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MADHUCA BUTYRACEA. CONSTITUENTS OF THE FRUIT-PULP AND THE BARK

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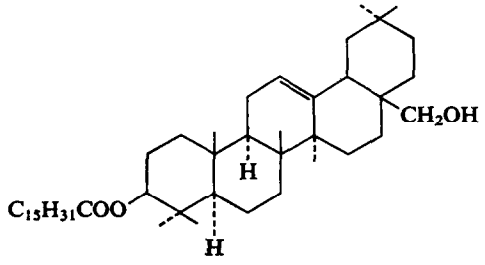
Abstract—Besides α-spinasterol, β-D-glucoside of β-sitosterol, acetates of α- and β-amyrins and hithertofore unreported 3β-palmitoxy-olea-12en-28-ol isolated from both the bark and the fruit-pulp of *Madhuca butyracea*, two other esters—the oleanolic acid palmitate and betulinic acid palmitate—have been isolated from the fruit-pulp and the bark respectively. Friedelin is also present in the bark. Chemical constituents isolated from the two species of *Madhuca*, i.e. *latifolia* and *butyracea*, indicate similar biosynthetic pathways apart from the chemotaxonomic significance.

Madhuca butyracea syn. *Diploknema butyracea* (family Sapotaceae) grows abundantly in the sub-Himalayan tract at altitudes ranging from 300 to 1500 m. Its bark, leaves, flowers, fruits and seeds find various uses in the indigenous system of medicine, and the plant is often identified under the name *Madhuca*[1] together with its sister species, *M. latifolia* (*Mahua*). Its seed kernel is rich in saponins,[2, 3] and the seed fat has a unique place amongst the vegetable fats for its highest palmitic acid content (56 per cent) ever recorded in a nut-fat.[4, 5]

In pursuance of the studies in the constituents of the sapotaceous plants of economic importance,[6–10] systematic chemical examination of the bark and fruit-pulp of *M. butyracea* has been carried out. Apart from α-spinasterol, β-D-glucoside of β-sitosterol, acetates of α- and β-amyrins, and hithertofore unreported 3β-palmitoxy-olea-12en-28-ol (I) obtained from



(I)

[1] B. MUKERJI, *Indian Pharmaceutical Codex*, Vol. I, p. 144. C.S.I.R. (India), New Delhi (1953).
[2] B. J. HEYWOOD, G. A. R. KON and L. L. WARE, *J. Chem. Soc.* 1124 (1939).
[3] B. J. HEYWOOD and G. A. R. KON, *J. Chem. Soc.* 713 (1940).
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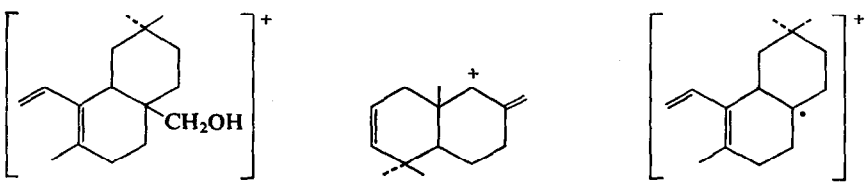
[5] W. J. BUSHELL and T. P. HILDITCH, *J. Soc. Chem. Ind.* **50**, 468 (1931).
[6] Y. C. AWASTHI and C. R. MITRA, *Phytochem.* **6**, 121 (1967).
[7] C. R. MITRA and G. MISRA, *Phytochem.* **4**, 345 (1965).
[8] G. MISRA and C. R. MITRA, *Phytochem.* **5**, 535 (1966).
[9] G. MISRA and C. R. MITRA, *Phytochem.* **6**, 453 (1967).
[10] G. MISRA and C. R. MITRA, *Phytochem.* **6**, 1309 (1967).

both the bark and the fruit-pulp, two other ester, the oleanolic acid palmitate and betulinic acid palmitate, have been isolated from the fruit-pulp and the bark respectively. These esters also do not appear to have been isolated earlier from plant source. Friedelin has also been isolated from the bark.

3β-Palmitoxy-Olea-12-en-28-ol

The neutral ester obtained by chromatography of the bark as well as the pulp extractive had γ_{max} 3484 (hydroxyl), 1700 (ester C=O) and 800, 815, 830 (trisubstituted double bond) cm^{-1}. On alkaline hydrolysis it yielded palmitic acid and erythrodiol. On oxidation of the ester with chromium trioxide and subsequent hydrolysis of the reaction product, oleanolic acid was obtained. This suggested the primary hydroxyl of erythrodiol at C-28 to be free, thereby indicating the ester linkage at C-3. This contention is supported by the presence of an AB type quartet centred at 6·7τ in the NMR spectra arising due to the two protons of methylene group of a free hydroxyl group.[11] The presence of an unresolved triplet at 5·45τ accounting for the 3α-proton of the 3β-acyloxy triterpenes[12] also favoured the placement of the ester grouping at C-3. An olefinic proton situated at a trisubstituted double bond appeared around 4·8τ in the NMR spectra as a broad signal with a poorly defined centre, while an intense signal at 1·3τ indicated the presence of a fairly large number of protons in the molecule situated in an aliphatic chain.[13]

The fragmentation pattern in the mass spectra of the ester provided the confirmation of the position of the ester linkage. The molecular ion (M^+=680 m/e) recorded a loss of 256 mass units (palmitic acid) to give sufficiently abundant peak at 424 m/e and a subsequent loss of 31 mass units (CH_2OH) was observed from this fragment resulting in a peak at 393 m/e. Further, the characteristic *retro*-Diels-Alder fragment[14] (II) arising due to the "right half" of Δ^{12}-oleanenes appeared at m/e 234 and the left half of the molecule provided the fragment (III) of almost equal abundance at m/e 189. A loss of 31 mass units from the *retro*-Diels-Alder fragment resulting in another abundant fragment (IV) at m/e 203, provides conclusive proof for the ester to be 3β-palmitoxy-olea-Δ^{12}-en-28-ol (I).



(II) (III) (IV)

Triterpene Acid Esters

The triterpene acid ester isolated from the bark extractive had γ_{max} 1695, 3200 (COOH) 1730 (ester C=O) and 1640, 885 (isopropylene) cm^{-1}. The two terminal protons of the isopropylene group appeared at 4·69 and 4·65τ in the NMR spectra whereas a single proton gave a signal at 4·2τ. The free carboxyl group of the acid could be titrated against dilute alkali and reacted spontaneously with diazomethane. On alkaline hydrolysis it gave palmitic

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[12] J. B. THRONTON, *Tetrahedron* **22**, 351 (1966).
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acid and betulinic acids. The acid isolated from the fruit-pulp was found to be oleanolic acid palmitate confirmed by saponification when quantitative amounts of oleanolic acid and palmitic acid could be isolated and identified.

The constituents of bark and fruit-pulp of *M. butyracea* (*D. butyracea*) are very similar and their marked resemblance with the constituents of *M. latifolia* fruit-pulp[6] and bark (unpublished) is of interest. It may be recalled that the nut-shell of both these plants contain quercetin and dihydroquercetin[15] and the saponins of the nut-kernel of both the species are also reported to have the common genin acid, bassic acid.[2,3] This remarkable similarity between the chemical constituents of the corresponding parts of these two species of the genus *Madhuca*, indicates comparable biosynthetic pathways and seems to be of significant chemo-taxonomic importance particularly in contradistinction to a comparatively recent nomenclature,[16] *D. butyracea* for *M. butyracea*. It may, therefore, be worthwhile to re-investigate if a change in the genus of *M. butyracea* is otherwise called for.

EXPERIMENTAL

Unless otherwise mentioned, optical rotations were measured in chloroform solution; melting points (uncorrected) were determined in open capillaries; hexane used was mostly *n*-hexane (b.p. 70°); i.r. spectra were recorded in KBr films; NMR spectra were recorded in $CDCl_3$ using TMS as internal reference at 60 m/c and alumina used for chromatography was neutral Brockman (E. Merck) quality.

Constituents of the Bark

The *Madhuca butyracea* bark was collected (in July) under personal supervision, air dried and freed of the extraneous materials. The coarsely powdered bark (6·3 kg) was exhaustively percolated with alcohol at room temperature (25–40°). The dark-brown sticky residue (1 kg), obtained by removal of the solvent at reduced pressure and finally *in vacuo*, was subsequently fractionated into hexane-soluble and hexane-insoluble fractions. The hexane-soluble fraction (110 g) was chromatographed over neutral Al_2O_3 (1·1 kg) using hexane, benzene, $CHCl_3$ and MeOH and mixtures thereof as eluents in succession with increments of 10 to 25 per cent of the following solvent after complete elution with the preceding one. The following constituents were isolated and characterized from the different fractions:

β-Amyrin acetate. The initial hexane eluent fractions, on cooling, deposited silky needles of β-amyrin acetate (2·5 g), m.p. and m.m.p. 240–242°; $(\alpha)_D^{24}$ +80°; γ_{max} 1720, 1245 cm^{-1}; identified as reported earlier.[6]

α-Amyrin acetate. Hexane eluent also yielded a small amount of α-amyrin acetate (40 mg) as long silky needles, m.p. and m.m.p. 222–224°; $(\alpha)_D$ +90°; γ_{max} 1734, 1238 cm^{-1}.

Friedelin. Subsequent hexane eluent fractions, on chilling, deposited friedelin (100 mg) as clusters of needles which, after crystallization (alcohol), had m.p. and m.m.p. 255–256°; $(\alpha)_D$ −20°; γ_{max} 1712 cm^{-1} (Found: C, 84·0; H, 12·35. Calc. for $C_{30}H_{50}O$: C, 84·5; H, 11·73 per cent); oxime, m.p. and m.m.p. 262°, and 2,4-dinitrobenzoate, m.p. and m.m.p. 294°. Its i.r. spectra was superimposable with that of an authentic sample of friedelin.[17]

Erythrodiol monopalmitate. The hexane:benzene (20:80) eluent fractions of the chromatogram, on cooling, deposited needles of an LB and Noller's positive neutral ester (1·5 g), which on repeated crystallizations and chromatography over acid-washed alumina gave shining flakes of erythrodiol monopalmitate, m.p. 115–116°; $(\alpha)_D$ + 48°; γ_{max} 3484, 1700, 830, 815, 800 cm^{-1}; NMR signals at 4·8, 6·7 (quartet) and 5·45 (unresolved triplet) τ and prominent peaks in the mass spectra at 680 (M^+), 424, 393, 256, 234, 203 and 189 m/e (Found: C, 81·43; H, 11·66. Calc. for $C_{46}H_{80}O_3$: C, 81·03; H, 11·76 per cent). On treatment with acetic anhydride in pyridine it gave an acetate as slimy semi-solid mass, γ_{max} 1245 cm^{-1}. When the ester (100 mg) was treated with $LiAlH_4$ (100 mg) in tetrahydrofuran (25 ml) the reaction product after usual processing gave erythrodiol (50 mg) as silky needles, m.p. and m.m.p. 228–230° and i.r. spectra superimposable. The ester (500 mg) on saponification with alcoholic KOH (50 per cent; 50 ml) gave, from the neutral fraction, erythrodiol (250 mg), m.p. and m.m.p. 230–232°; $(\alpha)_D$ +76°; γ_{max} 3332 cm^{-1} (Found: C, 80·9; H, 11·76. Calc. for $C_{30}H_{50}O_2$: C, 81·44; H, 11·3 per cent); diacetate, m.p. and m.m.p. 180–184°; diformate (formic acid, 100°, 30 min), m.p. 195° (lit. 195°). The acidic fraction of the hydrolysate yielded palmitic acid (100 mg), m.p. and m.m.p. 63–65°; neutralization equiv. 251; calc. 256 (Found: C, 75·06; H, 12·78. Calc. for $C_{16}H_{32}O_2$: C, 75·00; H, 12·50 per cent).

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[16] *Index Kewensis*, Suppl., Vol. VIII, p. 76 (1926–1930).
[17] S. K. Nigam and C. R. Mitra, *Indian J. Chem.* **2**, 378 (1964).

Oleanolic acid from the ester. The above ester (500 mg) was heated with CrO_3 (500 mg) in acetic acid (50 ml) on steam bath for 2 hr, and the reaction product obtained after usual processing was subsequently saponified (5 per cent alcoholic KOH). The acidic fraction of the hydrolysate on usual processing and repeated fractional crystallization (hexane) yielded oleanolic acid (20 mg), m.p. and m.m.p. 304–308°; $(\alpha)_D^{34°}$ +78°; i.r. superimposable with that of an authentic sample of oleanolic acid.[6]

Betulinic acid palmitate. The $CHCl_3$:MeOH (50:50) eluent fractions of the chromatogram yielded a microcrystalline low melting solid, m.p. 80–100°, which, on repeated chromatography over acid-washed alumina and crystallizations with different solvents, gave the acid ester, m.p. 95–100°; $(\alpha)_D$ +8° (Found: C, 79·74; H, 10·85. Calc. for $C_{46}H_{78}O_4$: C, 79·54; H, 11·20 per cent); γ_{max} 1720, 1690 cm^{-1}; NMR signal at 4·69, 4·65, 4·2τ. The ester (500 mg), on alkaline hydrolysis, gave palmitic acid (150 mg) and a triterpene acid (300 mg), m.p. 280–285°; $(\alpha)_D$ +10° which was methylated (CH_2N_2) and the methyl ester thus obtained yielded, on chromatography (alumina), methyl betulinate, m.p. and m.m.p. 223–224°; $(\alpha)_D$ +8° (Found: C, 79·2; H, 10·87. Calc. for $C_{31}H_{50}O_3$: C, 79·18; H, 10·68 per cent); methyl betulinate acetate, m.p. and m.m.p. 204–205°; i.r. spectra superimposable with that of an authentic sample.[10] Traces of another methyl ester, m.p. 130–180°, were also obtained from the chromatogram, which showed spots corresponding to a mixture of methyl betulinate and methyl ursolate in the TLC (silica gel g, MeOH:Bz::5:95, iodine vapours).

α-Spinasterol. The $CHCl_3$ eluent fractions of the chromatogram yielded needles (alcohol) of α-spinasterol (500 mg), m.p. and m.m.p. 168°; $(\alpha)_D$ ±0°; i.r. spectra superimposable; acetate, m.p. and m.m.p. 182°; benzoate, m.p. and m.m.p. 192°.

β-D-Glucoside of β-sitosterol was obtained as a microcrystalline solid (1·5 g), m.p. and m.m.p. 290–292°; $(\alpha)_D$ −9° (1, Py), from the middle layer during the partitioning of the alcohol extractive with hexane. On hydrolysis (5 per cent alcoholic HCl) it gave β-sitosterol, m.p. and m.m.p. 137°; acetate, m.p. and m.m.p. 128°; glucose was identified in the aqueous fraction through paper chromatography in two different solvent systems (*n*BuOH:AcOH; H_2O::5:1:4; *n*BuOH; Py:Bz:H_2O::5:1:3:3; aniline phthalate spray).

Constituents of the Fruit Pulp

Fresh ripe fruits of *M. butyracea* were collected in July under personal supervision and the pulp (moisture 55 per cent) was separated from the nuts. The pulp was immediately soaked in alcohol and percolated at room temperature (25–38°) and the dark-green sticky extractive (*ca.* 100 g), obtained on the removal of the solvent from the extract at reduced pressure (below 55°) and finally *in vacuo*, when examined systematically (cf. the bark extractive) yielded the following compounds:

β-Amyrin acetate. (0·8 g), m.p. and m.m.p. 242°; $(\alpha)_D$ +85°; identified as described above.

α-Amyrin acetate. (50 mg), m.p. and m.m.p. 220–224°; $(\alpha)_D$ +86°; identified as described above.

Erythrodiol monopalmitate. The analogous hexane:benzene eluent fractions (cf. bark extractive) of the chromatogram gave shining flakes (alcohol) of erythrodiol monopalmitate (0·5 g), m.m.p. 115–120°; $(\alpha)_D$ +48°; γ_{max} 3484, 1700 cm^{-1} (Found: C, 81·36; H, 11.87. Calc. for $C_{46}H_{80}O_3$: C, 81·03; H, 11·76 per cent); identified as described above.

Oleanolic acid palmitate. From the $CHCl_3$–MeOH (1:1) eluent fraction of the chromatogram an LB positive low-melting microcrystalline solid (1·5 g), m.p. 80–90°, was obtained. On repeated chromatography over neutral Al_2O_3 and crystallizations with different solvents the melting point did not register any rise and the compound gave a single spot in the TLC (silica gel g, Bz:MeOH::9:1, acidic $KMnO_4$). It readily reacted with CH_2N_2 but its methyl ester was obtained only as a slimy mass. When the ester (1 g) was saponified (5 per cent alcoholic KOH; 50 ml) it yielded oleanolic acid (600 mg), m.p. and m.m.p. 305–308°; $(\alpha)_D$ +75°; acetate, m.p. and m.m.p. 262°; methyl ester, m.p. and m.m.p. 200°; i.r. spectra superimposable with that of authentic sample.[6] Palmitic acid (300 mg) isolated from the reaction mixture melted at 62–64°; identified as recorded above.

α-Spinasterol. Silky needles of α-spinasterol (400 mg) were obtained from the chloroform eluent fractions, m.p. and m.m.p. 168°; $(\alpha)_D^{34°}$ ±0°; identified as described above.

β-D-Glucoside of β-sitosterol was obtained from the middle layer (cf. bark extractive) as micro-crystalline solid (400 mg), m.p. 292–296°.

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