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TRITERPENOIDS OF THE LEAVES OF *PSIDIUM GUAIJAVA**

A. M. OSMAN, M. EL-GARBY YOUNES† and A. E. SHETA

Department of Chemistry, Faculty of Science, University of Assiut, Assiut, Egypt, A.R.E.

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Key Word Index—*Psidium guaijava*; Myrtaceae, triterpenoids; 2α-hydroxyursolic acid.

The leaves of *Psidium guaijava* L. (Myrtaceae) are used medicinally for the treatment of throat and chest complaints. Soliman and Farid[1] isolated from the ether soluble fraction of the methanol extract of the defatted leaves a new triterpene acid "psidiolic acid". Arthur and Hui[2] reported that psidiolic acid is a mixture of four acids, oleanolic, ursolic, crategolic (maslinic) acids together with a new acid called "guaijavolic acid" ($C_{30}H_{48}O_4$).

Our primary aim in reinvestigating the leaves of the Egyptian *P. guaijava* was to elucidate the structure of guaijavolic acid. The occurrence of oleanolic acid with its ursane analogue, ursolic acid, led us to assume that guaijavolic acid may be the ursane analogue of crategolic acid, i.e. 2α-hydroxyursolic acid.

We followed the isolation procedure of El-Garby Younes *et al.*[3,4] The petrol. extract was steam distilled and the non-volatile material hydrolysed with ethanolic KOH (5%). The neutral portion was chromatographed on alumina with C_6H_6–Et_2O (1:1) giving sitosterol, identified by comparison IR, m.p. and m.m.p. 129°, $[\alpha]_D$ − 35° with authentic material and by conversion to sitosteryl acetate m.p. 119°, $[\alpha]_D$ − 38°.

The defatted leaves were further extracted with Et_2O, and the solvent concentrated and left at room temp. overnight. This gave a green solid which was methylated (CH_2N_2) and acetylated (Ac_2O/pyridine), followed by chromatography over alumina. Elution with petrol.–C_6H_6 (1:1) afforded fraction *A* as colourless plates, while elution with benzene and C_6H_6–Et_2O (1:1) yielded fraction *B* as yellowish gum.

Hydrolysis of fraction *A* with ethanolic KOH afforded a mixture of methyl ursolate and methyl oleanolate, benzoylation of which (benzoyl chloride/pyridine) and fractional crystallization (MeOH–$CHCl_3$) gave methyl ursolate benzoate in the least soluble fraction, m.p. and m.m.p. 215–216°, $[\alpha]_D$ + 51·2°, while methyl oleanolate benzoate was obtained in the more soluble fraction, m.p. 220–222°, $[\alpha]_D$ + 31·3°. Hydrolysis of both benzoate gave the corresponding alcohols, identified by comparison with authentic specimens.

Hydrolysis of fraction *B* with ethanolic KOH (5%) and benzoylation of the methyl esters at 0°, followed by chromatography over alumina, and elution with petrol. afforded a mixture of dibenzoate methyl esters. Fractional crystallization of which (MeOH–$CHCl_3$) gave methyl 2α,3β-dibenzoyloxyurs-12-en-28-oate, m.p. and m.m.p. 217°, $[\alpha]_D$ − 30° and methyl 2α,3β-dibenzoyloxy-olean-12-en-28-oate (as a gum). Hydrolysis of both dibenzoate methyl esters gave methyl 2α-hydroxyursolate (from C_6H_6–petrol), m.p. and m.m.p. 211°,

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† To whom inquiries should be addressed.

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$[\alpha]_D$ + 56°), and methyl maslinate (from C_6H_6–petrol., m.p. and m.m.p. 203°, $[\alpha]_D$ + 61°), respectively.

Further elution of the original column with C_6H_6–petrol. afforded the monobenzoate methy ester mixture, fractional crystallization of which (as before) gave colourless needles of methyl 2α-benzoyloxy-3β-hydroxy-urs-12-en-28-oate (m.p. and m.m.p. 225°, $[\alpha]_D$ + 3°) and methyl 2α-benzoyloxy-3β-hydroxyolean-12-en-28-oate (m.p. and m.m.p. 182°, $[\alpha]_D$ + 7·7°) as needles. Hydrolysis of both monobenzoate methyl esters, with ethanolic potassium hydroxide, yielded the corresponding diol methyl esters obtained above.

Thus from the above results we can conclude that "guaijavolic acid" is in reality 2α–hydroxyursolic acid.

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ISOLATION OF COCLAURINE FROM *ZIZYPHUS JUJUBA* BY DROPLET COUNTER-CURRENT CHROMATOGRAPHY

HIDEAKI OTSUKA, YUKIO OGIHARA and SHOJI SHIBATA

Faculty of Pharmaceutical Sciences, University of Tokyo, Bunkyo-ku, Tokyo, Japan

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Key Word Index—*Zizyphus jujuba* Mill. var. *inermis* Rehd; Rhamnaceae; frangulanine; adouétine X; coclaurine; peptide alkaloids; droplet counter-current chromatography.

From the basic fraction of methanolic extracts of the root bark of *Zizyphus jujuba* Mill. var. *inermis* Rehd., three new peptide alkaloids, *F*, *G* and *H* have been isolated besides two known peptide alkaloids, frangulanine[1] and adouétine X,[2] by the preparative TLC, while a benzylisoquinoline alkaloid, coclaurine, has been separated by the droplet counter-current chromatography (DCCC), which has been developed by Tanimura *et al.*[3] The structures of the peptides, *F*, *G* and *H* are now under investigation.

DCCC has been shown to be a very efficient method for the separation of water-soluble natural products. Coclaurine was obtained in a crystalline form, m.p. 217–218·5°, by this method.

EXPERIMENTAL

Isolation of peptide alkaloids. The alkaloid fraction was obtained by a usual method.[4] It was separated on preparative TLC (Kieselgel GF_{254}) to yield adouétine X, frangulanine substances, *F*, *G* and *H*.

Identification of adouétine X. Adouétine X, colourless needles, m.p. 278–280° from MeOH, IR_{max}^{KBr} cm^{-1}: 3250 (NH), 2790 (NCH_3), 1630 (CONH), 1238 (C–O–C); NMR ($CDCl_3$) δ 0·69 (3H, *d*, *J* 7 Hz), 0·86 (3H, *t*, *J* 7 Hz) 0·87 (3H, *d*, *J* 7 Hz), 0·94 (3H, *d*, *J* 7 Hz), 0·98 (3H, *d*, *J* 6 Hz), 1·27 (3H, *d*, *J* 6 Hz), 2·20 (6H, *s*, $N(CH_3)_2$); MS, *m/e* 500 (M^+), 114 (100%), 72 (11·3%). Amino acid analysis: Isoleucine, glycine, *erythro* and *threo*-β-hydroxyleucine.

Conditions of DCCC. The alkaloid fraction (970 mg) was developed on DCCC with the solvent system, benzene:$CHCl_3$:MeOH:H_2O = 5:5:7:2. Moving phase: upper layer; Stationary phase:lower layer; Number of glass tubes:500; Theoretical plates:1000. In one fraction 300 drops of eluated moving phase (*ca* 5 ml) were collected, and from the 69–75th fraction tubes fine needle shaped crystals of coclaurine were obtained.

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