Tetrahedron, 1965, Vol. 21, pp. 3197 to 3203. Pergamon Press Ltd. Printed in Northern Ireland

TERPENOIDS—LXXI*

CONSTITUENTS OF INDIAN BLACK DAMMAR RESIN

V. K. HINGE, A. D. WAGH, S. K. PAKNIKAR and S. C. BHATTACHARYYA
National Chemical Laboratory, Poona

(*Received* 13 *May* 1965)

Abstract—Some of the components of the Indian black dammar resin obtained from the tree *Canarium strictum* Roxb., have been isolated mainly by employing solvent partition and column chromatography. The petroleum ether extract of the methanol-soluble portion contains (+) junenol (III), a new monoethynoid sesquiterpene ketone canarone(IV), a liquid sesquiterpene alcohol *epi*-khusinol (V), α-amyrin(Ia), β-amyrin(IIa), β-amyrin acetate, ψ-taraxasterol (VIa), its related newly isolated diol ψ-epitaraxastane diol (VIIIa) and the new ketol (VII), along with another new triterpene diol very closely resembling, 11-β-hydroxy-α-amyrin (IXa). Petroleum ether extract of the methanol insoluble portion was found to contain α-amyrin, β-amyrin and 11-keto-α-amyrin (Xa).

BLACK DAMMAR[1] is a commercial name given to the resin which exudes from the plant *Canarium strictum* Roxb., a large tree found abundantly in the evergreen forests of South India. Although the timber is soft and of inferior quality, the black nodular lumps of the resin find many uses.[2,3]

Some work has been previously carried out on the "resin oil" obtained by destructive distillation of the resin.[4–6] During the present investigation some of the constituents of the parent resin have been isolated mainly by employing solvent partition and column chromatography over neutral alumina and the results reported in this communication.

The powdered resin was used for the determination of acid value, volatile oil content and solubility in various solvents. The acid value and essential oil contents were low. The resin is completely soluble in chloroform and carbon tetrachloride and partly soluble in other solvents. The details have been described in the experimental.

The coarsely powered resin was first extracted with acetone. The soluble portion recovered from acetone was re-extracted with ethanol. The ethanol extract was treated with methanol and separated into soluble and insoluble parts. The methanol-soluble part and the methanol-insoluble part were extracted separately with pet. ether and partitioned into corresponding soluble and insoluble portions. The components present in both the pet. ether extracts (A and B) were further resolved by chromatography over neutral alumina. The general scheme of solvent partition and chromatographic separation is given in the chart.

* Communication No. 780 from the National Chemical Laboratory, Poona-8, India.

[1] S. Dutt, *Indian Oil and Soap Journal* **26**, 123 (1960).
[2] H. Trotter, *Manual of Indian Forest Utilisation* p. 64. Oxford Univ. Press (1940).
[3] K. R. Kirtikar and B. D. Basu, *Indian Medicinal Plants, Lalit Mohan Basu, Allahabad* **1**, 531 (1903).
[4] K. L. Moudgill, *J. Soc. Chem. Ind.* (Trans), **44**, 169T (1925).
[5] R. C. Vasisth and S. M. Muthana, *J. Sci. Industr. Res. India*, **14B**, 632 (1955).
[6] R. C. Vasisth and M. S. Muthana, *J. Sci. Industr. Res. India*, **15B**, 25 (1956).

The substantial amount of solid material obtained together with fraction A_1, was found to contain two triterpene alcohols. These were separated by chromatography and identified as α-amyrin (Ia)[7] and β-amyrin (IIa)[8] from their physical properties, acetate and benzoate derivatives and comparative IR spectra.

The liquid component of fraction A_1 on extensive chromatography afforded in the pet. ether eluted fractions, together with sesquiterpenoids, a crystalline triterpene ester which after repeated crystallization from ethyl acetate has been identified as β-amyrin acetate (IIb).

The yellow viscous benzene eluted fractions of the above chromatography was distilled under vacuum and the distillate after elaborate column chromatography yielded in small amount, a new monoethynoid sesquiterpene ketone canarone (IV), followed by a crystalline substance. The IR spectrum of the latter showed the presence of —OH group (3500 cm^{-1}) and a methylenic double bond $>C=CH_2$ (1645 and 885 cm^{-1}). It was purified by crystallization from pet. ether and has been identified as the rare alcohol (+) junenol (III) from its physical properties, mixed m.p. with an authentic sample and comparative IR spectra. This alcohol and its optical antipode (−) junenol have been previously isolated from juniper berry oil and Indian vetiver oil by Sorm *et al.*[9] and Bhattacharyya *et al.*[10] respectively.

Canarone, $C_{15}H_{24}O$, $(\alpha)_D$ +34·25°, a new sesquiterpene ketone, in its IR spectrum showed bands at 1700 cm^{-1} characteristic of 2,2-dialkyl cyclohexanone and 1420 cm^{-1} due to —CO—CH_2— grouping. Bands at 3080, 1640 and 890 cm^{-1} indicated the presence of terminal methylene group. UV spectrum showed no characteristic absorption for α,β-unsaturated ketone. Canarone furnished a semicarbazone, m.p. 222–224°. On the basis of chemical degradations, IR, UV and NMR spectral studies and optical rotatory dispersion measurements* the absolute configuration of canarone† can be represented by the stereoformula (IV).

The tail benzene eluted fraction of the said chromatography after distillation under vacuum yielded a liquid component which on careful chromatography gave in small amount a sesquiterpene alcohol, $C_{15}H_{24}O$, b.p. 125° (bath)/0·5 mm, $(\alpha)_D$ −86·8°. It furnished a crystalline 3,5-dinitrobenzoate, $C_{22}H_{28}O_6N_2$, m.p. 186–187°. On the basis of the experimental evidences and its structural similarity with khusinol,[11] the liquid alcohol is considered to be epi-khusinol, being epimeric at C_3, and is represented by the stereoformula (V). This however could not be rigorously established due to paucity of material.

The chromatographic sub-fractions obtained from A_2 on further chromatography gave (i) ψ-taraxasterol (VIa),[12] (ii) possibly 11-β-hydroxy-α-amyrin (IXa), (iii) a new diol ψ-epi-taraxastanediol and a new ketol. The compounds IXa and the new diol were conveniently isolated as their acetates. The new ketol on reduction with LAH

* We are grateful to Prof. W. Klyne for the ORD measurements.

† Initially the location of the carbonyl group in canarone was assumed to be at C_1, but subsequent verification showed that it is located at C_2 and not at C_1 (*J. Org. Chem.* No. 8, **29**, 2479, 1964).

[7] J. L. Simonsen and W. C. J. Ross, *The Terpenes* Vol. IV, 116 (1957).

[8] D. C. Hibbit and R. P. Lenstead, *J. Chem. Soc.* 470 (1936).

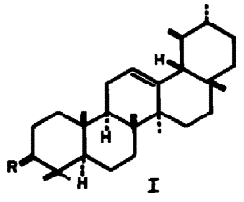
[9] V. Herout, O. Motl and F. Sorm, *Coll. Czech. Chem. Comm.* **19**, 990 (1954); **22**, 785 (1957).

[10] A. M. Shaligram, A. S. Rao and S. C. Bhattacahryya, *Tetrahedron* **18**, 969 (1962).

[11] A. A. Rao, K. L. Surve, K. K. Chakravarti and S. C. Bhattacharyya, *Tetrahedron* **19**, 233 (1963).

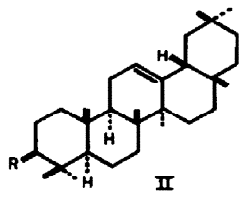
[12] J. L. Simonsen and W. C. J. Ross, *The Terpenes* Vol. IV; p. 162. (1957); G. Lardelli, Hs. K. Krusi, O. Jeger and L. Ruzicka, *Helv. Chim. Acta* **31**, 1159 (1948).

gave the new diol mentioned above. From the chemical and mass spectral data* these compounds were found to be derivatives of ψ-taraxasterol (VIa) and are represented by the structures* VII and VIIIa respectively. The compound VIa was isolated as the acetate and its identity was established by comparison of its physical properties, UV and IR spectra with an authentic sample. The compound IXa, also isolated as its monoacetate, had m.p. 187–188°, $(\alpha)_D$ +13·3°. Elemental analysis of the monoacetate suggested the formula, $C_{32}H_{52}O_3$. Catalytic hydrogenation using Adams' catalyst afforded α-amyrin acetate (Ib). Taking into consideration all these facts mentioned



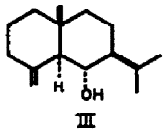
I

(I a, R=OH)
(I b, R=OAc)

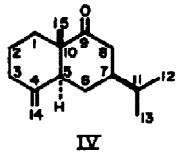


II

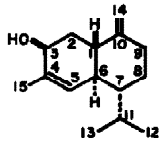
(II a, R=OH)
(II b, R=OAc)



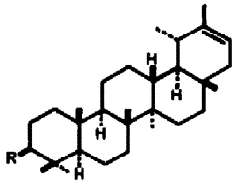
III



IV

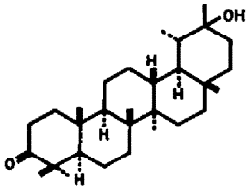


V

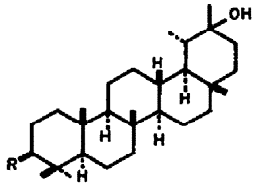


VI

(VI a, R=OH)
(VI b, R=OAc)

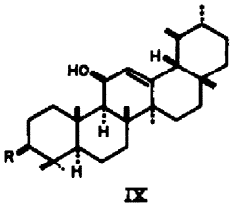


VII



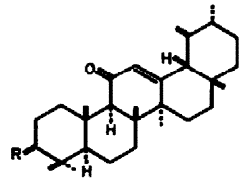
VIII

(VIII a, R=OH)
(VIII b, R=OAc)



IX

(IX a, R=OH)
(IX b, R=OAc)



X

(X a, R=OH)
(X b, R=OAc)

* The structural details and mass spectral data will be published in a separate communication. For the mass spectral data we are indebted to Dr. A. K. Bose and collaborators of the Stevens Institute of Technology, U.S.A.

above and other chemical data collected so far, this diol appeared to be 11-β-hydroxy α-amyrin* (IXa).

From the fraction B_2, two triterpene alcohols have been separated and characterized as α-amyrin (Ia) and β-amyrin (IIa).

The acetate of the fraction B_3, on chromatography afforded in small amount a pure crystalline substance, m.p. 275–276°, $(\alpha)_D$ +107°. Its UV spectrum gave absorption maximum at 247 mμ (log ϵ, 4·11). From its comparable IR spectra and physical properties with an authentic sample, it has been identified as 11-keto-α-amyrin acetate (Xb). The occurrance of 11-keto-α-amyrin (Xa) in this resin is worth noting, as this is only for the second time that its occurrence as a natural product is reported.[13] 11-keto-α-amyrin acetate on catalytic hydrogenation afforded α-amyrin acetate (Ib).

EXPERIMENTAL

All the m.ps are uncorrected. Specific rotations were determined in $CHCl_3$ solution unless otherwise stated. IR spectra were taken on a Perkin–Elmer (Model 137b) Infracord spectrophotometer and UV absorption spectra were measured in EtOH with a Beckman Model DK-2 spectrophotometer by Mr. Gopinath and Mr. Deshpande. Microanalyses were carried out by Mr. Pansare and colleagues.

Properties of the resin. The powdered resin was used for determining the properties; m.p. 120–125°, $(\alpha)_D$ +2·07° (*c*, 10·58, in CCl_4), the acid value was 8·7 and the volatile oil content 0·7%.

Solubility(%) in different solvents. The solubility of the resin in various solvents was determined by taking approx. 10 g samples of powdered resin in 100 ml of the solvent. The mixture was shaken thoroughly in a well-stoppered bottle for a period of 2 hr at the room temp of about 32°. The solubility data are as follows: MeOH-22, EtOH-27, acetic acid-38, acetone-41, ethyl acetate-50, dioxan-65, ether-80, pet. ether (60–80°)-84, benzene-90, pet.ether (40–60°)-97, CCl_4-100 and $CHCl_3$-100.

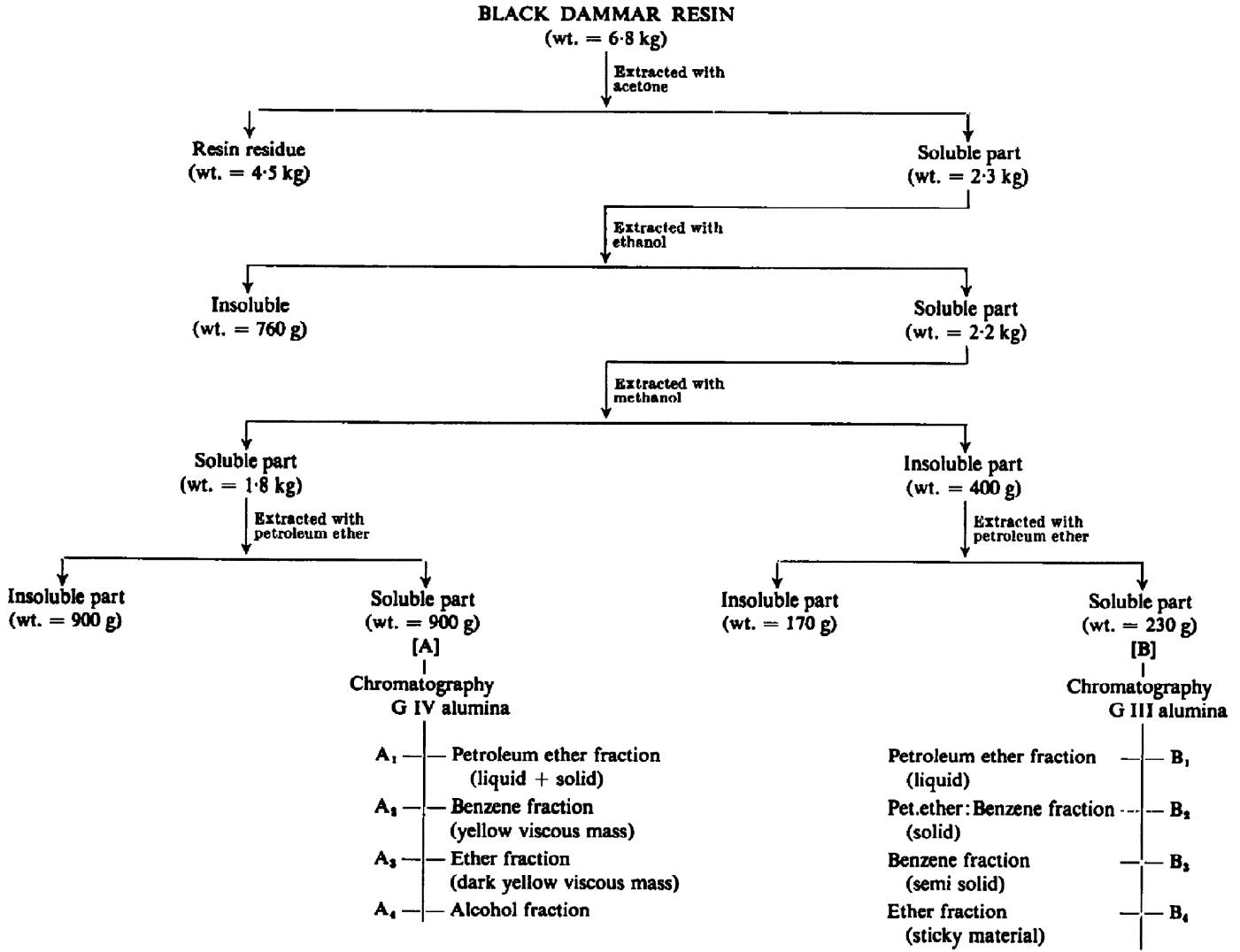
Isolation of α-amyrin (Ia). From the total solid material obtained together with fraction A_1, a portion (5 g) was chromatographed on neutral alumina (gr. III, 1:30). The first two fractions eluted with benzene gave α-amyrin (2 g) which was repeatedly crystallized from MeOH, m.p. 182–184°, $(\alpha)_D$ +91·2° (*c*, 1·87). (Found: C, 84·10; H, 11·36. $C_{30}H_{50}O$ requires: C, 84·44; H, 11·81%.) IR spectrum was identical with that of α-amyrin. The acetate prepared in the usual way by treating with pyridine–acetic anhydride at room temp for 24 hr was crystallized from ethyl acetate and had the following properties; m.p. 223–224°, $(\alpha)_D$ +82·2° (*c*, 2·25). (Found: C, 82·00; H, 11·00. $C_{32}H_{52}O_2$ requires: C, 81·99; H, 11·18%.) The benzoate derivative (benzoyl chloride–pyridine at room temp for 24 hr) was crystallized from a mixture of benzene and EtOH and had m.p. 192–194°, $(\alpha)_D$ +94°. (Found: C, 83·7; H, 10·4. $C_{37}H_{54}O_2$ requires: C, 83·72; H, 10·25%.) IR spectra of acetate and benzoate also confirmed the identity of α-amyrin.

Isolation of β-amyrin (IIa). The last benzene eluted fraction of the above chromatography after purification by repeated crystallization from MeOH gave β-amyrin (0·3 g), m.p. and mixed m.p. 197–198°, $(\alpha)_D$ +89° (*c*, 0·50). (Found: C, 84·16; H, 11·96. $C_{30}H_{50}O$ requires: C, 84·44; H, 11·81%.) The acetate derivative had m.p. and mixed m.p. 238–239°. The benzoate derivate had m.p. and mixed m.p. 232–233°. (Found: C, 84·30; H, 10·50. $C_{37}H_{54}O_2$ requires: C, 83·72; H, 10·25%.) IR spectra of β-amyrin, its acetate and benzoate derivatives confirmed the identity of β-amyrin.

Isolation of β-amyrin acetate (IIb). The liquid component of the fraction A_1 on chromatography (grade III, 1:30) gave some lower terpenic hydrocarbons followed by a solid material in the pet. ether eluted fractions. It was rechromatographed on grade III alumina and purified by crystallization

* In view of a recent publication on 11-hydroxy-α-amyrins (*Gazz. Chim. Itali*, 1378 (1964) further investigation will be required to establish the identity of our compound.

[13] Kazuyoshi Yagishita and Masanu Nashimura, *Agri. and Biol. Chem. Japan* **25**, 844 (1961). We are grateful to Dr. Kazuyoshi Yagishita, Department of Food Chemistry, Kumamoto Women's Univ., Ooemachi Kumamoto Japan, for supplying the authentic sample. These workers named it as "neoilexonol".


BLACK DAMMAR RESIN
(wt. = 6·8 kg)
Extracted with acetone
Resin residue
(wt. = 4·5 kg)
Soluble part
(wt. = 2·3 kg)
Extracted with ethanol
Insoluble
(wt. = 760 g)
Soluble part
(wt. = 2·2 kg)
Extracted with methanol
Soluble part
(wt. = 1·8 kg)
Insoluble part
(wt. = 400 g)
Extracted with petroleum ether
Extracted with petroleum ether
Insoluble part
(wt. = 900 g)
Soluble part
(wt. = 900 g)
[A]
Insoluble part
(wt. = 170 g)
Soluble part
(wt. = 230 g)
[B]
Chromatography
G IV alumina
Chromatography
G III alumina
A1 — Petroleum ether fraction (liquid + solid)
A2 — Benzene fraction (yellow viscous mass)
A3 — Ether fraction (dark yellow viscous mass)
A4 — Alcohol fraction
Petroleum ether fraction (liquid) — B1
Pet.ether:Benzene fraction (solid) — B2
Benzene fraction (semi solid) — B3
Ether fraction (sticky material) — B4

CHART

from ethyl acetate to give β-amyrin acetate, m.p. 240°, $(\alpha)_D$ +85° (*c*, 1·67). Mixed m.p. with an authentic sample remained undepressed. (Found: C, 82·00; H, 11·37. $C_{32}H_{52}O_2$ requires: C, 81·99; H, 11·18%.) IR spectrum was superimposable with that of β-amyrin acetate.

Isolation of junenol (III). The yellow viscous benzene eluted fractions of the above chromatography of the fraction A_1 was distilled under vacuum and the distillate on chromatography (grade II, 1:25) gave the crystalline substance junenol in the benzene eluted fraction. It was purified by crystallization from pet. ether followed by sublimation and had the following properties, m.p. 60°, $(\alpha)_D$ +52° (*c*, 3·35). Mixed m.p. with an authentic sample was 60°. (Found: C, 80·63; H, 11·51. $C_{15}H_{26}O$ requires: C, 81·02; H, 11·79%.) IR spectrum was identical with that of junenol available in our laboratory.

Isolation of canarone (IV). The combined pet. ether eluted fraction of the above chromatography (grade II, 1:25) on fractionation gave the ketone, canarone (1·8 g), b.p. 120–125° (bath)/1 mm, n_D^{28} 1·5020, $(\alpha)_D$ +34·78° (*c*, 3·45), d_{28}^{28} 0·9819. (Found: C, 81·00; H. 10·98. $C_{15}H_{24}O$ requires: C, 81·76; H, 10·98%.) The semicarbazone prepared by the acetate method had m.p. 222–224°. (Found: N, 15·58. $C_{16}H_{27}ON_3$ requires: N, 15·15%.) The ketone regenerated from the semicarbazone showed the properties as before.

Isolation of epi-khusinol (V). The tail benzene eluted fraction of the chromatography of A_1 was distilled, b.p. 140–160° (bath)/1·6 mm. The distillate (2·5 g) was further chromatographed (grade II, 1:25). The fraction eluted with benzene gave epi-khusinol (1·8 g), b.p. 120–125° (bath)/0·5 mm, n_D^{27} 1·5140, $(\alpha)_D$ −86·80° (*c*, 7·88). (Found: C, 81·40; H, 11·20. $C_{15}H_{24}O$ requires: C, 81·76; H, 10·90%.) It formed a 3,5-dinitrobenzenoate derivative which was crystallized from pet. ether, m.p. 186–187°. (Found: N, 6·91; $C_{15}H_{22}O$. $C_7H_8O_6N_2$ requires: N, 6·76%.)

Processing of the benzene fraction A_2. A portion of this fraction was acetylated in the usual way and chromatographed (grade III, 1:30) and eluted with pet. ether-benzene (1:1) mixture and large number of fractions collected. Further processing of these fractions gave the four compounds in the following order:

ψ-Taraxasterol acetate (VIb). It was repeatedly crystallized from ethyl acetate, m.p. 229°, $(\alpha)_D$ +52·15° (*c*, 0·79). (Found: C, 82·62; H, 11·62. $C_{32}H_{52}O_2$ requires: C, 81·99; H, 11·18%.) IR spectrum was identical with that of ψ-taraxasterol acetate.

*11-β-hydroxy-α-amyrin acetate** (IXb). It was purified by repeated crystallization from ethyl acetate, m.p. 187–188°, $(\alpha)_D$ +13·3° (*c*, 1·25). (Found: C, 79·4; H, 10·9. $C_{32}H_{52}O_3$ requires: C, 79·28; H, 10·81%.) Its identity was based on its oxidation with chromic acid to yield Xb, m.p. and mixed m.p. 275°.

Diol-monoacetate (VIIIb). It was crystallized from ethyl acetate, m.p. 265–267°, $(\alpha)_D$ +23·4° (*c*, 0·47). (Found: C, 78·11; H, 11·14. $C_{32}H_{54}O_3$ requires: C, 78·69; H, 11·18%.) On saponification it gave the parent diol (VIIIa) which was crystallized from MeOH, m.p. 261–263°, $(\alpha)_D$ ±0° (*c*, 0·23). On dehydration by treatment with $SoCl_2$ in pyridine, the diol monoacetate gave VIb, identified by m.p. and mixed m.p. and comparative IR spectra.

Ketol (VII). The keto alcohol was obtained from the tail chromatographic fraction. It was purified by crystallizations from MeOH, m.p. 257–259°, $(\alpha)_D$ +25° (*c*, 2·96). (Found: C, 81·53; H, 11·69. $C_{30}H_{50}O_2$ requires: C, 81·39; H, 11·38%.) This did not form an acetate showing thereby that the hydroxy group is tertiary. On reduction with LAH it gave VIIIa mentioned above.

Isolation of α-amyrin (Ia) *and β-amyrin* (IIa) *from fraction* B_3. The pet. ether–benzene fraction B_3 was chromatographed (grade III, 1:30) and the earlier solid material eluted with a mixture of pet. ether benzene (1:1) mixture was characterized as α-amyrin from its physical properties, acetate and benzoate derivatives and superimposable IR spectra.

The tail fractions eluted with a mixture of pet. ether–benzene (1:1) of the same chromatography was identified as β-amyrin from its physical properties, acetate and benzoate derivatives and comparative IR spectra.

Isolation of 11-keto-α-amyrin (Xa) *from fraction* B_3 *and its identification by conversion to 11-keto-α-amyrin acetate* (Xb). The acetylation product of benzene fraction B_3 was chromatographed (grade III, 1:30) and several fractions were collected. The product eluted with a mixture of pet. ether–benzene (4:1) was found to be a crystalline substance which on repeated crystallization from pet. ether gave pure Xb, m.p. and mixed m.p. with an authentic sample 275°, $(\alpha)_D$ +107° (*c*, 2·11). (Found: C,

* As mentioned earlier further confirmatory evidences will be required for establishing the identity of this material.

78·91; H, 10·40. $C_{32}H_{50}O_2$ requires: C, 79·60; H, 10·40%.) IR spectrum was superimposable with the IR spectrum of 11-keto-α-amyrin acetate.

Hydrogenation of 11-*keto-α-amyrin acetate* (Xb) *to α-amyrin acetate* (Ib). A solution of 11-keto-α-amyrin acetate (0·100 g) in acetic acid (20 ml) was hydrogenated over Pt catalyst for 6 hr. After removal of catalyst, the filtrate afforded the hydrogenated product (0·095 g) which on purification by chromatography followed by crystallization from ethyl acetate afforded α-amyrin acetate, identified from its physical properties and superimposable IR spectra.