TRITERPENES OF *POUTERIA TORTA* (SAPOTACEAE)

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Although the genus *Pouteria* embraces 435 or more species (1), phytochemical information pertaining to this taxon is limited. Lupeol, α-amyrin, erythrodiol and dammarenediol II have been isolated from an unsaponifiable fraction of *Pouteria camimito* Ruiz et Pav. fruits (2), and investigation of the bark yielded tarax-14-ene-3β-ol acetate, tarax-14-ene-3-one, tarax-14-ene-3β-ol and β-sitosterol (3). β-Amyrin acetate has been obtained from the bark of *P. tomentosa* (Roxb.) Baehni (4).

An alkaloid, tentatively identified as yohimbine, was isolated by Altman (5) from a plant believed to be a species of *Pouteria*. However, this finding was questioned by Stellfeld (6). In a field survey of New Guinea plants for alkaloids, Hartley *et al.* (7) found the leaf and bark of *P. luzoniensis* (Merr.) Baehni var. *popuana* Erlee, the leaf and fruit of *P. maclayana* (F. Muell.) Baehni and the leaf of *P. malaccensis* (Clarke) Baehni to be devoid of alkaloids. The bark of *P. malaccensis* gave an equivocal test for the presence of alkaloids (7), and the fruit of *P. caimito* was reported to be devoid of alkaloids (2).

We have been unable to find any references to previous phytochemical or pharmacologic literature on *P. torta* (Mart.) Radlk. A phytochemical evaluation of a methanol extract of the twigs of this plant in our laboratories showed alkaloids to be absent, but triterpenes to be present. The present study was undertaken to examine the triterpene constituents of this plant.

EXPERIMENTAL[1]

Plant material.—The twigs examined in this study were collected in Brazil in October, 1974.[2]

Extraction and fractionation.—The dried, milled plant material (55.0 kg) was extracted with petroleum ether (bp 60–80°) to yield fraction A (550 g). After being air-dried, the defatted plant material was exhaustively extracted with methanol. The petroleum ether and methanol extracts were screened for phytochemical constituents as previously described (8) and were found to contain triterpenes, but were devoid of alkaloids. The methanol extract was concentrated, *in vacuo*, to a syrupy consistency and partitioned between petroleum ether (3×2 liters) and water (3×2 liters). The methanolic aqueous phase was further extracted with chloroform (6×6 liters) to yield 212 g of chloroform-soluble fraction B.

A 100 g portion of fraction B was redissolved in chloroform and partitioned against 5% Na_2CO_3 solution (5×1 liter) to remove

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[1]Melting points were determined on a Kofler hot plate and are uncorrected. Ir spectra were determined using a Beckman model 18 spectrophotometer with polystyrene calibration at 1601 cm^{-1}. Pmr spectra were recorded on a Varian model T-60A instrument with a Nicolet TT-7 Fourier Transform attachment; samples were dissolved in $CDCl_3$ with tetramethylsilane as the internal standard. Low resolution mass spectra were obtained with a Hitachi Perkin Elmer, model RMU-6D, single-focusing spectrometer operating at 70 ev. A Perkin Elmer model 881 gas chromatograph equipped with a hydrogen flame ionization detector was employed for glc determinations on a 6 ft x ¼ in. O.D. spiral glass column packed with 3% OV-17 on Gas Chrom Q. Operating conditions were injector temperature, 370°; detector temperature, 310°; column temperature, 290°; flow rate of carrier gas (nitrogen) 20 ml/min.

[2]The plant material was collected and identified by the Economic Botany Laboratory, BARC-East, United States Department of Agriculture, Beltsville, MD, and funded by the National Cancer Institute. A voucher speciman representing the collection (PR-43914) is deposited in the Herbarium of the National Arboretum, USDA, Washington, D.C.

phenolic constituents. The chloroform extract was dried over anhydrous sodium sulfate, filtered and concentrated to yield 42 g of a viscous fraction C.

ISOLATION OF β-AMYRIN ACETATE.—A portion of fraction A (100 g) was chromatographed on silica gel PF–254[3] with benzene as the eluting solvent. Fractions 3–4 (500 ml each) from the column were combined, evaporated to dryness *in vacuo* and crystallized from petroleum ether-ether to afford crude β-amyrin acetate (2.0 g). Recrystallization from petroleum ether-ether yielded 1.52 g (0.0152%) of analytical sample, mp 235–236°, identical (pmr, ms, ir and tlc) with a reference sample.[4]

Concentration of the crude β-amyrin acetate mother-liquor to dryness *in vacuo* afforded a residue which was crystallized from methanol-acetone to yield 14.0 g of a mixture of triterpenes.

SEPARATION OF TRITERPENE MIXTURE AND ISOLATION OF α-AMYRIN ACETATE.—A 3 g portion of the triterpene mixture was rechromatographed on silica gel PF–254 with petroleum ether-benzene (2:1) as the solvent; fractions (20 ml each) were monitored by glc. Fractions 412–431 gave a single peak (R_t= 17 min 24 sec) identified as β-amyrin acetate by direct comparison with reference sample.[4]

Analysis of fractions 448–490 by glc showed a major component at R_t 20.0 min. Crystallization of the combined fractions from acetone-methanol afforded α-amyrin acetate (630 mg, 0.0294%), identical (pmr, ms, ir, tlc and glc) with a reference sample.[4]

ISOLATION OF BETULINIC ACID.—Fraction C (42 g) was chromatographed on MN silica gel P/UV–254[5] with chloroform. Combined fractions 204–297 afforded a crystalline compound from methanol and recrystallization from the same solvent yielded 414 mg of betulinic acid (0.0016%), mp 277–279; identical (pmr, ms, ir, and tlc) with an authentic sample.[4]

ISOLATION OF URSOLIC ACID.—Crystallization and recrystallization of the combined column fractions 430–497 ($CHCl_3$-MeOH, 98:2) from 95% ethanol afforded 16 mg (0.00006%) of ursolic acid, mp 283–285°, identical (pmr, ms, ir, and tlc) with an authentic sample.[4]

[3]E. Merck, Darmstadt, W. Germany.

[4]From our Laboratories.

[5]Macherey Nagel and Co., Doren, W. Germany.

DISCUSSION

The isolation of β-amyrin acetate, α-amyrin acetate, betulinic acid and ursolic acid from *Pouteria torta* is reported and adds further phytochemical information on this poorly-studied genus.

In view of the negative alkaloid test results and the isolation of triterpenes from *Pouteria* species in our laboratories and elsewhere, the reported occurrence of the indole alkaloid, yohimbine, in a species of this genus should be viewed with caution. Indeed, Altman (5) reported only the isolation of an alkaloid with a mp and chromogenic reaction similar to yohimbine from the bark of a plant believed to be a *Pouteria* species.

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