Phytochemistry, 1966, Vol. 5, pp. 535 to 538. Pergamon Press Ltd. Printed in England

SHORT COMMUNICATION

MIMUSOPS HEXANDRA—II.

CONSTITUENTS OF BARK AND SEED

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(*Received* 10 *September* 1965)

Abstract—Taraxerol, taraxeryl acetate, α-amyrin cinnamate, α-spinosterol and a triterpenoid acid have been isolated from the bark of *Mimusops hexandra*. Further investigation with the seed constituents showed the presence of dihydroquercetin and quercetin in the testa, and the sterol in the seed kernel has been identified as α-spinosterol and not ergosterol as reported in the past.

INTRODUCTION

IN CONTINUATION of the work reported earlier[1] the constituents of the inner bark of *Mimusops hexandra* were examined and taraxeryl acetate, free taraxerol, the cinnamic acid ester of α-amyrin and α-spinosterol apart from traces of a triterpenoid acid, m.p. 280–285°; $[\alpha]_D^{26} + 50°$ (methanol) have been isolated. Besides characterization of the products and their derivatives by their analytical, physico-chemical and spectrophotometric data, the identity of the products were confirmed by degradation. Taraxerol was finally identified by its conversion to β-amyrin via its acetate.[2]

The cinnamic acid ester of α-amyrin was obtained as an amorphous mass in comparatively good yield (*c.* 0·7 per cent of bark) from the *n*-hexane soluble portion of the alcoholic extractive of the bark. The alkali labile amorphous product on careful hydrolysis yielded α-amyrin and cinnamic acid almost in quantitative yield. Cinnamic acid was identified as its dibromide and its oxidation to benzoic acid. A cinnamic acid ester of α amyrin does not appear to have been previously isolated as a natural product.

While repeating the work[1] with a larger quantity of the testa, quercetin and dihydroquercetin have been isolated and characterized in addition to the constituents already reported. Isolation of the reduced flavonoid from *M. hexandra* seed coat extends confirmation of its presence in the Sapotaceous nut coats, as it has been earlier isolated in this laboratory from *Madhuca butyracea*[3] nut coat. The presence of dihydroquercetin was hitherto reported in several families, including coniferous heartwoods,[4,5] and therefore its isolation from the Sapotaceae is of much interest as a chemotaxonomic feature of this family of plants.

On further examination, the sterol isolated from the unsaponifiable portion of the seed kernel oil has been confirmed to be α-spinosterol and thus shows that the compound is present in different parts of the plant, unlike β-sitosterol which is only present in the glucosidal form in the testa and the seed kernel.[1] The reported presence of ergosterol[6] in the seed kernel or its fat could not, however, be confirmed.

[1] C. R. MITRA and G. MISRA, *Phytochem.* **4**, 345 (1965).
[2] J. M. BEATON, F. S. SPRING, R. STEVENSON and J. L. STEWART, *J. Chem. Soc.* 2131 (1955).
[3] Y. C. AWASTHI and C. R. MITRA, *J. Org. Chem.* **27**, 2636 (1962).
[4] J. C. PEW, *J. Am. Chem. Soc.* **70**, 3031 (1948).
[5] R. N. GOEL and T. R. SESHADRI, *Tetrahedron* **5**, 91 (1959).
[6] C. K. PATEL, *J. Indian Inst. Sci.* **7**, 71 (1924); *Chem. Abstr.* **18**, 3283 (1924).

EXPERIMENTAL

Optical rotations were recorded with 1% chloroform solution unless otherwise stated. Melting points were determined in open capillaries and were uncorrected. Infrared spectra were recorded in nujol in a Perkin–Elmer Infracord Spectrometer model 137 and u.v. spectra in ethanol in an Unicam spectrometer model 700. Alumina used for chromatography was neutral Brockman (E. Merck) quality.

Constituents of the Bark

The shade-dried, coarsely powdered bark (2·9 kg) was successively extracted with alcohol (36 l.) and acetone (12 l.) by percolation at ordinary temperature (20–32°). The combined dried extractives, on fractionation with different solvents, yielded the following constituents.

Taraxeryl acetate. The *n*-hexane-insoluble residue of the semi-solid extractive gave an L–B-positive triterpenoid fraction (1·12 g) which on successive crystallizations from boiling alcohol (700 ml) and hot pyridine (25 ml) yielded shining crystals of taraxeryl acetate, m.p. 300–302° (lit[7] 304–305°); $[\alpha]_D^{20}+10°$ (lit[7] +12°); ν_{max} 1724 cm^{-1} (OAc). (Found: C, 82·69; H, 11·58. Calc. for $C_{32}H_{52}O_2$: C, 82·06; H, 11·02%.) Hydrolysis of taraxeryl acetate (60 mg) with KOH (alcoholic; 10 per cent; 3 ml; 30 min at 100°) gave on usual working and crystallization (hot pyridine) taraxerol (41 mg), m.p. and mixed m.p. with an authentic sample, 283–285° (lit[7] 282–283°); ν_{max} 3333 cm^{-1} (OH). Acetic acid was detected[8] in the distillate of the aqueous hydrolysate indicating that the parent compound was taraxeryl acetate. Its identity was further confirmed by i.r. spectra.

Acetylation of taraxerol with acetic anhydride and pyridine gave taraxeryl acetate, m.p. 298–300°, identical with the natural product (mixed m.p. 300–302°).

Conversion to β-amyrin.[2] Treatment of taraxeryl acetate (200 mg) with alcoholic hydrochloric acid (5%; 150 ml) for 3 hr at 100° under reflux, gave on usual working a neutral product (150 mg) which after successive crystallization from alcohol (25 ml) and alcohol–ether (1:1; 5 ml) melted at 190–192°; $[\alpha]_D^{22·5}+83°$; ν_{max} 3333 cm^{-1} (OH). (Found: C, 84·19; H, 11·99. Calc. for $C_{30}H_{50}O$: C, 84·50; H, 11·73%.) It was identified and confirmed as β-amyrin by the fact that the i.r. spectra was identical with that of an authentic sample.[1]

Acetylation of β-amyrin with acetic anhydride and sodium acetate gave β-amyrin acetate m.p. 225–231°; mixed m.p. 230–233°. (Found: C, 82·50; H, 11·85. Calc. for $C_{32}H_{52}O_2$: C, 82·06; H, 11·02%.)

The yellow amorphous residue (20 g) from the *n*-hexane-soluble portion of the bark extract was passed through an alumina column (200 g; *n*-hexane, 700 ml) and the resultant micro-crystalline product (17·5 g) obtained after removal of the solvent, finally *in vacuo*, was hydrolysed with alcoholic KOH (10%, 250 ml), when α-amyrin and cinnamic acid were isolated from the hydrolysate.

α-Amyrin. The neutral product of hydrolysis after usual working and crystallization (*n*-hexane) yielded an L–B-positive triterpenoid alcohol (12 g) which on successive crystallization from *n*-hexane and alcohol and chromatography over alumina (250 g; eluent: hexane, 1 l.), melted at 184–186°; $[\alpha]_D^{26}+70°$; ν_{max} 3226 cm^{-1} (OH). (Found: C, 84·07; H, 12·08. Calc. for $C_{30}H_{50}O$: C, 84·50; H, 11·73%.) It was identified and confirmed as α-amyrin by its mixed melting point (185–186°) and superimposable i.r. spectra with that of an authentic sample.

[7] J. SIMONSEN, *The Terpenes*, Vol. IV, p. 278. Cambridge, University Press London (1957).
[8] F. FEIGL, *Spot Tests*, Vol. II, p. 247. Elsevier, London (1954).

Benzoylation in pyridine with benzoyl chloride at room temperature yielded α-amyrin benzoate as shining needles, m.p. 194–196° (lit.[9] 195–196°); $[\alpha]_D^{34}+97°$ (lit.[9] +94·6°). Found: C, 82·96; H, 10·52. Calc. for $C_{37}H_{54}O_2$: C, 83·77; H, 10·19%.

Cinnamic acid. The aqueous hydrolysate on acidification and extraction with ether yielded on usual working an acid (2 g) which on successive crystallization from *n*-hexane–ether (1:1; 50 ml) and dilute alcohol (50%) gave rhomboidal crystals of cinnamic acid, m.p. 132–134°; mixed m.p. 133–135° (lit.[10] 133°); neutr. equiv., 146 (calc. 148). (Found: C, 73·34; H, 6·04. Calc. for $C_9H_8O_2$: C, 72·97; H, 5·41%.)

Dibromide of cinnamic acid. The dibromide was prepared in the usual way, giving white rhomboidal crystals, m.p. 204–205° (lit.[10] 203–204°). Found: Br, 52·06. Calc. for $C_9H_8O_2Br_2$: Br, 51·95%.

Oxidation of cinnamic acid to benzoic acid. Oxidation with potassium permanganate gave benzoic acid, m.p. 119–120° (lit. 121°); mixed m.p. 120°. Its identity was further confirmed by i.r. spectra.

Taraxerol. The tail fractions obtained after chromatography of the *n*-hexane soluble bark extractive, from which the cinnamic acid ester of α-amyrin was separated earlier, yielded on maceration with ether (200 ml) rhomboidal crystals of taraxerol (690 mg), m.p. 284–286° (lit.[7] 283°); mixed m.p. 283–285°; $[\alpha]_D^{20}\pm0°$ (lit.[7] ±0°); ν_{max} 3448 cm^{-1} (OH). (Found: C, 84·38; H, 11·84. Calc. for $C_{30}H_{50}O$: C, 84·50; H, 11·73%.) Its identity was finally confirmed by i.r. spectra. Taraxeryl acetate, prepared by refluxing with acetic anhydride and pyridine, melted at 303–305°; mixed m.p. 302–304°; $[\alpha]_D^{16}+7°$. (Found: C, 82·99; H, 11·32%.)

α-Spinosterol. The L–B-positive crystallizate (178 mg) from the last chromatographic fraction was combined with that obtained from the mother liquor of taraxerol (*vide supra*) and saponified (alcoholic KOH, 10%; 25 ml). The unsaponifiable neutral component obtained after usual working and chromatography (alumina, 10 g; eluent: *n*-hexane; 150 ml) and subsequent crystallizations from *n*-hexane and alcohol yielded shining flakes of α-spinosterol[1] (150 mg), m.p. and mixed m.p. 166–168°; $[\alpha]_D^{30}\pm0°$ (*c*, 0·5%; lit −2°). Its identity was confirmed through its acetate, prepared with acetic anhydride and fused sodium acetate, m.p. 174–176° (lit 174°) and their comparable i.r. spectra.[1]

Constituents of the Testa

The residue from the alcoholic extract of the coarsely powdered testa (2·2 kg) after separation of quercitol and the β-D-glucoside of β-sitosterol[1] was extracted with *n*-hexane to remove the fat (42 g). The ether soluble portion of the fat-free residue on usual working gave a light yellow flavonoid (Mg-HCl positive) which on fractional crystallization (dilute alcohol, 75 per cent; 25 ml) finally yielded dihydroquercetin and quercetin.

Dihydroquercetin. The comparatively less soluble crystallizate on repeated crystallizations yielded light lemon-yellow silky needles of dihydroquercetin (2·32 g), m.p. 236–238°; $[\alpha]_D^{16}+41°$ (methanol; lit[3] +36°) (Found: C, 58·8; H, 4·1. Calc. for $C_{15}H_{12}O_7$: C, 59·2; H, 3·9%). It was confirmed by its mixed m.p. (235–237°), i.r. spectra and paper co-chromatography (*n*-BuOH–AcOH–H_2O, 4:1:5; developer, ammonia) with an authentic sample[3] and its characteristic u.v. absorption at 288 ($\log\epsilon$ 4·17) and 322 ($\log\epsilon$ 4·11) mμ.[3]

[9] J. SIMONSEN, *The Terpenes*, Vol. IV, p. 117. Cambridge University Press, London (1957).
[10] E. H. HUNTRESS and S. P. MULLIKEN, *Identification of Pure Organic Compounds, Order I*, p. 150 Chapman. & Hall, London (1953).

Reduction of dihydroquercetin to eriodictyol was carried out[3] and the resultant product on crystallization (alc. 60%) melted at 270–271° (Found: C, 63·3; H, 5·0. Calc. for $C_{15}H_{12}O_6$: C, 62·5; H, 4·2%). Its identity was confirmed by paper co-chromatography and the superimposable i.r. spectra.

Quercetin. The mother liquor of dihydroquercetin on keeping in cold yielded pale yellow needles of quercetin (5 mg), m.p. 305–308°; mixed m.p. 310–315°, confirmed by i.r. spectra and paper co-chromatography (*loc. cit.*).

Sterol from the Kernel Fat

α-Spinosterol. The purified and refined kernel fat (670 g) on saponification and usual working gave an L–B-positive crystallizate (614 mg) as unsaponifiable matter which on repeated crystallizations from alcohol and *n*-hexane and subsequent chromatography (alumina, 20 g; eluent: chloroform–methanol, 1:1; 200 ml) yielded needles of α-spinosterol, m.p. 160–162°; mixed m.p. 163–164°; $[\alpha]_D^{30} \pm 0°$ (*c*, 0·5%). Its identity was confirmed by conversion to the acetate, m.p. 173–174° and i.r. spectra.

Acknowledgements—Authors' thanks are due to Mr. J. Saran for microanalyses and to Mr. K. G. Deshpande for the u.v. spectra of dihydroquercetin and to Dr. (Mme) J. Polonsky for kindly supplying the i.r. spectra of taraxerol.