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MADHUCA LATIFOLIA.

CONSTITUENTS OF FRUIT PULP AND NUT-SHELL

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Abstract—A number of triterpenoids including α- and β-amyrin acetates, 3β-monocaprylic ester of erythrodiol, 3β-capryloxy oleanolic acid and an acetate, have been isolated from the mesocarp of *Madhuca latifolia* fruit. The other constituents isolated and characterized are *n*-hexacosanol, β-D-glucoside of β-sitosterol and free β-sitosterol. The nut-shell extract yielded β-D-glucoside of β-sitosterol, quercetin and dihydroquercetin.

Madhuca latifolia syn. *M. indica* (Sapotaceae) is an important economic plant growing throughout the sub-tropical region of the Indian sub-continent. Its flowers, rich in sugars[1] and yeast[2] are employed for the production of country liquors; the nut is rich in fat[3,4] (40–45 per cent on the weight of nut kernel) which is used for soap making, but not for edible purposes due to the presence of toxic saponins[5,6] in the kernel.

In continuation of our work on sapotaceous fruits,[7] a systematic chemical examination of the mature, fresh fruit-pulp and the nut-coat of *Madhuca latifolia* has been carried out. No work appears to have been reported with these materials to date.

The alcoholic extractive of the pulpy mesocarp of the fresh fruit (60 per cent on the weight of fruit; moisture content, 60 per cent) on fractionation with hexane and subsequent column chromatography, yielded the hitherto unreported 3β-monocaprylic esters of erythrodiol and oleanolic acid, along with α- and β-amyrin acetates. Erythrodiol caprylic ester was shown to have a free hydroxyl as indicated in its i.r. spectra, and on oxidation with chromium trioxide and subsequent hydrolysis of the reaction product yielded oleanolic acid indicating that the free primary hydroxyl group was at C-28 and the capryl chain was attached to the secondary hydroxyl of the erythrodiol at C-3. A monostearate of erythrodiol has been reported from nature.[8]

In the other triterpene ester, the long chain attached to C-3 of the oleanolic acid imparted significant properties to the compound particularly its poor solubility in polar organic solvents and in weak alkali. Stronger alkali, however, degraded the product giving rise to oleanolic acid. Its i.r. spectra (chloroform) showed the presence of a broad band at 3200 cm^{-1} region (associated carboxylic OH) and two sharp peaks at 1720 and 1693 cm^{-1}, the former accounting for the ester carbonyl and the latter for a free carboxyl group. The NMR spectra

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of the compound showed a characteristic signal at 5·5 τ (triplet) for the α-proton at C-3 in 3-acyloxy triterpene molecules.[9] The NMR peak at 4·7 τ confirms the presence of Δ^{12} double bond in the amyrin series. The mass spectra of the compound shows the molecular ion peak at m/e 582 which is in agreement with the molecular formula, $C_{38}H_{62}O_4$, the number of protons, 62, being accounted for by the NMR spectra. The molecule also gives fragments like oleanolic acid, having a base peak corresponding to retro Diels-Alder fragment[10] at m/e 248, and the subsequent peaks at m/e 204 and 203 are indicative of loss of CO_2 and COOH respectively. The peak at m/e 438 confirms the caprylic acid moiety attached to 3β-OH.

The free carboxylic group at C-17 could be easily methylated with diazomethane. Reduction of the parent ester with $LiAlH_4$ yields erythrodiol. With acetyl chloride in pyridine, the ester isomerizes to 3β-capryloxy oleanolic acid lactone a characteristic of acids of the amyrin series.[11] Both oleanolic and caprylic acids were isolated from the alkaline hydrolysate of the ester.

Apart from the triterpenic constituents characterized, the chromatogram fractions yielded another triterpene, $C_{32}H_{52}O_2$, m.p. 189°, $(\alpha)_D^{36}+54°$, i.r. bands at 1710 and 1242 cm^{-1} (acetoxy). That it was an acetate was confirmed when on alkaline hydrolysis it gave an alcohol, m.p. 176–178°, $(\alpha)_D^{36}+50°$, yielding on acetylation the original product.

The β-D-glucoside of β-sitosterol,[12] as well as a free sterol, and hexacosanol have also been isolated from the mesocarp extractive. On usual characterization through its derivatives and paper co-chromatography, the free sterol appeared to be β-sitosterol. The mother liquor of the hexane eluates of the chromatogram yielded a mixture of carotenoid pigments (*c.* 0·05 per cent on the weight of fresh mesocarp) consisting mostly of β-carotene characterised by comparable u.v. spectra.

The β-D-glucoside of β-sitosterol has also been obtained from the nut-coat extract, the ether soluble fraction of which showed the presence of quercetin and dihydroquercetin, a chemotaxonomic character of the sapotaceous nut-coats.[7, 13]

EXPERIMENTAL

Unless otherwise stated, optical rotations were measured in chloroform solution; melting points (uncorrected) were determined in open capillaries; hexane used was mostly *n*-hexane, a petroleum cut, b.p. 70°; i.r. spectra were recorded in nujol and u.v. spectra in ethyl alcohol; NMR spectra was taken in $CDCl_3$ using TMS as internal reference at 60 M/C; alumina used for chromatography was neutral Brockman (E. Merck) quality.

Constituents of the Fruit Pulp

Fresh ripe fruits were crushed and the nuts (40 per cent of the whole fruit) separated from the pulp. The pulp (22 kg) was extracted with alcohol (160 l.) in cold. The semi-solid residue obtained after removal of the solvent at reduced pressure and finally in *vacuo* was subsequently fractionated into, hexane soluble and hexane insoluble and water soluble fractions. The hexane-soluble fraction (*c.* 100 g), neutral in character, was chromatographed (alumina, 1000 g) using hexane (12·3 l.) benzene (6·8 l.), chloroform (6·8 l.) and methanol (4·3 l.) in

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succession as eluents with 10 increments of the following solvent after elution with the preceding one. The following constituents were isolated from the different eluent fractions and characterized:

β-amyrin acetate. The bright orange coloured semi-solid residue (*c.* 20 g) on removal of the solvent from the hexane eluents deposited, on cooling its hexane solution, silky needles of β-amyrin acetate (5 g) which after repeated crystallizations (alcohol) melted at 240–242°; $(\alpha)_D^{34}+83{\cdot}5°$; ν_{max} 1723 and 1242 cm^{-1}. (Found: C, 82·07; H, 11·02. Calc. for $C_{32}H_{52}O_2$: C, 82·04; H, 11·02%). The acetate on alkaline hydrolysis gave β-amyrin m.p. and mixed m.p. 194°; $(\alpha)_D^{34}+85°$; ν_{max} 3330 cm^{-1}, which yielded the acetate, m.p. and mixed m.p. 242°.

α-amyrin acetate. The mother liquor of β-amyrin acetate on keeping gave shining needles (50 mg) m.p. and mixed m.p. 222–224°; $(\alpha)_D^{34}+83°$; ν_{max} 1740 and 1245 cm^{-1} and its identity was confirmed by i.r. spectra.

Carotenoid fraction. Bright orange-coloured residue (*c.* 15 g) left after the separation of the amyrin acetates responded to the colour reaction of carotenoids (Conc. H_2SO_4 and $SbCl_3$)[14] and had a u.v. spectrum, ($\lambda_{max}^{cyclohexane}$ 330, 371, 428, 450, 478, nm) like that of β-carotene.[15]

A neutral triterpenoid. Subsequent hexane eluents after separation of the above constituents yielded a Noller's reagent[16] and TNM positive compound (1 g, needles), m.p. 189°; $(\alpha)_D^{34}+52°$; R_f 0·36 (TLC; silica gel G; BzCl: AcOH, 9:1; spray: vapours of 10% $SnCl_4$ in $SOCl_2$); ν_{max} 1720, 1245 (OAc) and 815 (trisubstituted double bond) cm^{-1} (Found: C, 82·07; H, 11·14. $C_{32}H_{52}O_2$ required: C, 82·04; H, 11·02%). The compound (200 mg) refluxed (2 hr) with alcoholic KOH (5%, 20 ml) yielded the corresponding alcohol (needles), m.p. 176–178°; $(\alpha)_D^{36}+50°$; ν_{max} 3500 cm^{-1} (Found C, 84·22; H, 11·91. Calc. for $C_{30}H_{50}O$: C, 84·5; H, 11·74%); acetate, m.p. and mixed m.p. 189°; benzoate, m.p. 240–242°; $(\alpha)_D^{36}+58°$ (Found: C, 83·16; H, 10·38. Calc. for $C_{37}H_{54}O_2$: C, 83·73; H, 10·2%).

Erythrodiol monocaprylate. The hexane-benzene (30:70 to 10:90) eluent fractions of the chromatogram yielded still another triterpenoid (Noller's and TNM positive) which on repeated crystallizations (alcohol) gave erythrodiol monocaprylate (3 g; shining flakes), m.p. 152–156°; $(\alpha)_D^{26}+62°$; ν_{max} 3325 (OH), 1710 (ester carbonyl) and 815 cm^{-1} (Found: C, 80·43; H, 11·27. $C_{38}H_{64}O_3$ required: C, 80·0; H, 11·27%). The ester (450 mg) was refluxed (2 hr; 100°) with alcoholic potash (5%; 50 ml) yielded erythrodiol (350 mg) as shining needles (alcohol), m.p. 230–232° (*lit*[17] 232°); $(\alpha)_D^{34}+75°$ (*lit* +75°); ν_{max} 3332 cm^{-1} (OH) (Found: C, 80·80; H, 11·48. Calc. for $C_{30}H_{50}O_2$: C, 81·4; H, 11·3%); diacetate m.p. 182–184°, (*lit*[17] 179–184°), $(\alpha)_D^{26}+63°$ (*lit* 58°), and diformate m.p. 195° (*lit* 196°). The acidic fraction of the hydrolyzate yielded caprylic acid as a viscous liquid (congealed needles below 10°). It was soluble in $NaHCO_3$ solution (5%) and showed no unsaturation (TNM and $KMnO_4$); neutr. equ. 144 (Calc. for $C_8H_{16}O_2$: 144).

Oleanolic acid from erythrodiol caprylic ester. The ester (500 mg) was heated (4 hr, 100°) with CrO_3 (400 mg) in acetic acid (50 ml) and the reaction product hydrolysed with alcoholic potash (5%; 50 ml). The acidic fraction of the hydrolyzate on repeated crystallization from ether-alcohol (1:1), yielded oleanolic acid as needles (15 mg) m.p. and mixed m.p. 308–310°; i.r. spectra super-imposable with that of an authentic sample.

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3β-capryloxy oleanolic acid. The chloroform–methanol (30:70 to 50:50) eluent fractions of the chromatogram yielded oleanolic acid caprylate (*c.* 10 g) as silky needles (alcohol), m.p. 148°; $(\alpha)_D^{34}+44°$; R_f. 0·52 (TLC, Silica gel G, BzCl: AcOH, 9:1; developer, 10% $SnCl_4$ in $SOCl_2$); λ_{max} 214 mμ; $\nu_{max}^{CHCl_3}$ 3226 (OH), 1720 (ester carbonyl), 1693 (carboxyl), 1155, 1122 and 795 cm^{-1}; mol. wt. 582 (mass spectra); NMR signals at 4·7, 5·5 (triplet) and 7·7 τ (Found: C, 78·7; H, 11·0. $C_{38}H_{62}O_4$ requires: C, 78·4; H, 10·7%). The ester on treatment with diazomethane (ether) gave the 3β-capryloxy methyl oleanolate, m.p. 78–82°; $(\alpha)_D^{36}+40°$ (Found: C, 78·42; H, 10·99. $C_{39}H_{64}O_4$ requires: C, 78·52: H, 10·73%). When the ester (500 mg) was treated with $LiAlH_4$ (500 mg) in tetrahydrofuran (50 ml) it gave erythrodiol, m.p. and mixed m.p. 230–232°; $(\alpha)_D^{26}+76°$; diacetate (Ac_2O–pyridine) m.p. and mixed m.p. 182–184°. The identity was confirmed by i.r. spectra.

3β-capryloxy oleanolic acid lactone. The parent ester (500 mg) when treated with acetyl chloride (0·5 ml) in pyridine (0·5 ml) at 0° gave after crystallization (alcohol) the lactone (silky needles), m.p. 186–188°; $(\alpha)_D^{34}+36°$; ν_{max} 1727 (ester carbonyl) and 1780 (γ-lactone) cm^{-1} (Found: C, 78·17; H, 10·78 $C_{38}H_{62}O_4$ requires: C, 78·4; H, 10·7%).

Hydrolysis of 3β-Capryloxy-oleanolic Acid

Oleanolic acid and caprylic acid. The ester (500 mg) was refluxed (8 hr) with alcoholic potash (15%) over a steam bath. The hydrolyzate gave oleanolic acid, (400 mg) m.p. and mixed m.p. 308–310; $(\alpha)_D^{34}+80°$; acetate, m.p. 262° (*lit*[17] 262°), $(\alpha)_D^{26}+68°$; methyl ester, m.p. 200° (*lit*[17] 201°) and methyl ester acetate, m.p. 220–221° (*lit*[17] 222°). The identity of oleanolic acid was finally confirmed by superimposable i.r. spectra. From the mother liquor of oleanolic acid, caprylic acid (50 mg) was obtained as viscous liquid (N.E. 144), and its identity confirmed (*vide supra*).

n-*hexacosanol.* The hexane–benzene (40:60) eluent fraction of the chromatogram yielded on recrystallization (alcohol), *n*-hexacosanol (250 mg) m.p. and mixed m.p. 78°; ν_{max} 3332 cm^{-1} (OH); mol. wt. (Rast) 378. (Found: C, 81·51; H, 13·95. Calc. for $C_{26}H_{54}O$: C, 81·75; H, 14·14%).

β-sitosterol. From the chloroform eluent of the chromatogram the sterol (240 mg) was obtained as long needles (alcohol), m.p. 148° $(\alpha)_D^{34}-29°$ (Found: C, 83·6; H, 12·5. Calc. for $C_{29}H_{50}O$: C, 84·0; H, 12·07%). The acetate m.p. and mixed m.p. 127°; the benzoate had m.p. and mixed m.p. 142–144°.

β-D-glucoside of β-sitosterol. The micro-crystalline deposit (1 g) separated as middle layer during partitioning of the alcoholic extractive with hexane, on recrystallization gave β-D-glucoside of β-sitosterol, m.p. and mixed m.p. 298–300° On hydrolysis (5% alcoholic-HCl), it yielded β-sitosterol, m.p. and mixed m.p. 137–138°; $(\alpha)_D^{34}-30°$; acetate, m.p. 127°. Glucose was confirmed in the aqueous fraction of the hydrolyzate by paper co-chromatography in two different solvent systems (*n*-BuOH:AcOH:H_2O::4:1:5 and *n*-BuOH:H_2O:Bz:Py:: 5:3:3:1; aniline pthalate spray).

Constituents of the Nut-Shell

β-D-glucoside of β-sitosterol. The coarsely powdered nut-shell (800 g) were extracted with alcohol (4 l.) at room temperature (20–30°) and the extractive (100 g) thus obtained on maceration with hexane (250 ml), yielded from the middle layer β-D-glucoside of β-sitosterol (60 mg), m.p. and mixed m.p. 294–296° (confirmed as usual, *vide supra*).

Quercetin and dihydroquercetin. The ether soluble fraction (Mg-HCl positive) of the alcoholic extractive, on paper chromatography in two different solvent systems (*n*-BuOH: AcOH:H_2O::4:1:5; BzOH:H_2O::4:1), showed the presence of quercetin (R_f 0·84; bright yellow fluorescence in u.v.) and dihydroquercetin (R_f 0·91; dull violet-red fluorescence in u.v.).

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