107. *Extractives from Woods. Part III.* Extractives from* Manilkara bidentata.

By Wesley Cocker and S. J. Shaw.

The heartwood of *Manilkara bidentata* (*Mimusops globosa*) contains n-alkanes from tridecane to tetracosane inclusive, palmitic and stearic acid, β-amyrin, bassic acid, cyclolaudenol, and α-spinasterol.

The hardwood, *Manilkara bidentata*, known also as *Mimusops globosa* (family Sapotaceae) was obtained from Trinidad. Several species of *Mimusops* have been investigated previously; *e.g.*, *M. djave* Engl.,[1] *M. elengi* L.,[2] *M. heckelii* Pierre,[3] and *M. hexandra* Roxb.[1] have been shown to contain bassic acid, a characteristic triterpene of the family Sapotaceae.[1] *M. heckelii* also contains hexadec-9-enoic acid,[4] whilst β-amyrin and a lupeol ester [5] have been isolated from *M. globosa*.

The neutral fraction of the ligroin extract of the wood was a gum from which α-spinasterol[6] was isolated. Hydrolysis of the gum followed by chromatography on alumina gave (*a*) a mixture of approximately equal amounts of palmitic and stearic acid which were identified by gas–liquid chromatography of their methyl esters, (*b*) a mixture of all the normal alkanes from tridecane to tetracosane, identified by gas–liquid chromatography, (*c*) β-amyrin, and (*d*) an alcohol fraction. The last fraction was acetylated and chromatographed, giving an acetate fraction, $C_{33}H_{54}O_2$, m. p. 108°, $[\alpha]_D$ +60°. Hydrolysis of the latter gave the corresponding alcohol fraction, $C_{31}H_{52}O$, m. p. 118°, $[\alpha]_D$ +30°, from which a benzoate mixture, m. p. 130°, $[\alpha]_D$ +55°, was prepared. These characteristics are closely related to those of three reported alcohols and their derivatives, namely, balataresinol

* Part II, 1962, 5194.

[1] Heywood and Kon, *J.*, 1940, 713.
[2] Van der Haar, *Rec. Trav. chim.*, 1929, **48**, 1155.
[3] Sandermann and Barghoorn, *Holzforschung*, 1955, **9**, 112 (*Chem. Abs.*, 1955, **49**, 13,646).
[4] Karrer, " Konstitution und Vorkommen der Organischen Pflanzenstoffe," Birkhäuser Verlag, Basel und Stuttgart, 1958, p. 303.
[5] Cohen, *Arch. Pharm.*, 1908, **246**, 510.
[6] Barton and Cox, *J.*, 1948, 1354.

previously isolated [7] from *M. globosa* and formulated as $C_{27}H_{46}O_2$ or $C_{27}H_{44}O_2$ [8] or $C_{30}H_{50}O$,[9] balatol isolated [10] from balata resin and formulated as $C_{32}H_{52}O_2$, and resiniferol, $C_{30}H_{50}O$ isolated [11] from *Euphorbia resinifera*. The identity of these materials has been suggested,[11] and the substance now described is probably coidentical. It is a mixture, and its spectrum and that of several derivatives suggested that it was mainly cyclolaudenol.[12] In particular, the maximum it shows at 885 cm.$^{-1}$ is characteristic of the RR'C=CH_2 group; ozonolysis of the corresponding mixture of ketones afforded formaldehyde. The spectrum of the mixed acetates shows a peak at 3040 cm.$^{-1}$. Cyclolaudenol [12] exhibits this peak which comes from the methylene group of a cyclopropane ring.[13] A peak at 990 cm.$^{-1}$ exhibited by the mixed acetates is also found in cycloartanyl acetate,[14] and likewise arises from the cyclopropane ring.

Attempted separation of cyclolaudenol from the mixture of alcohols by crystallisation or column chromatography failed. Thin-layer chromatography on silica gel and gas–liquid chromatography on three columns revealed two components, the major (85%) being cyclolaudenol. Owing to shortage of material the minor component has not been identified, but gas–liquid chromatography shows that it is not α- or β-amyrin, cycloartenol, cycloartenone, lupeol, β-sitosterol, or α-spinasterol. Cyclolaudenol was obtained from the mixture by chromic acid oxidation to a mixture of ketones from which pure cyclolaudenone was obtained; reduction with sodium in propan-2-ol gave cyclolaudenol.

The acetone extract of *Manilkara bidentata* afforded a saponin from which bassic acid, glucose, rhamnose, and xylose were isolated. This accords with the saponin isolated by King, Baker, and King [15] from *M. heckelii*.

Experimental

Ultraviolet spectra were measured for ethanolic solutions, infrared spectra for Nujol suspensions, and $[\alpha]_D$ for chloroform solutions. Gas–liquid chromatography was carried out on an Aerograph Hi Fi 600 instrument (Wilkens Instrument and Research Inc.).

Wood shavings (30 lb.) were extracted continuously with ligroin (b. p. 60—80°) (10 l.) for 48 hr. The extract was filtered whilst hot, concentrated to 2 l., and washed with 5% aqueous sodium hydrogen carbonate (3 × 500 c.c.) and 5% aqueous sodium hydroxide (3 × 500 c.c.). The washings were extracted with ether and the extracts added to the ligroin. The alkaline wash-liquors were acidified, yielding acids (brown oil, 16 mg.) and phenols (dark gum, 2·4 g.). Removal of the solvents gave the neutral fraction as a gum (41·5 g.).

The gum was steam-distilled and the residue extracted with 1 : 1 ether–chloroform, yielding a brown gum (22·5 g.). Dissolved in ligroin, this was run on to a column of neutral alumina. Elution with ligroin gave a yellow gum, and with 1 : 1 ligroin–benzene gave α-spinasterol (500 mg.) (needles from ligroin), m. p. 162° not raised by further crystallisation, $[\alpha]_D$ 0° ± 1° (*c* 0·4), λ (end-absorption only) log ε 2·43 and 2·15 at 2150 and 2220 Å, $\nu_{max.}$ 3509 (OH), 1653 (C=C), 970 (*trans*-RCH=CHR), 833 (R_2C=CHR), 800, 781 cm.$^{-1}$ (Found: C, 82·5, 82·4; H, 11·7, 11·25. Calc. for $C_{29}H_{48}O,\frac{1}{2}H_2O$: C, 82·7; H, 11·6%). Its acetate (prisms from ethanol) had m. p. and mixed m. p. 180°, $[\alpha]_D$ −7° (*c* 0·2) (lit.[6] m. p. 185°, $[\alpha]_D$ −5°), $\nu_{max.}$ 1739 (ester), 1242 cm.$^{-1}$ (acetate) (Found: C, 81·6; H, 11·0. Calc. for $C_{31}H_{50}O_2$: C, 81·9; H, 11·1%). Hydrolysis of the acetate with 5% methanolic potassium hydroxide gave α-spinasterol, m. p. and mixed m. p. 173° (lit.[6] m. p. 167—168°, $[\alpha]_D$ −3°). Its benzoate (needles from ethanol) had m. p. and mixed m. p. 200°, $[\alpha]_D$ +2° (lit.[6] m. p. 201°, $[\alpha]_D$ +2°). γ-Spinastenyl acetate

[7] Tschirch and Muller, *Arch. Pharm.*, 1905, **243**, 114.
[8] Tschirch and Schereschewski, *Arch. Pharm.*, 1905, **243**, 358.
[9] Cohen, *Arch. Pharm.*, 1907, **245**, 245; 1908, **246**, 510.
[10] Tanaka, Kuwata, and Suzuki, *J. Soc. Chem. Ind., Japan*, 1935, **38**, 504*B*.
[11] Dupont, Julia, and Wragg, *Bull. Soc. chim. France*, 1953, **852**, 504*B*.
[12] Bentley, Henry, Irvine, Mukerji, and Spring, *J.*, 1955, 596; Henry, Irvine, and Spring, *J.*, 1955, 1607.
[13] Cole, *Chem. and Ind.*, 1953, 946; *J.*, 1954, 3810.
[14] Barton, *J.*, 1951, 1444.
[15] King, Baker, and King, *J.*, 1955, 1338.

(plates from methanol) had m. p. 157°, $[\alpha]_D$ +14° (lit.[6] m. p. 156—157°, $[\alpha]_D$ +8°). γ-Spinastenol, prepared by hydrogenation of α-spinasterol over Adams catalyst in ethanol, had m. p. 140° (plates from methanol), $[\alpha]_D$ +5° (*c* 0·1) (lit.[6] m. p. 144—145°, $[\alpha]_D$ +11°), λ (no max.) 2150 Å (log ε 2·5) (Found: C, 83·6; H, 12·1. Calc. for $C_{29}H_{50}O$: C, 84·0; H, 12·15%). Acetylation gave γ-spinastenyl acetate, m. p. and mixed m. p. 157°. α-Spinastenyl acetate (plates from methanol) had m. p. 114°, $[\alpha]_D$ +9° (lit.[6] m. p. 116—117°, $[\alpha]_D$ +12°).

The yellow gum (20 g.) was refluxed for 13 hr. with 8% ethanolic potassium hydroxide (200 c.c.) containing benzene (25 c.c.). The mixture was acidified, diluted, and extracted with ether. Solvent was removed from the dried extract, and the residue, dissolved in ether, was run on to a column of neutral alumina and eluted in 100-c.c. fractions with (*a*) light petroleum (b. p. 40—60°) (fractions 1—10), (*b*) 1 : 1 light petroleum–benzene (fractions 11—13), (*c*) 1 : 1 benzene–acetone (fractions 14—18), and (*d*) 1 : 1 acetone–1% formic acid (fractions 19—22). Fractions 1—4 were clear gums (1 g. in all) with the infrared spectra of long-chain alkanes, 5—10 had the spectrum of an alcohol, 11—13 yielded more α-spinasterol (0·8 g.), 15—18 gave a yellow gum (3 g.) which could not be resolved, and 19—22 consisted of fatty acids.

Fractions 1—4 were analysed by gas–liquid chromatography (Table).

Composition of mixture of hydrocarbons.

Column, Silicon S.E. 30; Temp. 205° ± 1°; N_2 15 c.c./min.; H_2 25 c.c./min. A = authentic specimen used as marker.

Peak no.	Relative retention time (docosane = 1)	Retention vol. (c.c.)	Hydrocarbon	Approx. quantity (%)
1	0·0451	22·3	Dodecane	0 A
2	0·0539	28·5	Tridecane	1·7 A
3	0·0682	35·5	Tetradecane	1·7
4	0·0910	46·5	Pentadecane	1·9
5	0·1222	63·7	Hexadecane	2·3
6	0·1734	90·0	Heptadecane	7·2
7	0·2415	127·5	Octadecane	10·9
8	0·3419	180·0	Nonadecane	14·3
9	0·4887	255·0	Eicosane	13·9
10	0·7015	367·5	Heneicosane	9·1
11	1·0	525·0	Docosane	8·6 A
12	1·1452	755·0	Tricosane	3·6 A
13	1·2011	1172·0	Tetracosane	0 A

By using a Quadrol S.A.I.B. column the composition of the mixture of hydrocarbons was verified.

Fractions 19—22 with fresh diazomethane gave an amorphous solid, m. p. 55° (from ethanol, $\nu_{max.}$ 1730 and 1176 (ester), 730, 719 cm.$^{-1}$ (CH_2). This was chromatographed at 195° on a D.E.G.S. column, 5 ft. × 1/8 in., N_2 26 c.c./min., H_2 22 c.c./min. Two peaks (T_R 8·45 and 17·33 min.) corresponding to methyl palmitate and methyl stearate, respectively, were produced.

Fractions 5—10 were acetylated with cold acetic anhydride and pyridine. The dried product, in light petroleum, was run on to a column of neutral alumina, and eluted in 100-c.c. fractions with light petroleum. Fractions *a*—*c* consisted of more hydrocarbons (not investigated). Fractions *d*—*f* yielded β-amyrin acetate (1·5 g.), m. p. and mixed m. p. 231° (from ethanol), $[\alpha]_D$ +91° (*c* 0·19), $\nu_{max.}$ 1730, 1653, 1242 cm.$^{-1}$, identical with the spectrum of an authentic specimen.

The mixture of fractions *g*—*l* was spotted on a thin layer of silica gel, developed with cyclohexane–ethyl acetate (17 : 3), and sprayed with 30% antimony trichloride in chloroform. The plate was heated at 100° for 2 min., revealing two spots of cyclolaudenyl acetate (R_F 0·64) and a minor component (R_F 0·663).

Removal of solvent from fractions *g*—*l* gave a mixture of acetates, m. p. 108° (needles from ethanol), $[\alpha]_D$ +60° (*c* 0·1) (cf. refs. 7—11), $\nu_{max.}$ 1724 (ester), 1639 (C=C), 1250 (acetate), 990 (cyclopropane), 885 cm.$^{-1}$ (RR'C=CH_2) (Found: C, 81·75; H, 11·1; Ac, 9·4. Calc. for $C_{33}H_{54}O_2$: C, 82·1; H, 11·3; Ac, 8·9%).

The mixture of acetates (106·2 mg.) was refluxed for 2·5 hr. with potassium hydroxide (4 g.) in methanol (50 c.c.), giving an alcohol mixture (90 mg.), m. p. 118°, $[\alpha]_D$ +30° (*c* 0·1)

(cf. refs. 7—11). It did not crystallise and had $\nu_{max.}$ 3344 (OH), 1639 (C=C), 1000 (cyclopropane), 885 cm.$^{-1}$ (C=C). Its benzoate (needles from methanol) had m. p. 130°, $[\alpha]_D$ +55°, $\nu_{max.}$ 1724 (C=O), 1639, 1000, 885 cm.$^{-1}$ (Found: C, 83·1; H, 9·7. Calc. for $C_{38}H_{56}O_2$: C, 83·8; H, 10·4%).

Cyclolaudenone.—The alcohol mixture (170 mg.) in acetone (100 c.c.) was oxidised for 0·5 hr. with chromium trioxide (2·6 g.) in sulphuric acid (2·3 c.c.) and water (10 c.c.), then heated at 40—50° for 5 min. The product (39 mg.) (plates after several crystallisations from methanol) had m. p. (and mixed m. p. with cyclolaudenone) 115°, $[\alpha]_D$ +25° (*c* 0·24) (lit.[12] m. p. 115°, $[\alpha]_D$ +19°, for cyclolaudenone), $\lambda_{max.}$ 2800 Å (log ε 2·1), $\nu_{max.}$ 1715 (C=O), 1639 (C=C), 885 cm.$^{-1}$ (RR'C=CH_2) (Found: C, 84·65; H, 10·9. Calc. for $C_{31}H_{50}O$: C, 84·9; H, 11·5%). Reduction of the ketone (50 mg.) with an excess of sodium in boiling propan-2-ol (20 c.c.) for 3 hr. gave cyclolaudenol (20 mg.), m. p. and mixed m. p. 123° (lit.[12] m. p. 125°, $[\alpha]_D$ +46°, for cyclolaudenol). Its benzoate had m. p. and mixed m. p. 186° (lit.[12] m. p. 194°, $[\alpha]_D$ +63°). Gas–liquid chromatography of cyclolaudenol and cyclolaudenone on silicone rubber, silicone S.E., and poly-(*m*-phenyl ether) columns showed one discrete band in each case. The conditions for the silicone rubber column were as follows: column, 5 ft. × 1/8 in.; temp. 260° ± 1°; N_2 26 c.c./min.; H_2 24 c.c./min. Cyclolaudenone gave T_R 8·1 min. and cyclolaudenol T_R 8·4 min.

Acetone Extract.—Continuous extraction of the ligroin-extracted wood with acetone (10 l.) gave a water-insoluble fraction (lignin) and a water-soluble fraction. The latter was heated for 3 hr. with an excess of 3% hydrochloric acid, and extracted with butanol. The aqueous layer was chromatographed on paper in two systems. (1) Pyridine–ethyl acetate–water (2 : 8 : 1 v/v), with solvent front descending, for 40 hr. When the paper was sprayed with a mixture prepared from phthalic acid (3·3 g.), acetone (90 c.c.), aniline (2 c.c.), and water (5 c.c.), and heated for 5 min., three spots were detected, corresponding to glucose (R_G 1), xylose (R_G 3·29), and rhamnose (R_G 6·58). (2) Butanol–acetic acid–water (4 : 1 : 5 v/v) for 18 hr., when sprayed as for system (1), gave three spots corresponding to glucose (R_F 0·12), xylose (R_F 0·22), and rhamnose (R_F 0·34).

The butanol layer was extracted with 10% aquous sodium hydroxide leaving a neutral fraction which was refluxed for 3 hr. with 6% methanolic hydrochloric acid. The mixture was filtered giving insoluble material (14 g.) which was not further investigated. The methanol was removed from the filtrate, giving bassic acid (10 g.), m. p. and mixed m. p. 290° (from aqueous ethanol), $[\alpha]_D$ +80° (pyridine) (lit.[15] m. p. 290°, $[\alpha]_D$ +82°, for bassic acid monohydrate), $\nu_{max.}$ 3448 (OH), 1695 cm.$^{-1}$ (CO_2H). Its methyl ester (needles from aqueous methanol) had m. p. 210°, $[\alpha]_D$ +61° (lit.[1] m. p. 212°, $[\alpha]_D$ +56°).

The authors thank Professor E. R. H. Jones, F.R.S., and Dr. J. McLean for specimens of cyclolaudenol, The Tropical Products Institute of the Department of Scientific and Industrial Research and The Conservator of Forests, Trinidad, for providing the wood. The authors are particularly indebted to the U.S. Army through its European Research Office for supporting this investigation under contract.

UNIVERSITY CHEMICAL LABORATORY, TRINITY COLLEGE, DUBLIN,
REPUBLIC OF IRELAND.

[*Received, July 11th,* 1962.]