Phytochemistry, 1968, Vol. 7, pp. 2173 to 2176. Pergamon Press. Printed in England

MIMUSOPS HEXANDRA—III.

CONSTITUENTS OF ROOT, LEAVES AND MESOCARP

G. MISRA and C. R. MITRA

Utilization Research Laboratory, National Botanic Gardens, Lucknow, India

(*Received* 1 *May* 1968)

Abstract—The cinnamic acid ester of α- and β-amyrins along with taraxerol, α-spinasterol, quercitol and β-D-glucoside of β-sitosterol have been isolated from the roots. The leaves yielded cinnamic acid, hentriacontane, a triterpene ketone, a terpenic hydrocarbon besides taraxerol and quercitol. The triterpene acid of the mesocarp[1] has since been identified as ursolic acid.

INTRODUCTION

WHILE pursuing the investigation with the different parts of *Mimusops hexandra* tree (Sapotaceae), chemical examination of the root and the leaves was carried out. The former yielded cinnamic acid ester of α- and β-amyrins along with taraxerol, α-spinasterol, β-D-glucoside of β-sitosterol and quercitol and the latter yielded besides taraxerol and quericitol, cinnamic acid, hentriacontane, a triterpene ketone, m.p. 318–320°; $[\alpha]_D^{22}+65°$, a terpenic hydrocarbon, m.p. 54–55°; $[\alpha]_D^{21}-2{\cdot}5°$ and a thermoplastic resinous product. Besides characterization of the products and their derivatives by analytical, physicochemical and spectrophotometric methods, the identity of the esters were confirmed by chemical degradation and mass spectra as well.

The cinnamic acid esters of the amyrins appear to have been isolated for the first time from the Sapotaceae. The isolation of cinnamic acid along with α- and β-amyrins[2] from plants of this family as well as from the bark[3] of this plant have already been recorded in literature but the ester as such was not isolated. Both the esters were obtained from the *n*-hexane-soluble fraction of the alcoholic extractive of the root by chromatography followed by crystallization. The i.r. spectra of the esters are similar, showing, apart from usual peaks of triterpenes, the characteristic peak of an α,β-unsaturated ester carbonyl[4] at 1710 cm^{-1} supported by one at 1180 cm^{-1} with absorption frequencies between 1600–1450 cm^{-1} for the presence of a phenyl substituent[4] in the compound. They also showed u.v. absorption at 217 nm ($\Delta^{\alpha,\beta}$).[4,5]

The fragmentation pattern in the mass spectra of both the esters (Fig. 1 & 2) was similar to those of the derivatives of Δ^{12}-oleanene/ursene.[6,7] In the present cases the molecular ion, $M^+=556$ *m/e*, underwent a loss of 148 mass units ($C_6H_5{\cdot}CH{=}CH{\cdot}COOH$) to give an

[1] C. R. MITRA and G. MISRA, *Phytochem.* **4**, 345 (1965).
[2] R. F. A. ALTMAN, *Bol. Tec. Inst. Agron. Norte* (*Belem Brazil*) **31**, 81–95 (1956); *Chem. Abstr.* **52**, 506 (1958).
[3] G. MISRA and C. R. MITRA, *Phytochem.* **5**, 535 (1966).
[4] A. K. BARUA, S. P. DUTTA and B. C. DASS, *Tetrahedron* **24**, 1113 (1968).
[5] C. N. R. RAO, *Ultraviolet and Visible Spectroscopy*, p. 25, Butterworth, London (1961).
[6] Y. C. AWASTHI and C. R. MITRA, *Phytochem.* **7**, 637 (1968).
[7] H. BUDZIKIEWICZ, J. M. WILSON and C. DJERASSI, *J. Am. Chem. Soc.* **85**, 3688 (1963).

abundant peak at 408 *m/e*. While the peaks at 337 and 218 *m/e* were due to the *retro*-Diels-Alder fragmentation,[7] characteristic of the left and right half arising from compounds having Δ^{12}-oleanene/ursene structure, the peak at 190 (337–147) *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 position 3.

Alkaline hydrolysis of the esters gave cinnamic acid along with the corresponding amyrins.

In addition to the constituents already reported[1] the *M. hexandra* mesocarp yielded on re-investigation an ester, presumably of ursolic acid, m.p. 118–120°; ν_{max} 1730 and 1686 cm^{-1} (ester and acid carbonyls respectively) and no hydroxyl band, along with β-D-glucoside of β-sitosterol and ursolic acid, recorded earlier,[1]

The acid, m.p. 280–285°; $(\alpha)_D + 50°$ reported from the bark[2] has since been identified as ursolic acid by co-TLC and i.r. spectra.

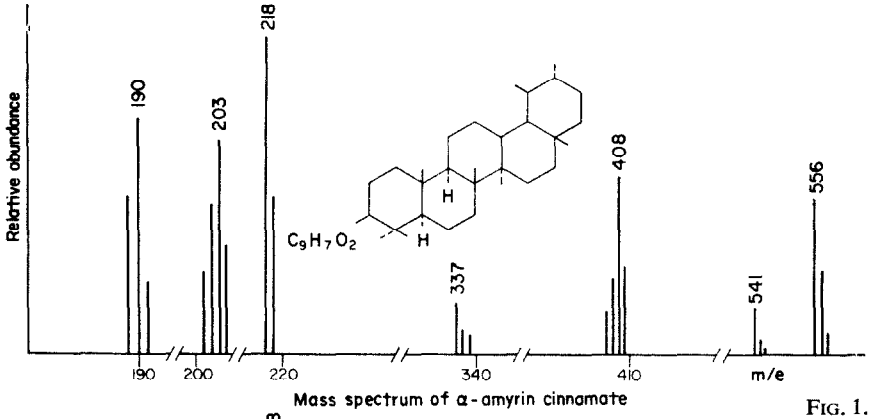

FIG. 1.

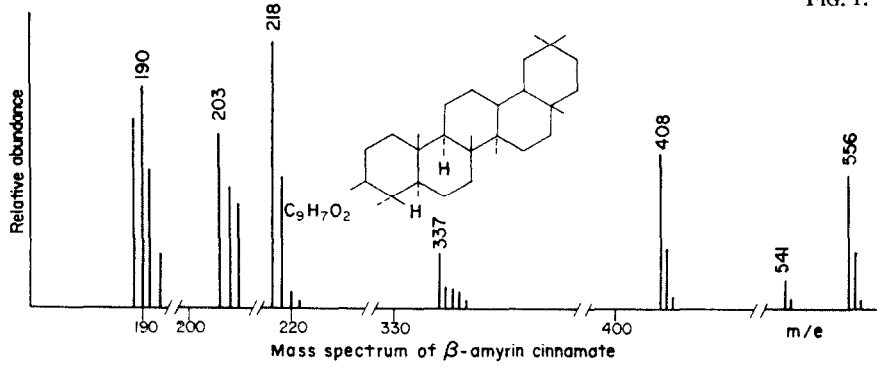

FIG. 2.

EXPERIMENTAL

Unless otherwise mentioned, optical rotations were measured in $CHCl_3$ (1%); melting points determined in open capillaries are uncorrected; i.r. spectra were recorded in KBr films and the alumina used for chromatography was neutral Brokmann (E. Merck) quality.

Constituents of the Root

The concentrate of the alcoholic percolate (6 × 5 l.) of the air-dried powdered root (2·9 kg) of *Mimusops hexandra* deposited on keeping in cold a microcrystalline powder which after separation and recrystallization

(50% alcohol) yielded *Quercitol* (13·4 g), m.p. and mixed m.p. 235–236°; $[\alpha]_D^{22}+27°$ (*c.* 1·0; water), confirmed through its benzoate, m.p. 154–155°; $[\alpha]_D^{34}+58°$.

The mother liquor of quercitol was exhaustively extracted with hexane (2·5 l.) when a hexane-soluble fraction (17 g), responding to Lieberman-Burchard (L-B) and Noller's tests, was obtained and subjected to alumina (260 g) column chromatography using successively hexane, benzene, chloroform and methanol as eluents. The initial fractions of hexane eluate yielded a golden yellow viscous liquid (12 g) from which α- and β-amyrin cinnamates (m.p. 150–160°; 6·9 g and m.p. 220–225°, 2·2 g respectively) were obtained by rechromatography (alumina, 1:100) followed by repeated crystallizations.

α-Amyrin cinnamate. The product melting at 150–160° on repeated crystallizations from alcohol finally furnished a pure compound (TLC), m.p. 177–178°; (lit.[8] 179°); $[\alpha]_D^{34}+68°$ (lit.[8] +79°); ν_{max} 1710, 1630, 1570, 1478, 1450, 1380, 1362, 1180, 765 and 706 cm^{-1} and prominent peaks in the mass spectra at 556 (M^+), 541, 408, 337, 218, 205, 204, 203, 192, 191, 190 and 189 *m/e* (Found: C, 83·56; H, 10·72. $C_{39}H_{56}O_2$ required: C, 84·16; H, 10·08%).

The ester (640 mg) on hydrolysis with alcoholic KOH (2%; 100 ml) gave α-amyrin (474 mg), m.p. 177–178°; $[\alpha]_D^{34}+88°$; ν_{max} 3228 cm^{-1} (OH), confirmed by mixed[3] m.p. (181–182°), superimposable i.r. spectra and by prepn. of its acetate, m.p. 210–212°. The acid fraction of the hydrolysate yielded cinnamic acid (150 mg) as needles, m.p. and mixed m.p. 130–131° (Found: C, 73·04; H, 5·61. Calc. for $C_9H_8O_2$: C, 72·97; H, 5·41%); neutr. equiv. 147 (calc. 148), confirmed by superimposable i.r. spectra, and by prepn. of the dibromide, m.p. 202–203° (lit.[9] 203–204°) (Found: Br. 51·80. Calc. for $C_9H_8O_2Br_2$: Br, 51·95%).

β-Amyrin cinnamate. The product melting at 220–225° on repeated crystallizations from ether–alcohol and alcohol furnished β-amyrin cinnamate, single spot in TLC, m.p. 230° (lit.[10] 236°); $[\alpha]_D^{34}+63°$; ν_{max} 1709 (CO), 1635, 1570, 1495, 1478, 1450, 1380, 1362, 766 and 706 cm^{-1} and prominent peaks in the mass spectra at 556 (M^+), 408, 337, 218 and 190 *m/e* (Found: C, 84·86; H, 10·78. $C_{39}H_{56}O_2$ required: C, 84·16; H, 10·08%).

The ester (120 mg) on alkali hydrolysis gave the neutral fraction (68 mg), confirmed as β-amyrin, m.p. 192–193°; mixed [1] m.p. (194°); $[\alpha]_D^{34}+90°$; ν_{max} 3320 cm^{-1} (OH); superimposable i.r. spectra and co-TLC, and the acidic fraction (25 mg), confirmed as cinnamic acid (*vide supra*).

Taraxerol. Another L-B positive compound from the later hexane eluates on crystallization (hexane–benzene) furnished taraxerol (162 mg), m.p. 280–282° (lit.[3] 283°); $[\alpha]_D^{22}+2°$ (lit.[3] ±0°); ν_{max} 3440 cm^{-1} (OH); (Found: C, 84·26; H, 12·01. Calc. for $C_{30}H_{50}O$: C, 84·50; H, 11·73%); confirmed by mixed[3] m.p. (281–282°); superimposable i.r. spectra and acetate, m.p. 302–304° (lit.[3] 302°); $(\alpha)_D^{22}+11°$ (lit.[3] +10°).

α-Spinasterol. The L-B and Tortelli–Jaffe positive product from the mother liquor of taraxerol, on crystallization yielded α-spinasterol (290 mg), m.p. 163–165°; $(\alpha)_D^{22}-5·5°$ (lit.[11] −4°); confirmed through mixed[3] m.p. (164–166)°; co-TLC; i.r. spectra and acetate, m.p. 176–178° (lit. 176–177°); $(\alpha)_D^{22}-3·8°$ (lit. −4°).

β-D-Glucoside of β-sitosterol (1 g), obtained from the chloroform–methanol eluate, after crystallization (excess alcohol) melted at 286–288°; $[\alpha]_D^{22}-32°$; identity confirmed through mixed m.p.; i.r. spectra and its tetra-acetate, m.p. 168–169°; on acid hydrolysis it furnished β-sitosterol and glucose.

Constituents of the Leaves

The *M. hexandra* leaf powder (4·15 kg) was successively percolated with hexane (10 × 5 l.) and alcohol (10 × 8 l.). The hexane concentrate (500 ml) on cooling deposited an L-B positive yellow product (10 g) which on chromatography (alumina, 160 g; eluent, hexane–benzene, 1:1) yielded: (i) *a terpenic hydrocarbon* (2·4 g) which on crystallization from hexane gave needles, m.p. 54–55°; $[\alpha]_D^{21}-2·5°$; ν_{max} 1667 and 806 cm^{-1} (di- or trisubstituted double bond)[12], 1439, 1379 and 1212 cm^{-1} (methyl and *gem*-dimethyl), no absorption in the hydroxyl region (Found: C, 88·53; H, 12·15. Calc. for $(C_5H_8)_n$: C, 88·23; H, 11·76%) and (ii) *Taraxerol* (6 g), m.p. 282–284°; $[\alpha]_D^{22}+1°$; ν_{max} 3448 cm^{-1} (OH); formate, m.p. 238–240° (lit.[13] 247–249°); $(\alpha)_D^{22}+0·5°$ (Found: C, 82·60; H, 11·68. Calc. for $C_{31}H_{50}O_2$: C, 81·94; H, 11·01%); confirmed as described earlier (*vide supra*).

The sticky yellow compound (20 g) from the mother liquor of the above crystallisate was saponified (10% alc. KOH; 1·5 l.) and the hydrolysate on usual working yielded from the hexane-soluble neutral fraction (alumina chromatography), (iii) *hentriacontane* (5 g), m.p. 65°; ν_{max} 727 cm^{-1} (alkane chain)[14] (Found: C, 85·69; H, 14·45. Calc. for $C_{31}H_{64}$: C, 85·29; H, 14·68%); identified through mixed[14] m.p. and superimposable i.r. spectra and (iv) *a triterpene ketone* (70 mg), m.p. 318–320°; $[\alpha]_D^{22}+65°$; ν_{max} 1690 cm^{-1} (CO) and other characteristic peaks of triterpenes (Found: C, 85·53; H, 11·48. $C_{30}H_{48}O$ required: C, 84·90; H, 11·32%). The hexane-insoluble neutral fraction (7 g) furnished (v) *a hard thermoplastic white product* (cf.

[8] J. SIMONSEN and W. C. J. ROSS, *The Terpenes*, Vol. IV, p. 117, Cambridge University Press, London (1957).
[9] E. H. HUNTRESS and S. P. MULLIKEN, *Identification of Pure Organic Compounds, Order I*, p. 150, Chapman and Hall, London (1953).
[10] W. KERN and W. HASELBECK, *Arch. Pharm.* **280**, 277 (1942).
[11] L. F. FIESER and M. FIESER, *Steroids*, p. 352, Chapman and Hall, London (1959).
[12] L. J. BELLAMY, *The Infrared Spectra of Complex Molecules*, p. 34, Methuen, London (1962).
[13] J. SIMONSEN and W. C. J. ROSS, *The Terpenes*, Vol. IV, p. 278, Cambridge University Press, London (1957).
[14] G. MISRA and C. R. MITRA, *Phytochem.* 7, 501 (1968).

gutta percha) softening at 50–60°; soluble in CS_2, $CHCl_3$, CCl_4, C_6H_6 and hot hexane; and the acidic fraction, (vi) *Cinnamic acid* (110 mg), m.p. and mixed m.p. 133–134°.

The alcoholic extract of the leaves on concentration and cooling yielded (vii) *Quercitol* (82 g; 2% of the leaves), m.p. 234–226° and its mother liquor showed presence of *glucose* (paper co-chromatography).

Constituents of the Mesocarp

Ursolic acid. To isolate the triterpene acid reported earlier,[1] the *M. hexandra* mesocarp (40 kg) was successively extracted with alcohol (6 × 2 l.) and hexane (10 × 8 l.) yielding a dark-coloured semi-solid residue (600 g). A portion (200 g) of the hexane-soluble part of it was separated into acidic (20 g) and neutral (170 g) fractions. The acidic fraction (10 g) after alkali purification and chromatography over acid-washed alumina (200 g) gave ursolic acid (TLC) as the main constituent, m.p. 285–286°; $[\alpha]_D^{39}+56°$ (pyridine); ν_{max} 3390 (OH), 1695 (COOH), 1389, 1379, 1361 and 1307, 1274, 1250 (ursolic acid),[15] 833 and 812 cm^{-1} (trisubstituted double bond). (Found: C, 78·96; H, 10·83. Calc. for $C_{30}H_{48}O_3$: C, 78·94; H, 10·52%); identity confirmed via *methyl ester*, m.p. 166–167°; $[\alpha]_D^{38}+68°$; ν_{max} 1724 cm^{-1} (ester) (Found: C, 78·85; H, 10·93. Calc. for $C_{31}H_{50}O_3$: C, 79·15; H, 10·64%); *acetate*, m.p. 290–293°; $[\alpha]_D^{38}+75!$; ν_{max}^{nujol} 1708 and 1242 cm^{-1} (acetate) (Found: C, 76·89; H, 10·20. Calc. for $C_{32}H_{50}O_4$: C, 77·10; H, 10·04%) and *methyl ester acetate*, m.p. 238–240°; $[\alpha]_D^{38}+71°$; ν_{max} 1724 cm^{-1} (Found: C, 77·05; H, 10·56. Calc. for $C_{33}H_{52}O_4$: C, 77·34; H, 10·15%).

Ursolic acid was finally confirmed through mixed m.p., superimposable i.r. spectra and its reduction ($LiAlH_4$) to *uvol*,[16] m.p., 228–230° (lit.[16] 233°); $[\alpha]_D^{39}+75°$ (lit.[16] 74·4°); ν_{max} 3333 cm^{-1} (OH) (Found: C, 80·92; H, 11·78. Calc. for $C_{30}H_{50}O_2$: C, 81·44; H, 11·31%).

Another portion (100 g) of the hexane-soluble residue on chromatography (alumina, methanol) yielded, besides the constituents already reported,[1] *β-D-Glucoside of β-sitosterol* (1·3 g) as hexane-insoluble residue and *a hexane-soluble triterpene ester* which on chromatography and subsequent crystallization furnished an L-B positive compound (108 mg), m.p. 118–120°; ν_{max} 1730 and 1686 cm^{-1} (ester and acid) and no peak in the hydroxyl region. Ursolic acid (m.p., mixed m.p. and co-TLC) only was obtained after alkaline hydrolysis of the ester.

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

[15] G. SNATZKE, F. LAMPERT and R. TSCHESCHE, *Tetrahedron* **18**, 1417 (1962).
[16] L. RUZICKA and A. MARXER, *Helv. Chim. Acta* **23**, 144 (1940).