

Hydrolysis and benzoylation : The colourless powder (2.75 g.) was saponified and benzoylated in the same manner as fraction II of Alstonia yielding the mixed benzoates (3 g.) as yellow-brown solid. The material was dissolved in light petroleum (100 c.c.) and adsorbed on a column (22.5 x 3 cm.) of alumina and developed and eluted as shown in the table below. The volume of each fraction was 100 c.c.

		After Removal of Solvent		
Fraction	Eluant	Weight	Product	m.p.
1-9	light petroleum	0.0 g.		
10	light petroleum 95; benzene 5	.279 g.	solid crystallized from ethanol-benzene	182-190°
11-14	do.	.526 g.	do.	186-194°
15-18	do.	.455 g.	do.	190-198°
19-24	do.	.384 g.	do.	188-196°
25-32	do.	.322 g.	do.	220-230°
33-37	do.	.269 g.	do.	222-231°
38-55	benzene	.211 g.	yellow gum	
56-61	benzene 50 ethanol 50	0.0 g.		

Based on the melting points the fractions were combined to form new fractions as follows :-

Fractions 10-14 to form fraction K (0.516 g.)

Fractions 15-24 to form fraction L (0.621 g.)

Fractions 25-37 to form fraction M (0.402 g.)

Fraction K

After fractional crystallization from ethanol-benzene the material was separated into two crops :-

Crop	I	II
Weight	0.226 g.	0.140 g.
m.p.	229°	190-192°
$[\alpha]_D^{18}$ in chloroform	+ 93.1° (c=1.573)	+96° (c= .928)

Crop I after further crystallization from ethanol-benzene afforded plates of β -amyrin-benzoate, m.p. 232°, $[\alpha]_D^{18}$ + 97.7°, (c = 1.305 in chloroform). A mixed m.p. with authentic β -amyrin-benzoate showed no depression.

Found :- C , 83.86; H , 10.38%

Calc. for $C_{37}H_{54}O_2$:- C , 83.73; H, 10.3%

Crop II on crystallization yielded prisms of α -amyrin-benzoate m.p. 198°, $[\alpha]_D^{18}$ + 94.2 (c = 1.286 in chloroform). A mixed m.p. with authentic α -amyrin-benzoate showed no depression.

Fraction L

Fractional crystallization from ethanol-benzene mixture resulted in three crops of crystals as follows :

Crop	I	II	III
Weight	0.460 g.	0.040 g.	0.02 g.
m.p.	227-229°	190-192°	192-194°
$[\alpha]_D^{18}$ in chloroform	+ 91.2° (c= .505)	+ 93.8° (c= .905)	+ 92.9° (c=1.103)

Crop I. The solid after two more crystallizations from ethanol-benzene afforded platelets of β-amyrin-benzoate, m.p. 232-233°, $[\alpha]_D^{18}$ + 98.3° (c= 1.442 in chloroform). A mixed m.p. with an authentic specimen of β-amyrin-benzoate showed no depression.

Found :- C , 83.74; H , 10.4%

Calc. for $C_{37}H_{54}O_2$:- C , 83.73; H , 10.3%

Crops II and II, were combined and crystallized from ethanol-benzene mixture affording prisms of α-amyrin-benzoate, m.p. 197° $[\alpha]_D^{18}$ + 94.3° (c = .984 in chloroform). A mixed m.p. with an authentic specimen of α-amyrin-benzoate showed no depression.

Fraction M

Three crops of material were obtained on fractionation from ethanol benzene mixture.

Crop	I	II	III
Weight	0.213 g.	0.051 g.	0.027 g.
m.p.	258-259°	255-256°	255-257°
$[\alpha]_D^{18}$ in chloroform	+ 62.1° (c=1.03)	+ 63.7° (c= .585)	+ 63.1° (c=1.131)

In view of the similarity of properties the crops were combined and re-crystallized from ethanol-benzene yielding colourless plates of lupeol-benzoate, m.p. 262°

$[\alpha]_D^{18}$ + 63.2° (c = .806 in chloroform). A mixed m.p. with authentic lupeol-benzoate showed no depression.

Found :- C, 83.70; H, 10.7%

Calc. for $C_{37}H_{54}O_2$:- C, 83.73; H, 10.3%

Part II

Isolation of β-Amyrin Benzoate from Manila Elemi Resin

The solid Manila Elemi resin (800 g.), which had been steam-distilled to remove volatile oils, was dissolved in ether (2500 c.c.) and washed successively with sodium hydroxide solution (10%; 2 x 750 c.c.), hydrochloric acid (10%; 750 c.c.) and water (2 x 750 c.c.). The ethereal solution was dried (sodium sulphate), filtered and the solvent was removed. The light coloured gummy residue was then shaken with cold ethanol (400 c.c.) for thirty minutes and filtered. The residue was again treated with ethanol in the same way giving a crude mixture of α- and β -amyrin as a colourless solid (200 g.).

The crude mixed amyrins (200 g.) were dissolved in pyridine (130 c.c.) and benzoyl chloride (140 c.c.) was added dropwise to the stirred solution at 100°C over a period of 90 minutes. After the addition the reaction mixture was heated with stirring for a further six hours. The dark reddish-brown mixture was allowed to cool and diluted with benzene (625 c.c.). The solution was washed with hydrochloric acid (10%; 2 x 400 c.c.), then with sodium hydroxide solution (10%; 400 c.c.) and finally with brine solution (4%; 2 x 400 c.c.). The benzene

solution was dried (sodium sulphate), filtered and concentrated under vacuum to about 300 c.c. Boiling ethanol was added to the solution until slightly turbid, and on cooling mixed α - and β -amyrin benzoates separated as a crystalline mass, which was filtered, washed with cold ethanol and dried at 100°.

The crude mixed benzoates (130 g.) were then placed into a wide necked stoppered bottle fitted with a tap for the relief of pressure, and shaken with ether (140 c.c.) for 10 minutes. The undissolved solid was collected. Since the mixture melted over a considerable range, the final clearing point was determined. The process was then repeated a further four times after which the clearing point was above 210°. The residue after repeated crystallization from an acetone-benzene mixture yielded pure β -amyrin benzoate as small colourless plates, m.p. 233°, $[\alpha]_D^{18}$ + 98.4° (c = 1.04 in chloroform), 30 g.

β -Amyrin (β -amyrenol):- β -Amyrin benzoate (42 g.) was dissolved in benzene (300 c.c.) and a solution of potassium hydroxide (50 g.) in aqueous ethanol (water 50 c.c. and ethanol distilled over potassium hydroxide 1100 c.c.) was added. The mixture was refluxed for 18 hours and concentrated until solid began to appear. The solution was then poured into water (1600 c.c.) and the solid which

precipitated was collected, washed with water and dried at 100° giving β-amyrin, m.p. 195° (34 g.)

β-Amyrin-acetate :- β-Amyrin (30 g.) was dissolved in pyridine (90 c.c.) and was heated with acetic anhydride (145 c.c.) on a steambath for two hours. The solution was allowed to cool and crystalline solid which separated was filtered off and washed with a little glacial acetic acid. Crystallization from a chloroform-methanol mixture yielded needles of β-amyrin-acetate, m.p. 240°, $[\alpha]_D^{18}$ + 80.6° (c = 1.14 in chloroform). Yield 30 g.

β-Amyranonyl-acetate :- (cf. Spring, 17) β-Amyrin-acetate (15 g.) was dissolved in glacial acetic acid (1250 c.c.) with vigorous stirring on a steambath at 100° and a solution of hydrogen peroxide (100 vols.; 80 c.c.) in glacial acetic acid (80 c.c.) was added dropwise during 30 minutes. Stirring was continued at 100° for a further two hours when a solution of hydrogen peroxide (100 vols.; 40 c.c.) in glacial acetic acid (40 c.c.) was added dropwise during 15 minutes. After stirring for a further hour water was added to the hot solution until a faint opalescence appeared. The solution was allowed to stand overnight after which the product was filtered and washed with a little methanol. Crystallization from a chloroform-methanol mixture afforded colourless plates of β-amyranonyl-acetate

m.p. 192-193° (7 g.)

β-Amyranol:- (cf. Ruzicka and Jeger, 18). β-Amyranonyl-acetate (13 g.) and sodium (13 g.) dissolved in n-amyl alcohol (260 c.c.), were heated for 16 hours with anhydrous hydrazine (40 c.c.; Org. Synth., 1944, 24, 53), at 180° in an autoclave. The mixture was allowed to cool and poured into water (1500 c.c.), acidified with dilute hydrochloric acid and extracted with ether (4 x 150 c.c.). The ether extract was washed with water (200 c.c.), dried (sodium sulphate) and filtered. The ether and amyl alcohol were removed by vacuum distillation, leaving a dark gum. This was dissolved in benzene (20 c.c.), decolourised (charcoal) and methanol (20 c.c.) was added to the boiling solution but the product did not crystallise.

The solvents were removed under vacuum and the residue was treated with pyridine (30 c.c.) and acetic anhydride (45 c.c.) on a steambath for two hours. The solution was allowed to cool, diluted with benzene (150 c.c.) and washed with hydrochloric acid (10%; 2 x 100 c.c.), water (100 c.c.) and dried (sodium sulphate), filtered and finally the benzene was removed. The acetylated gums (12 g.) were dissolved in light petroleum (200 c.c.) and filtered through a column (15 x 2.5 cm.) of alumina. The chromatogram was eluted with benzene (300 c.c.). The

eluant was distilled off yielding a gum. On crystallization from a chloroform-methanol mixture β-amyranyl-acetate crystallized in small colourless plates, m.p. 182° (5 g.)

The β-amyranyl-acetate was saponified by dissolving it in benzene (15 c.c.) and heating it under reflux with 5% ethanolic potassium hydroxide solution (150 c.c.) for 3 hours. The cooled solution was poured into water (250 c.c.) and extracted with ether (3 x 60 c.c.). The extract was dried (sodium sulphate) filtered and the solvent was removed. The residue was crystallized from methanol yielding β-amyranol, m.p. 186-187°, $[\alpha]_D^{18}$ + 17.9° (c = 1.34 in chloroform). The substance was sublimed for analysis at 160°/1 x 10^{-4} mm.

Found :- C, 83.76; H, 12.03%

Calc. for $C_{30}H_{52}O$:- C, 84.04; H, 12.23%

β-Amyrene VI :- Phosphorus pentachloride (1.8 g.) was covered with dry light petroleum (b.p. 40-60°; 65 c.c.) and β-amyranol (2.3 g.) was added to the mixture in small portions during 4 hours. The β-amyranol dissolved slowly with the evolution of hydrogen-chloride gas. When the addition was complete the mixture was refluxed gently for 30 minutes and only a very small amount of phosphorus pentachloride remained undissolved. The solution was

allowed to stand overnight and was then extracted with water (10 x 50 c.c.), dried (sodium sulphate), filtered and the solvent was removed. The residue, a yellow gum, was dissolved in benzene (50 c.c.) and filtered through a column (12 x 2 cm.) of alumina. The product, after removal of the solvent, was crystallized from ethyl-acetate yielding β-amyrene VI m.p. 169-170°, $[\alpha]_D^{18}$ + 69.3° (c = .695 in chloroform), as needles. Yield (0.380 g.). The substance gave a yellow colour with tetranitromethane in chloroform.

Found :- C, 87.93; H, 12.03%

$C_{30}H_{50}$ requires :- C, 87.80; H, 12.20%

β-Amyran II:- β-Amyrene VI (0.250 g.) was dissolved in a mixture of pure ethyl acetate (20 c.c.) and stabilised acetic acid (10 c.c.) and the solution was added to a suspension of prereduced platinium oxide catalyst (0.1 g.) in glacial acetic acid (10 c.c.). The solution was shaken with hydrogen for four hours at room temperature and atmospheric pressure. The addition of fresh catalyst did not induce a further uptake of hydrogen. 14.6 c.c. Of hydrogen (corrected to N.T.P.) were absorbed. The volume of hydrogen required to saturate one double bond was 13.6 c.c. (at N.T.P.).

The catalyst was filtered off and the solvents were distilled off under vacuum leaving a colourless gum. This

was dissolved in benzene 20 c.c. and filtered through a column (5 x 1 cm.) of alumina. The column was washed with benzene (50 c.c.) and the combined benzene solutions were evaporated. The residue was crystallized repeatedly from a chloroform-methanol mixture which gave small needles of β-amyran II m.p. 110°, $[\alpha]_D^{28}$ + 17.1 (c = .690 in chloroform). The substance gave no colour with tetranitormethane in chloroform.

Found :- C, 86.93; H, 12.50%

$C_{30}H_{52}$ requires C, 87.29; H, 12.71%

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