Phytochemical Study on *Randia siamensis*

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**Abstract** From the roots of *Randia siamensis*, D-mannitol, a mixture of *β*-sitosterol and campesterol, oleanolic acid acetate, oleanolic acid-3-α-L-arabinoside and mesembryanthemoidigenic acid as a sapogenin were isolated and characterized.

**Keywords** *Randia siamensis*, Rubiaceae, D-Mannitol, Sterol mixture, Oleanolic acid acetate, Oleanolic acid-3-α-L-arabinoside, Mesembryanthemoidigenic acid.

*Randia siamensis* (Rubiaceae) is an erect shrub distributed in Thailand, the roots of which have been used in a folkloric medicine for inducing abortion. In the course of search for the active substances, five compounds I-V were isolated and characterized.

On concentration of the MeOH extract of the roots, compound I, mp 166-7°, \([\alpha]_D^{20}=+10.16^\circ\) (c= 2.07, H_2O), was crystallized, which was identified as D-mannitol by direct comparison with an authentic sample (mmp, co-TLC). Column chromatography of the CHCl_3 soluble fraction of the MeOH extract on silica gel using a solvent (CHCl_3→EtOAc, gradient) to give compounds II, III & IV.

Compound II, mp 138-40°, was identified as a mixture of *β*-sitosterol (64.5%) and campesterol (35.5%) (MS, GLC).

Compound III, mp 248-50°, \([\alpha]_D^{20}=+103.07^\circ\) (c=0.28, MeOH) showed positive Liebermann-Burchard test and strong absorption bands at 3200(OH), 1740, 1260 (acetate) and 1695 cm\(^{-1}\) (acid) in its IR. Its MS showed a molecular ion peak at m/z 498 (0.8%) and other peaks at m/z 452 [M\(^+\)--(COOH+H), 1.47], 438 (M\(^+\)--CH_3COOH, 4.39), 248 (RDA, 100) and 203 (RDA-COOH, 75.4). The \(^1\)H NMR (60 MHz, CDCl_3, TMS) exhibited signals at δ0.88-1.13 (7×Me), 2.03 (3H, s, OAc), 4.60 (IH, m, H-3), and 5.30 (IH, m, H-12). These spectral data were in agreement with those for the structure of oleanolic acid acetate. It was confirmed by deacetylation of III to give oleanolic acid, mp 209-10° (mmp, co-TLC).

Compound IV, mp 242-4°, \([\alpha]_D^{20}=+47.5^\circ\) (c=0.04, MeOH), gave positive reaction in Liebermann-Burchard and Molisch tests and showed strong absorption bands at 3400 (OH), 1700 (acid) and 1000-1100 cm\(^{-1}\) (glycoside). Acid hydrolysis of IV gave oleanolic acid (mmp, co-TLC) and L-arabinose (co-TLC, GLC). Methanalysis of permethylated product of IV, mp 170-2° (M^+ 644) gave methyl oleanolate, mp 201-2° (mmp, co-TLC) and methyl-2, 3, 4-tri-O-methyl-Î±-l-arabinopyranoside (GLC).

The a-orientation of the glycosidic linkage was suggested not only from the J value (J=6Hz) of the anomeric proton signal of its peracetylated product, but also from the molecular rotation difference (+12.1°) between IV and oleanolic acid ([M]_D of methyl-α-l-arabinopyranoside=+28.37°, that of β-form=
Therefore the structure of IV was elucidated as 3-O-α-l-arabinopyranosyl oleanolic acid, which is a rare natural compound. The presence of IV in the plants has previously been only reported in Fatsia japonica and Patrinia scabiosae folia.

Acid hydrolysis of the BuOH soluble fraction of the MeOH extract, column chromatography on silica gel and elution with C₆H₆-EtO (4:1) gave compound V, mp 335-8 °C: 347.2 °C (c =0.18, MeOH) which showed positive Liebermann-Burchard test and strong absorption bands at 3400 (OH) and 1700 cm⁻¹ (acid) in its IR.

Methylation of V with CH₃NO₂ and subsequent acetylation with Ac₂O and pyridine gave a methylester, mp 209-10 °C and a methylester diacetate, mp 234-7 °C respectively.

Oxidation of the methylester diacetate with SeO₂ yielded a heteroannular diene compound, which showed triple UV maxima at 241, 251, and 261 nm (log ε, 4.18, 4.23 and 4.15), typical of 11:12, 13:18 diene of the oleanane series. The MS of V showed a molecular ion peak at m/z 472 (1.57%) and other peaks at m/z 454 (M+-H₂O, 1.76), 441 (M+-CH₂OH, 2.17), 264 (RDA, 72.4), 233 (RDA-CH₂OH, 100), 201 (RDA-(COOH+H₂O), 35.39), indicating that V has an α-amyrin skeleton with one hydroxyl group and one carboxyl group at rings D/E and one hydroxyl group at rings A/B.

The appearance of the base peak at m/z 233 which corresponded the loss of a CH₂OH group from RDA fragment with D/E ring and saponification rate (33%) of V methylester by 10% KOH in EtOH for 8 hr suggested that a CH₂OH group preferred to be located at C-17, strongly supported the presence of a COOH group at C-17 in V.

Treatment of V methylester with LiAlH₄ gave triol, mp 250-7 °C which was identified as a 28,29-dihydroxy-β-amyrin by direct comparison with an authentic sample (mmp, co-TLC). From the above results, the structure of V was elucidated as 3β,29-dihydroxy-olean-12-en-28-oic acid. A direct comparison (mmp, co-TLC and MS) with an authentic sample of mesembryanthemoidigenic acid, kindly supplied by Dr. Daloze of Universite Libre de Bruxelles, confirmed the identity of these two terpenoids.

**EXPERIMENTAL METHODS**

**Isolation**

The roots of Randia siamensis were extracted with hot MeOH. Concentrating and cooling the hot MeOH extract gave a precipitate which was recrystallized from MeOH to give compound I (d-mannitol) as colorless needles, mp 166-7 °C (C= 2.07, H₂O) (mmp, co-TLC). The filterate after separation of I was partitioned between equal volumes of n-hexane and H₂O. The aqueous layer was extracted with CHCl₃ and subsequently with BuOH.

The CHCl₃ extract was evaporated and chromatographed on a Si gel and eluted with CHCl₃ -EtOAc (gradient) to give compounds II, III and IV.

**Compound II** (a mixture of β-sitosterol and campesterol)

Colorless needles from MeOH, mp 138-40 °C, LB; positive (pink−→blue), IR ν max cm⁻¹; 3400 (OH), 790-840 (trisubstituted double bond); MS