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MIMUSOPS HEXANDRA—I.

CONSTITUENTS OF FRUIT AND SEED

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Abstract—A number of triterpenoids including α- and β-amyrin acetates, a tetra-hydroxy alcohol, a monohydroxy monocarboxylic acid and α-spinosterol have been isolated from the mesocarp of *Mimusops hexandra* fruit. Only glucose has been found in the mesocarp, along with the usual amino acids. Seed kernel and the testa yielded the β-D-glucoside of β-sitosterol and quercitol. The sugar moiety of the kernel saponin consists of xylose, arabinose, rhamnose and glucose.

INTRODUCTION

Mimusops hexandra is a tropical tree bearing edible but highly astringent fruits (oval, 1·5 cm long; average wt. 2·1 g) which mature in April–June. The analysis of the fruit as a food material, the physicochemical constants of the seed kernel oil[1] and the identification of the seed sapogenin as bassic acid[2] have been reported earlier. During the present investigation, fresh ripe fruits were collected locally and the fleshy mesocarp along with the skin were separated from the seed. The mesocarp, the testa and the kernel were separately examined.

The mesocarp yielded five triterpenoids: α-spinosterol, α- and β-amyrin acetates, a tetrahydroxy alcohol and a monohydroxy monocarboxylic acid. The acid yielded a methyl-ester acetate on further characterization; the alcohol, however, did not give a pure acetate but it could be characterized as its benzoyl derivative. It did not yield any derivative with carbonyl reagents and showed no carbonyl band absorption in its i.r. spectra. In addition to the products noted above, traces of another Liebermann–Burchard (L–B)-positive alcohol, m.p. 290–300°, presumably a triterpenoid, was also isolated. While glucose was found to be the only sugar, sixteen common amino acids were identified in the acid hydrolysate of the mesocarp; in addition, tyrosine, aspartic and glutamic acids were present in the free state.

The testa and the kernel yielded the β-D-glucoside of β-sitosterol and quercitol. The sugar moiety of the kernel saponin consisted of xylose, arabinose, rhamnose and glucose, not reported earlier.

EXPERIMENTAL

Optical rotations were generally measured in 1% chloroform solution unless otherwise mentioned. Melting points were determined in open capillaries and are uncorrected. Petroleum ether was of 40–60° boiling range. Infrared spectra were recorded in nujol and u.v. spectra in ethanol. Alumina used for chromatography was neutral Brockman (E. Merck) quality.

[1] *The Wealth of India, Raw Materials*, Vol. VI, p. 298, C.S.I.R., New Delhi, India (1962).
[2] A. W. VAN DER HAAR, *Rec. trav. chim.* **49**, 1155 (1929); *Chem. Abstr.* **24**, 857 (1930).

The fresh ripe fruits (9·46 kg; moisture content 71·6 per cent of the mesocarp) were macerated with alcohol and the amber-coloured oval seeds (1·1 kg) were separated from the pulpy mesocarp and the thin lemon-yellow skin.

Constituents of the Mesocarp

The pasty mesocarp (8·31 kg) was successively extracted with alcohol (60 l.; in the cold) and ether (5 l.; Soxhlet) and the extracts, after removal of the solvents, were examined separately and the constituents characterized as noted below.

β-Amyrin acetate. The ether-soluble fraction of the precipitate from the alcoholic extract yielded, on further purification and crystallization from alcohol, an L-B and tetranitromethane-positive product (1·55 g) as white needles, m.p. 240–242°; $(\alpha)_D^{30}$ +90°; ν_{max} 1727 cm^{-1} (Found: C, 82·33; H, 11·43. Calc. for $C_{32}H_{52}O_2$: C, 82·06; H, 11·02%). Alkaline hydrolysis of the compound (225 mg) gave β-amyrin (181 mg) as silky needles, m.p. 192–196°; $(\alpha)_D^{17}$ +87·5° (*lit* +86°); ν_{max} 3333 cm^{-1} (Found: C, 84·48; H, 12·19. Calc. for $C_{30}H_{50}O$: C, 84·50; H, 11·73%). Acetic acid was found in the distillate of the aqueous hydrolysate,[3] indicating that the parent compound was β-amyrin acetate.

Acetylation of β-amyrin (50 mg) with acetic anhydride and fused sodium acetate gave β-amyrin acetate (45 mg), m.p. 225–235 (*lit* 240°); ν_{max} 1730 cm^{-1} (Found: C, 82·15; H, 11·92%) identical with the isolated product (mixed m.p. 225–235°).

β-Amyrin acetate was further identified by treating it with selenium dioxide when it yielded the oxidation product,[4] $C_{32}H_{50}O_2$, m.p. 175–178°; $(\alpha)_D^{28}$ +23° having characteristic u.v. absorption at 242 ($\log_\epsilon$ 4·02), 250 ($\log_\epsilon$ 4·07) and 259 ($\log_\epsilon$ 3·90) mμ. Found: C, 81·46; H, 11·09. Calc. C, 82·40; H, 10·73%.

The residue from the ethereal mother liquor of β-amyrin acetate yielded a micro-crystalline deposit, on keeping its petroleum ether solution in the cold. The deposit, on extraction with aqueous alkali, gave an acidic and a neutral component noted below.

A triterpenic monohydroxy monocarboxylic acid. The acidic component on successive crystallization from alcohol and chloroform:methanol (1:1) yielded an L-B and tetranitromethane-positive hydroxy acid (1 g), m.p. 263–266°; $(\alpha)_D^{26}$ +55° (pyridine); ν_{max} 3488 and 1684 cm^{-1} (Found: C, 78·41; H, 11·47. $C_{30}H_{50}O_3$ required: C, 78·60; H, 10·91%). The acid sublimed unchanged at 235–240°/2 mm and the sublimed product (m.p. 260°) did not depress the melting point of the original compound.

The methyl ester acetate of the acid. The methyl ester of the acid, prepared by diazomethane in methanol–ether, after crystallization from chloroform–methanol, melted at 125–130°; $(\alpha)_D^{30}$ +60°. Acetylation of this methyl ester (100 mg) with acetic anhydride–pyridine (2:1; 1·5 ml) under reflux, followed by chromatography (alumina: ether–petroleum ether) and crystallization (petroleum ether) yielded the methyl ester acetate as needles, m.p. 215–230°; $(\alpha)_D^{32}$ +58° (Found: C, 77·24; H, 10·75. $C_{33}H_{54}O_4$ required: C, 77·04; H, 10·50%).

Triterpenic tetra-hydroxy alcohol. The neutral residue, left after complete removal of the acidic component, on crystallization from hot alcohol yielded a tetra-hydroxy alcohol (950 mg), m.p. 215–220° (Found: C, 75·78; H, 10·88. $C_{30}H_{50}O_4$ required: C, 75·95; H, 10·55%). Benzoylation of the alcohol (50 mg) with benzoyl chloride and pyridine yielded after chromatography (alumina; benzene–chloroform) and crystallization (alcohol) the tetra-

[3] F. FEIGL, *Spot Tests*, Vol. II, p. 247, Elsevier Publishing Co., London (1954).
[4] A. SANDOVAL, A. MANJARREZ, P. R. LEEMING, G. H. THOMAS and C. DJERASSI, *J. Am. Chem. Soc.* **79**, 4468 (1957).

benzoate (78 mg) melting at 254–258° (Found: C, 78·38; H, 10·8. $C_{58}H_{66}O_8$ required: C, 78·20; H, 7·4%).

α-Amyrin acetate. The semi-solid residue obtained on removal of the solvent from the mother liquor of the β-amyrin acetate fraction was chromatographed on alumina (250 g; petroleum ether) when α-amyrin acetate (39 g) was isolated as white needles, m.p. 220–222°; $(\alpha)_D^{17}$ +82·3°; $\nu_{max.}$ 1745 cm^{-1} (Found: C, 81·90; H, 10·80. Calc. for $C_{32}H_{52}O_2$: C, 82·06; H, 11·02%). Alkaline hydrolysis (1 g) gave α-amyrin (800 mg) as silky needles, m.p. 182–184° (*lit* 184°); $(\alpha)_D^{20}$ +80° (*lit* +84°); $\nu_{max.}$ 3333 cm^{-1} (Found: C, 84·13; H, 12·37. Calc. for $C_{30}H_{50}O$: C, 84·50; H, 11·73%). Acetic acid was found in the distillate of the aqueous hydrolysate,[3] thus establishing the parent compound to be α-amyrin acetate. Treatment of α-amyrin (50 mg) with acetic anhydride and fused sodium acetate gave the acetate (47 mg), m.p. 195–205° (*lit* 210–224°); $\nu_{max.}$ 1730 cm^{-1} (Found: C, 81·62; H, 11·48%) identical with the isolated product (mixed m.p. 210–215°).

α-Spinosterol. Further petroleum ether elution of the alumina column yielded α-spinosterol (380 mg), m.p. 165–167° (*lit* 165°); $(\alpha)_D^{27}$ −9° (*c*, 1·1; *lit* −4°).[5] It was identified by its derivatives and by comparing its i.r. spectra with that of an authentic sample.[6] α-Spinosterol acetate, prepared with acetic anhydride and fused sodium acetate, crystallized (alcohol) as silky needles, m.p. 173–174° (*lit* 174°). α-Spinosterol benzoate, prepared with benzoyl chloride and pyridine at 100°, was obtained as needles, m.p. 192–194° (*lit* 194°).

Sugar and amino acids. The aqueous fraction of the mesocarp, after paper chromatography, showed the presence of glucose, and of tyrosine, aspartic and glutamic acids as free amino acids. The acid hydrolysate of the extracted mesocarp, after two-dimensional chromatography (nBuOH:AcOH:H_2O,4:1:1; BzOH:H_2O,4:1) showed the presence of sixteen common amino acids, including methionine and hydroxy-proline.

Constituents of the Testa

β-D-Glucoside of β-sitosterol. The alcoholic extractive of the powdered testa (550 g) yielded the β-D-glucoside of β-sitosterol[7] (60 mg), m.p. 290° (mixed m.p. with an authentic sample 290–293°).

Quercitol. The alcoholic concentrate, freed of the sterol-glucoside, gave on cooling, quercitol (950 mg), m.p. 236–238°; mixed m.p. with an authentic sample 237°.

Constituents of the Seed Kernel

β-D-glucoside of β-sitosterol. Ths alcoholic extract of the kernel (558 g) yielded, from its petroleum ether soluble portion, the β-D-glucoside of β-sitosterol (200 mg), m.p. 286°. Acid hydrolysis of the glucoside (70 mg) yielded glucose, confirmed by paper chromatography, and β-sitosterol, m.p. 138°, confirmed by its i.r. spectra being identical to that of an authentic sample.[8]

Quercitol. The alcoholic extract, freed of the glucoside, on refrigeration deposited quercitol (2·4 g), m.p. and mixed m.p. 238°; $(\alpha)_D^{30}$ +25° (*c*, 1·0; water; *lit* +24°) (Found: C, 43·87; H, 7·70. Calc. for $C_6H_{12}O_5$: C, 43·90; H, 7·32%). The benzoate of quercitol melted at 153–155° (*lit* 155°); $(\alpha)_D^{30}$ +50° (*lit* +61·4°).

[5] L. F. Fieser and M. Fieser, *Steroids*, p. 352, Chapman & Hall, Ltd., London (1959).
[6] G. Misra, C. R. Mitra and K. N. Kaul, *J. sci. ind. Research (India)*, **21B**, 238 (1962).
[7] G. Brochere-Ferrol, J. Polonsky and C. R. Mitra, *Compt. Rend.* **246**, 3082 (1958).
[8] G. Misra, V. N. Sharma, C. R. Mitra and K. N. Kaul, *Soap, Perfumery and Cosmetics*, **34**, 761 (1961).

Sugar moiety of the kernel saponin. An aliquot portion of the purified saponin (yield 66 g: m.p. 230–235°), on acid hydrolysis, gave xylose, arabinose, rhamnose and glucose, as detected by paper chromatography.

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