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Phytochemical Study on *Randia siamensis*

Pattra Lapikanon, Phichai Tovivich, Won Sick Woo* and Jae Sue Choi*

*Department of Chemistry, Faculty of Science, Chulalongkorn University, Bangkok, Thailand and *Natural Products Research Institute, Seoul National University, Seoul 110, Korea*

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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°, $[\alpha]_D^{23}=+10.16°$ ($c=2.07$, 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 ($CHCl_3 \rightarrow$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°, $[\alpha]_D^{23}=+103.07°$ (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 ^1H NMR (60 MHz, $CDCl_3$, TMS) exhibited signals at δ0.88-1.13 (7×Me), 2.03 (3H, s, OAc), 4.60 (1H, m, H-3), and 5.30 (1H, 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 309-10° (mmp, co-TLC).

Compound IV, mp 242-4°, $[\alpha]_D^{23}=+47.5°$ (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). Methanolysis 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-L-arabinopyranoside (GLC).

The α-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=

+402.62°)[1]. 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*[2] and *Patrinia scabiosaefolia*[3].

Acid hydrolysis of the BuOH soluble fraction of the MeOH extract, column chromatography on silica gel and elution with C_6H_6-Et_2O (4:1) gave compound V, mp 335-8°, $[\alpha]_D^{23}$ +47.2° (c =0.18, MeOH) which showed positive Liebermann-Burchard test and strong absorption bands at 3100 (OH) and 1700 cm^{-1} (acid) in its IR.

Methylation of V with CH_2N_2 and subsequent acetylation with Ac_2O and pyridine gave a methylester, mp 209-10° and a methylester diacatate, mp 234-7°, respectively.

Oxidation of the methylester diacetate with SeO_2 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[4]. The MS of V showed a molecular ion peak at *m/z* 472 (1.57%) and other peaks at *m/z* 454 (M^+-H_2O, 1.76), 441 (M^+-CH_2OH, 2.17), 264 (RDA, 72.4), 233 (RDA-CH_2OH, 100), 201 [RDA-(COOH+H_2O), 35.39], indicating that V has an β-amyrin skeleton with one hydroxyl group and one carboxyl group at rings D/E and one hydroxyl group at rings A/B[5].

The appearance of the base peak at *m/z* 233 which corresponded the loss of a CH_2OH 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 CH_2OH group preferred to be located at C-17[5,6].

However, the formation of a monobromo γ-lactone by treatment of V with Br_2 HOAc and the appearance in NMR of V mentylester diacetate of the highest angular methyl signal in upfield region from 0.77 ppm[7] strongly supported the precence of a COOH group at C-17 in V.

Treatment of V methylester with $LiAlH_4$ gave triol, mp 250-7° 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[8,9], 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°, $[\alpha]_D^{23}$ = +10.16° (c=2.07, H_2O) (mmp, co-TLC).; acetate, mp 118-120° (mmp, co-TLC, NMR).

The filterate after separation of I was partitioned between equal volumes of n-hexane and H_2O. The aqueous layer was extracted with $CHCl_3$ and subsequently with BuOH.

The $CHCl_3$ extract was evaporated and chromatographed on a Si gel and eluted with $CHCl_3$-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°, LB; positive (pink→blue), IR ν_{max}^{KBr} cm^{-1}; 3100 (OH), 790-840 (trisubstituted double bond); MS

m/z(rel. int.); 414 (M_1^+, 100), 400 (M_2^+, 37.8), 329 (M_1-C_5H_9O, 100), 315 (M_2-C_5H_9O, 27.1), 303 (M_1-$C_7H_{11}O$, 47.6), 289 (M_2-$C_7H_{11}O$, 20.4), 275 (M_1-$C_9H_{15}O$, 22.7), 273 (M-side chain, 79.6), 261 (M_2-$C_9H_{15}O$, 11.1), 255 (M-side chain-H_2O, 76.9), which was found to consist of β-sitosterol (64.5%, T_R 3.2 min) and campesterol (35.5%, T_R 2.8 min) by GLC (column, 3% OV-I, 60-80 mesh, 4mm×1.5m; column temp., 270°; injector temp., 300°; N_2, 45 ml/min).

Acetate, mp 118-20°, NMR ($CDCl_3$, 60 MHz, TMS): δ0.67-1.0 (6×Me), 2.02 (s, 3H, OAc), 4.60 (1H, br s, H-3), 5.40 (m, 1H, olefin).

Compound III (oleanolic acid acetate)

Colorless needles from MeOH, mp 248-50° $[\alpha]_D^{23}$=+103.07° (c=0.28, MeOH).

Compound IV (oleanolic acid-3-α-L-arabinoside)

Colorless needles from MeOH, mp 242-4°, $[\alpha]_D^{23}$=+47.5° (c=0.04, MeOH).

Deacetylation of III

A sample (10mg) of III was heated in 10% NH_4OH in MeOH (5ml) for 5 hr. After concentration, it was crystallized from MeOH to yield colorless needles, mp 309-10°, identified as oleanolic acid (mmp, co-TLC).

Acid Hydrolysis of IV

A solution of IV (8mg) in 5% H_2SO_4 in EtOH (5ml) was refluxed for 5 hr. and concentrated under reduced pressure to remove EtOH. After addition of H_2O, the resulting ppt. was filtered and crystallized from MeOH to afford colorless needles, mp 309-10°, identified as oleanolic acid (mmp, co-TLC).

The filtrate was neutralized with $BaCO_3$, filtered and concentrated under reduced pressure.

The residue was found to be L-arabinose by TLC (cellulose plate, pyridine-EtOAc-HOAc-H_2O =36 : 36 : 7 : 21, Rf 0.38) and GLC of the TMS derivative (column OV-I (3%), 60-80 mesh, 1.5×4mm; column temp., 160°; injector temp., 180°; N_2 45ml/min; T_R 3.6).

Permethylation of IV Followed by Methanolysis

Permethylation of IV (30mg) according to the method described by Brimacombe, *et al.*[10] followed by purification by column chromatography with C_6H_6-Et_2O-MeOH (8 : 2 : 0.5) and crystallization from MeOH yielded prisms, mp 170-2°, MS *m/z*(%) : 644 (M^+, 0.2), 453 (M-PM Ara. 61.6), 262 (RDA, 47.4), 203 (RDA-$COOCH_3$, 100), 175 (PM Ara. 33.4).

The permethylether (10mg) was refluxed in 5% HCl-MeOH (3ml) for 5hr. The reaction mixture was poured onto ice and filtered and the ppt was crystallized from MeOH to give methyloleanolate, mp 201-2° (mmp, co-TLC). The filtrate was extracted with $CHCl_3$. The $CHCl_3$ solution was evaporated. The residue was found to be methyl-2,3-4-tri-O-methyl-L-arabinopyranoside by GLC (column, 5% NPGS 4mm×1.5m; colum temp., 180°; injector temp., 200°; N_2, 45ml/min; T_R 1.8).

Acetylation of IV

A sample (30mg) of IV was acetylated with acetic anhydride and pyridine in the usual way. The reaction product was crystallized form MeOH to give colorless needles, mp 148-50°, NMR ($CDCl_3$, 60 MHz, TMS): δ0.84-1.15 (7×Me), 2.05(6H, s, 2×OAc), 2.08(3H, s, OAc) 4.21 (1H, d, J=6, anomeric H).

Compound V (mesembryanthemoidigenic acid)

Hydrolysis of the BuOH extract gave a mixture of sapogenins, which was chromatographed on a Si gel column and eluted with C_6H_6-Et_2O (1 : 1) to give V as colorless needles from MeOH, mp 335-8°, $[\alpha]_D^{23}$+47.2° (c=0.18, MeOH).

Methylation of V

A sample (30mg) of V was esterified when dissolved in MeOH and treated with CH_2N_2.

The product was crystallized from MeOH as colorless needles, mp 209-10°, IR $\nu_{max}^{KBr}cm^{-1}$:1725 (ester).

Acetylation of V-methylester

A sample (30mg) of V-methylester was acetylated as above and crystallized from MeOH to give colorless needles, mp 234-7°, IR ν_{max}^{KBr} cm^{-1}: 1725 (ester), 1230 (acetate); NMR ($CDCl_3$, 80 MHz, TMS): δ0.72-1.10 (6×Me), 2.0 (3H, s, OAc), 2.03 (3H, s, OAc), 3.60 (3H, s, OMe), 4.15 (1H, t, H-3), 5.25 (1H, m, H-12).

SeO_2 Oxidation of V-methylester Acetate

A solution of V-methylester (5mg) and freshly prepared SeO_2 (5mg)in HOAc (1ml) was heated for 2hr. The solution was filtered, diluted with H_2O and extracted with Et_2O. The residue was chromatographed to give a diene as colorless material. UV λ_{max}^{EtOH} nm(logε): 241 (4.18), 251(4.23), and 261(4.15).

Saponification of V-methylester

A solution of V-methylester (10mg) in 10% KOH in EtOH was refluxed for 8hr and concentrated under reduced pressure to remove EtOH. After addition of H_2O, extracted with Et_2O (ester, 67%). The aqueous mother liquor neutralized with 5% HCl and extracted with Et_2O (acid, 33%).

$LiAlH_4$ Reduction of V

A sample of V (10mg) was dissolved in dry THF (50ml) and $LiAlH_4$ (50mg) was added slowly with stirring and then refluxed for 3hr. The reaction mixture was filtered and concentrated to give 28, 29 dihydroxy-β-amyrin, mp 250-7° (mmp, co-TLC).

Monobromo-γ-Lactone of V

To a solution of V (5mg) and NaOAc (2mg) in HOAc (1ml) was added dropwise a solution of bromine in HOAc (3%, 1ml). The reaction mixture was kept at room temp. for 3hr. and then poured into H_2O (5ml) containing $Na_2S_2O_3$ (500mg) to discharge excess bromine. The ppt was filtered, washed with thoroughly with H_2O, dried and crystallized from MeOH to give V-bromolactone, IR ν_{max}^{KBr} cm^{-1}: 1800 (γ-lactone).

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