Phytochemistry, 1969, Vol. 8, pp. 249 to 252. Pergamon Press. Printed in England

MIMUSOPS MANILKARA, CONSTITUENTS OF FRUIT AND SEED

G. MISRA and C. R. MITRA

Utilization Research Laboratory National Botanic Gardens, Lucknow, India

(*Received* 11 *June* 1968)

Abstract—The mesocarp yielded caprylic acid esters and acetates of α- and β-amyrins, α-spinasterol and β-D-glucoside of β-sitosterol; the seed coat constituents including dihydroquercetin and quercetin were the same as those present in the seed coats of the other two spp. of *Mimusops*; xylose has also been found to be a constituent sugar of the seed kernel saponin besides those reported in the past.

INTRODUCTION

Mimusops manilkara[1] syn. *Achras sapota* (Sapotaceae), commonly known as *Sapota* or *Sapodilla*, is a tropical tree, cultivated for its sweet tasty fruits (oval berry; 5–10 cm dia.; wt. 60–90 g) which mature during March–September. The fruit, seed and the bark are recorded to have medicinal uses[2] and the latter produces chicle gum[1] (cf. gutta percha). Sporadic work on the analysis of the fruit (as a food material),[1] the physico-chemical constants of the seed kernel fat[3] and the identification of the seed sapogenin as bassic acid,[4] is reported in literature.

In pursuance of the studies[5–7] in the constituents of sapotaceous plants, systematic chemical examination of the mesocarp (with the skin), the seed coat and the kernel of *M. manilkara* has been carried out. The mesocarp yielded caprylic acid ester of α- and β-amyrins along with their acetates, α-spinasterol and β-D-glucoside of β-sitosterol. The capyrlates of α- and β-amyrins appear to have been isolated for the first time from nature while the presence[8] of myristate, palmitate and stearate of β-amyrin in the plants are already recorded. Recently palmitic and oleic acid esters of β-amyrin[9] and caprylic acid[10] ester of erythrodiol have been isolated from the plants of this family besides the reported presence of cinnamates of the amyrins,[6,7] fatty acid ester of a sterol,[6] caprylate[10] of oleanolic acid and palmitate[11] of erythrodiol, betulinic and oleanolic acids. The analogy in regard to the presence of the different constituents in the plants of this family add to its chemotaxonomic features.

[1] *The Wealth of India, Raw materials*, Vol. 1, p. 23, CSIR, New Delhi, India (1948).
[2] W. DYMOCK, *Pharmacographia Indica*, Vol. 2, p. 365. Kegan Paul, Trench, Trübner, London (1891).
[3] N. L. VIDYARTHI and M. V. MALLYA, *J. Indian Chem. Soc.* **16**, 443 (1939).
[4] B. J. HEYWOOD and G. A. R. KON, *J. Chem. Soc.* 713 (1940).
[5] C. R. MITRA and G. MISRA, *Phytochem.* **4**, 345 (1965).
[6] G. MISRA and C. R. MITRA, *Phytochem.* **5**, 535 (1966); **6**, 453 and 1309 (1967); **7**, 501 (1968).
[7] G. MISRA and C. R. MITRA, *Phytochem.* **7**, 2173 (1968).
[8] J. L. SIMONSEN and W. C. J. ROSS, *The Terpenes*, Vol. 4, p. 174, Cambridge University Press, London (1957).
[9] G. WEISSMANN and W. SANDERMANN, *Phytochem.* **7**, 467 (1968).
[10] Y. C. AWASTHI and C. R. MITRA, *Phytochem.* **6**, 121 (1967).
[11] Y. C. AWASTHI and C. R. MITRA, *Phytochem.* **7**, 637 (1968).

α- and β-Amyrin Caprylates

The esters were isolated from the *n*-hexane soluble fraction of the alcohol extract of the mesocarp by chromatography followed by crystallization. α-Amyrin caprylate, $C_{38}H_{64}O_2$, melted at 158–160°; $(\alpha)_D + 76{\cdot}5°$; and the β-amyrin caprylate melted at 97–98°; $(\alpha)_D + 70{\cdot}5°$. The i.r. spectra of both the esters had similar absorption pattern and showed, apart from usual peaks of the triterpenes, the characteristic peak of an ester carbonyl[12] at 1735 cm^{-1} supported by a peak at 1180 cm^{-1}. The unusually strong absorption at 1180 cm^{-1} was indicative of the esters being of higher homologue than propionic acid.[12]

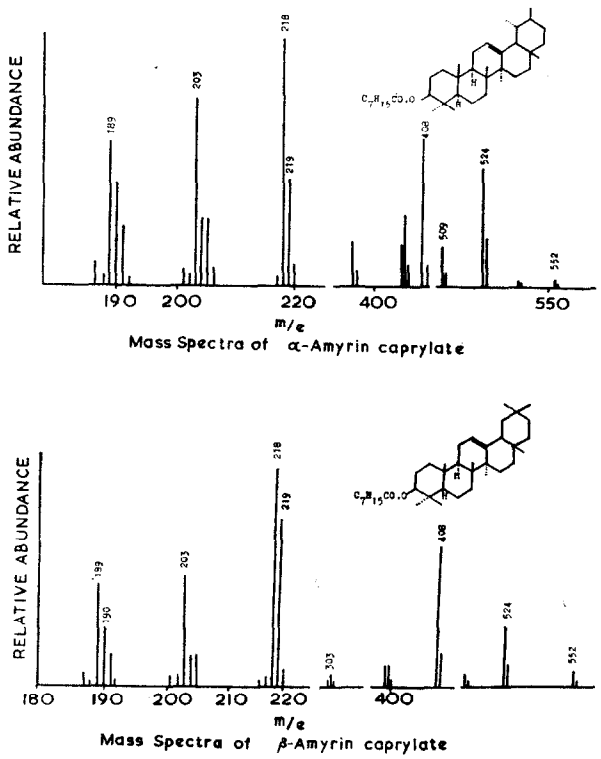

FIG. 1. *Mimusops manilkara.*

The fragmentation pattern in the mass spectra (Fig. 1) of both the esters was similar to those of the Δ^{12}-oleanane/ursane series of triterpenoids.[7,13] In the present cases the molecular ion, $M^+ = 552$ *m/e* underwent a loss of 144 (caprylic acid) mass units to give a peak at 408 *m/e*. While the peaks at 333 and 218 (base peak) *m/e* were due to the *retro*-Diels-Alder fragmentation[13] with the usual hydrogen transfer, characteristic of the left and right half arising from triterpenes having Δ^{12}-oleanane/ursane structure; the peak at 190 (333–143) *m/e* was due to the loss of the ester moiety from the left half providing conclusive proof for the attachment of the ester grouping at C^{-3}.

Alkaline hydrolysis of the esters yielded in both cases, caprylic acid, neutr. equiv. 146 (Calc. 144) along with the corresponding neutral triterpene alcohols, (i) $C_{30}H_{50}O$, m.p.

[12] L. J. BELLAMY, *The Infra-red Spectra of Complex Molecules*, p. 179, Methuen, London (1962).
[13] H. BUDZIKZEWIC, J. M. WILSON and C. DJERASSI, *J. Am. Chem. Soc.* **85**, 3688 (1963).

182°; $(\alpha)_D$+82°; acetate, m.p. 222°; $(\alpha)_D$+65° and (ii) $C_{30}H_{50}O$, m.p. 186°; $(\alpha)_D$+84° and acetate, m.p. 224°; $(\alpha)_D$+68°. However, selenium dioxide oxidation of the acetates distinguished the former as α- and the latter as β-amyrin acetate (characteristic u.v. absorption for heteroannular $\Delta^{11,13(18)}$-diene)[14] and thus, the esters were identified as α- and β-amyrin caprylates respectively.

The seed coat yielded all the constituents including dihydroquercetin and quercetin reported from that of *Mimusops hexandra*[6] and *M. elengi*.[6] The same flavonoids have also been reported from *Madhuca butyracea*[15] and *M. latifolia*[10] (Sapotaceae) seed coats which further extends support to this chemotaxonomic characteristic of the Sapotaceae family.

Xylose has also been found to be a constituent sugar of the kernel saponin in addition to glucose, arabinose and rhamnose reported in the past.[16] α-Spinasterol was identified in the unsaponifiable matter of the kernel fat.

EXPERIMENTAL

The melting points (uncorrected) were determined in open capillaries; optical rotations were measured in chloroform solution (1%); i.r. spectra were recorded in K^{Br} films; the alumina used for chromatography was neutral Brokmann (E. Merck) quality and the *n*-hexane was the petroleum cut b.p. 70°

The ripe fruits (19·6 kg) of *M. manilkara* were bruised to separate the pulpy mesocarp (19 kg; moisture 72 per cent) from the amber coloured oval seeds (530 g) which were decorticated to yield the seed coat (272 g) and the kernel (252 g).

Constituents of the Mesocarp

The mesocarp (along with the skin) was successively extracted with alcohol (10 l. × 4) and hexane (10 l. × 6) and the extracts after removal of the solvents were examined separately.

β-D-Glucoside of β-sitosterol. The LB positive microcrystalline deposit (1·56 g), separated during partitioning of the aqueous alcoholic extract with hexane, on crystallization from excess alcohol melted at 286–288°; $(\alpha)_D^{20}$ −33° (*c*, 1·0 per cent; pyridine); identified through mixed m.p., superimposable i.r. spectra and acid hydrolysis to *β-sitosterol* and *glucose*.

A portion (30 g) of the semi-solid hexane extract (250 g) on chromatography over alumina (1 :20) gave from the initial fractions of the hexane eluate a yellow sticky mass (29 g) and from the latter fractions a white crystallisate (635 mg).

α-Spinasterol. The LB and Tortelli-Jaffe positive crystallisate on purification through chromatography followed by crystallisation yielded α-spinasterol, m.p. 164–165°; $(\alpha)_D^{22}$ −3·5°; identified through mixed[6] m.p.; co-TLC; superimposable i.r. spectra and benzoate, m.p. 193–194° (*lit.* 194°).

The yellow sticky mass on crystallization from ether-alcohol (1 :1) gave a product (10 g) which on chromatography over alumina (1 :50) furnished two fractions, one (8·5 g), m.p. 98–120° and the other (800 mg), m.p. 205–210°. On fractional crystallizations (alcohol) the major fraction (m.p. 98–120°) yielded two crystalline products, m.ps. 150° and 97° when the minor fraction (m.p. 205–210°) gave two more products, m.p.s 230° and 220° respectively.

α-Amyrin caprylate. The substance (3·4 g), m.p. 150° from the major fraction on purification through chromatography followed by crystallizations gave silky needles, m.p. 158–160°; $(\alpha)_D^{34}$+76·5°; ν_{max} 1715, 1176 (ester carbonyl), 1460, 1399, 1379, 1364, 1333, 1290, 1232, 1212, 836, 822 and 725 (−$(CH_2)_4$—or more) cm^{-1} and prominent peaks in the mass spectra at 552 (M^+), 537, 524, 509, 408, 333, 218, 205, 204, 203, 192, 191, 190 and 189 *m/e* (Found: C, 82·91; H, 12·09. $C_{38}H_{64}O_2$ required: C, 82·60; H, 11.59 per cent).

The ester (800 mg) on hydrolysis with alcoholic KOH (4%; 100 ml) gave *α-amyrin* (599 mg), m.p. 181–182°; $(\alpha)_D^{34}$ 82°; ν_{max} 3238 cm^{-1} (OH); identified by mixed m.p. (182–183°), superimposable i.r. spectra and its acetate, m.p. and mixed m.p. 220–222°; $(\alpha)_D^{34}$+65° which remained unchanged on SeO_2 oxidation. The acid fraction (178 mg, viscous liquid) of the hydrolysate after usual purification identified as *caprylic acid*, neutr. equiv. 146 (Calc. for $C_8H_{16}O_2$: 144).

β-Amyrin caprylate. The product (3·6 g), m.p. 97° from the major fraction on usual purification (*vide supra*) yielded shining needles of β-amyrin caprylate, m.p. 97–98°; $(\alpha)_D^{34}$+70·5; ν_{max} 1735 and 1180 cm^{-1} (ester

[14] J. SIMONSEN and W. C. J. ROSS, *The Terpenes*, Vol. 4, p. 196, Cambridge University Press, London (1957).
[15] Y. C. AWASTHI and C. R. MITRA, *J. Org. Chem.* **27**, 2636 (1962).
[16] A. W. VAN DER HAAR, *Rec. Trav. Chim.* **41**, 784 (1922); and **48**, 1155 (1929); *Chem. Abstr.* **24**, 857 (1930).

carbonyl) and prominent peaks in the mass spectra at 552 (M^+), 408, 333, 218 and 190 *m/e* (Found: C, 82·42; H, 12·02. $C_{38}H_{64}O_2$ required: C, 82·60; H, 11·59 per cent).

The ester (1·08 g) on alkali hydrolysis yielded the neutral (724 mg) as *β-amyrin*, m.p. and mixed m.p. 184–186°; $(\alpha)_D^{34}$ +84°; ν_{max} 3262 cm^{-1} (OH); superimposable i.r. spectra; co-TLC and its acetate, m.p. 222–224°; $(\alpha)_D^{38}$ 68° which on SeO_2 oxidation furnished the heteroannular $\Delta^{11,13(18)}$-diene[14] having u.v. (alcohol) maxima at 242, 251 and 261 mμ (log ε 4·12, 4·36 and 3·98 respectively) and the acid part (185 mg) as *caprylic acid.*

β-Amyrin acetate. The compound (80 mg), m.p. 230° from the minor fraction (*vide supra*) on crystallization from alcohol gave needles of β-amyrin acetate, m.p. 232–234°; $(\alpha)_D^{34}$ +86°; ν_{max} 1730 and 1248 cm^{-1} (acetate) (Found: C, 82·28; H, 11·49. Calc. for $C_{32}H_{52}O_2$: C, 82·06; H, 11·02 per cent); identity confirmed by mixed m.p., superimposable i.r. spectra and alkali hydrolysis to *β-amyrin*, m.p. and mixed m.p. 190–191°; $(\alpha)_D^{34}$ +81°; ν_{max} 3320 cm^{-1} (OH) and *acetic acid* (positive lanthanum nitrate-iodine test).[17]

α-Amyrin acetate. The compound (180 mg), m.p. 220° from the minor fraction on usual purification gave fine needles of α-amyrin acetate, $C_{32}H_{52}O_2$, m.p. 223–225°; $(\alpha)_D^{34}$ +80·5°; ν_{max} 1728 and 1250 cm^{-1} (acetate); identified through mixed m.p., i.r. spectra and alkali hydrolysis to α-amyrin, m.p. 180–182° and *acetic acid.*

Constituents of the Seed Coat

β-D-Glycoside of β-sitosterol. The alcoholic extract of the powdered seed coat when partitioned with hexane yielded the sterol glucoside as a microcrystalline deposit (28 mg), m.p. 280–282°, identified as described earlier.

Dihydroquercetin and quercetin. The ether soluble fraction (310 mg; Mg–HCl positive) of the alcoholic extract on paper chromatography (*n*-BuOH:AcOH:H_2O, 4:1:5; BzOH:H_2O, 4:1) showed the presence of two flavonoids, R_f 0·90 (violet red in u.v.) and 0·83 (bright yellow in u.v.) respectively; identified and confirmed by co-chromatography and u.v. absorption at 287, 322 and 258, 376 nm respectively.

Quercitol. The alcohol extract, freed of the above constituents, on cooling deposited quercitol (300 mg), m.p. and mixed m.p. 235–236°.

Constituents of the Seed Kernel

The alcoholic extract of the kernel yielded besides *quercitol*[16] and bassic acid *saponin*,[16] *β-D-glucoside of β-sitosterol* (identified as described earlier). The hexane extract of the alcohol extracted kernel yielded the fixed fat[3] (11 per cent of the kernel).

Sterol from the Kernel Fat

α-Spinasterol. The unsaponifiable matter (402 mg) of the purified and refined fat (23 g) on chromatography followed by crystallization (hexane) yielded shining needles of α-spinasterol (108 mg), m.p. and mixed m.p. 162–164°; $(\alpha)_D^{34}$ −2·8°; TLC single spot; identified as described earlier.

Acknowledgements—Authors' thanks are due to Mr. J. Saran for microanalyses, to Dr. K. G. Dass for recording the mass spectra and to Dr. L. B. Singh, Director, National Botanic Gardens, Lucknow for his interest in the work.

[17] F. FEIGL, *Spot Tests*, Vol. 2, p. 247, Elsevier, London (1954).