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A Sapogenin of *Randia siamensis*

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Abstract □ From the roots of *Randia siamensis* (Rubiaceae), 3β, 30-dihydroxy-urs-12-en-28-oic acid (30-hydroxy ursolic acid) was isolated and characterized by spectral data.

Keywords□ *Randia siamensis*, Rubiaceae, 30-hydroxy-ursolic acid, ^{13}C NMR.

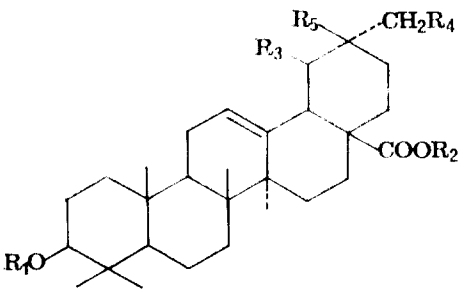
In the previous paper[1] the isolation of D-mannitol, sterol mixture, oleanolic acid acetate, oleanolic acid-3-α-L-arabinoside and mesembryanthemoidigenic acid as a sapogenin from the roots of *Randia siamensis* (Rubiaceae) was reported. In a course of continuous work on the plant, another sapogenin was isolated from the hydrolysate of butanol soluble fraction of methanol extract.

The sapogenin, mp 282-3°, $[\alpha]_{24}^{D}$+56° (c=1.0, EtOH) showed positive Liebermann-Burchard test and strong absorption bands at 3400 (OH) and 1685cm^{-1}(acid) in its IR spectrum. Methyation with CH_2N_2 of the compound and subsequent acetylation with Ac_2O and pyridine gave a methylester (II), mp 197-8° and a methylester diacetate (III), 176-8°, respectively.

The MS spectrum of the compound (I) showed a molecular ion at m/z 472 and fragment ions at m/z 264 and 207, suggesting that it is a compound having Δ^{12}-oleanene or Δ^{12}-ursene skeleton with one hydroxyl group and one carboxyl group at rings D and E and one hydroxyl group at rings A and B.[2]

The NMR spectrum of the methylester diacetate (III) showed five tertiary methyl signals at δ 0.75(3H), 0.86(6H), 0.95(3H), 1.06(3H), one secondary methyl signal at 0.91(3H, d, J=7.2Hz), two acetyl signals at 2.04(6H), one carbomethoxyl signal at 3.61(3H), a doublet centered at 2.25(1H, J=11Hz) assignable to allylic 18β-proton of ursene series[3,4], a double doublet centered at 4.5(1H, J=8 and 10Hz) due to H-3 and a multiplet centered at 5.26(1H) due to H-12.

The signals for the non-equivalent methylene protons further coupled with an adjacent proton appeared at δ 3.96(1H, dd, J=6.6 and 10Hz) and 4.18(1H, dd, J=1.6 and 10Hz), indicating



	R_1	R_2	R_3	R_4	R_5
I	H	H	Me	OH	H
II	H	Me	Me	OH	H
III	Ac	Me	Me	OAc	H
IV	Ac	Me	Me	H	H
V	Ac	Me	H	H	CH_2OAc
VI	Ac	Me	H	OAc	Me

Table I: Carbon 13 chemical shifts (δ_c) of methyl 30-hydroxyursolate acetate (III) and related compounds in $CDCl_3$.

Carbon	III	IV [a]	V [b]	VI
C-1	38.5	38.3	38.1	38.3
C-2	23.7	23.6	23.4	23.5
C-3	81.0	80.7	80.7	80.9
C-4	37.8	37.6	37.7	37.8
C-5	55.5	55.3	55.2	55.5
C-6	18.4	18.1	18.2	18.3
C-7	33.1	32.8	32.6	32.8
C-8	39.8	39.5	39.3	39.5
C-9	47.7	47.4	47.5	47.7
C-10	37.0	36.8	36.9	37.0
C-11	23.5	23.2	23.4	23.2
C-12	126.4	125.4	122.7	122.9
C-13	137.8	138.0	142.9	143.3
C-14	42.2	41.9	41.4	41.7
C-15	29.8	28.1	27.5	27.7
C-16	24.4	24.2	23.4	23.5
C-17	48.0	48.0	46.4	46.9
C-18	52.9	52.8	40.7	40.5
C-19	34.1	38.9	41.4	40.3
C-20	43.5	38.9	33.7	34.4
C-21	25.1	30.7	29.3[c]	28.7[d]
C-22	36.2	36.6	31.7[c]	31.4[d]
C-23	28.2	28.1	28.0	28.1
C-24	16.8	16.9	16.7	16.7[e]
C-25	15.6	15.5	15.4	15.4
C-26	16.8	16.9	16.7	16.9[e]
C-27	21.1	23.6	25.9	25.9
C-28	177.5	177.6	177.4	177.8
C-29	17.0	17.1	27.8	74.6
C-30	67.7	21.2	67.7	19.3
CO_2Me	51.5	51.3	50.2	51.5
COMe	21.2	21.2	21.2	21.2
	20.9		20.9	20.8
COMe	171.1	170.5	170.9	171.0
	170.8		170.6	170.8

a, b: Data taken from ref. 5 and 6, respectively,
c, d, e: Assignments may be reversed.

that CH_2OAc was not attached to C-17 but to C-19 or C-20. Therefore the presence of C-28 carboxyl group was suggested. On the basis of the above data, the compound is supposed to be 29(or 30)-hydroxyursolic acid.

Finally, the point of attachment of the primary alcohol group was established by ^{13}C-NMR data. The ^{13}C chemical shifts of the methylacetate (III) are given in Table together with those of methylursolate acetate (IV)[5], methylgueretaroate acetate (V)[6] and methyl mesembryanthemoidigenate acetate (VI). The ^{13}C signals of III and VI were assigned by means of attached proton test experiment[7] and by comparison of δ values with these of the compounds included in Table I.

Making a comparison between the spectra of III and IV, considerably large differences in the chemical shifts were discernible for C-19 (−4.8ppm), C-20(+4.6ppm), C-21(−5.6ppm), and C-30(+46.5ppm). Such differences clearly show the presence of the primary alcohol at C-20.

Unexpectedly, C-15 was deshielded by 1.7ppm and C-27 shielded by 2.5ppm in III compared to IV. These differences can be explained by the conformational change in the E ring caused by the spatially close proximity of the acetoxyl methyl group at C-30 to C-15 and C-27, which is accordingly accompanied with that in the D ring.

In the spectrum of III, as expected[4], carbon signals for C-13, C-19, C-21 and C-27 appeared at higher fields and those for C-12, C-17, C-18, C-20 and C-22 appeared at lower fields as compared with those of oleanene isomers, V and VI.

Therefore, the sapogenin is 3β, 30-dihydroxyurs-12-en-28-oic acid (rubifolic acid), previously known only from *Rubia cordifolia*.[8]

A direct comparison (mmp, co-TLC and MS) with an authentic sample kindly supplied by Dr. B. Talapatra of University College of Science, Calcutta, India, confirmed the identity of these two terpenoids.

EXPERIMENTAL METHODS

The mps were taken on a Mitamura-Riken apparatus and are uncorrected. The IR spectra were determined in KBr tablets on a Perkin-Elmer Model 281-283 IR spectrometer. ^{1}H-NMR (80MHz) and ^{13}C-NMR (20MHz) were recorded with a Varian FT-80A in $CDCl_3$ containing TMS as an internal standard, and chemical shifts are given as δ(ppm). Mass spectra were obtained with a Hewlett Packard Model 5985B GC/MS spectrometer. Optical rotations were measured on a Rudolph Autopol® III automatic polarimeter.

Isolation of Sapogenin (I)

The roots of *Randia siamensis* were extracted with hot MeOH. The MeOH extract was partitioned with hexane, $CHCl_3$, ethylacetate and BuOH sucessively. The BuOH soluble portion was hydrolyzed with 5% H_2SO_4 for 5 hr. After cooling, the precipitate was filtered, washed with water and dried to give a brown solid, which was chromatographed over SiO_2 column eluting with benzene: ether (4:1) to give I as colorless prisms from $CHCl_3$-MeOH. mp 282-3°, $[\alpha]_D^{24}$+56°(c=1.0, EtOH); LB : positive $IR\nu_{max}^{KBr}$ cm^{-1}: 3400(OH), 1685(acid); MS m/z (rel. int.) 472[M]$^+$(1.9), 454[M−H_2O]$^+$(0.7), 264[D/E ring]$^+$(100), 246[264 −H_2O]$^+$(10), 233[264 −CH_2OH]$^+$(14), 207[A/B ring]$^+$(93.7), 201[246 −CO_2]$^+$(76.9), 189[207 −H_2O]$^+$(14.8).

Methylation of I

A sample (30mg) of I was esterified when dissolved in MeOH and treated with CH_2N_2. The product was crystallized from MeOH as colorless needles, mp 197-8°, $IR\nu_{max}^{KBr}$ cm^{-1} 1725(ester); MS m/z(rel. int.) 486[M]$^+$(2.5), 455[M −CH_2OH]$^+$(0.7), 426[M −($COOCH_3$+H)]$^+$(1.4), 278[D/E ring]$^+$(67.2), 247[D/E ring −CH_2OH]$^+$(15.4), 219[D/E ring −$COOCH_3$]$^+$(7.0), 201[D/E ring −($COOCH_3$+H_2O)]$^+$(100).

Acetylation of II

A sample (20mg) of II was acetylated with Ac_2O (2ml) and pyridine (1ml) in the usual way. The reaction product was crystallized from MeOH-$CHCl_3$ to give colorless plates, mp 176-8°, $IR\nu_{max}^{KBr}$ cm^{-1}, 1728, 1235(ester); HMR ($CDCl_3$) see text; CMR($CDCl_3$) see Table; MS m/z(rel. int.) 570[M]$^+$(1.2), 511[M −OAc]$^+$(1.9), 510[M −HOAc]$^+$(4.3), 495[M −(HOAc +CH_3)]$^+$(1.2), 450[M −2×HOAc]$^+$(0.3), 320[D/E ring]$^+$(3.8), 260[320 −HOAc]$^+$(14.4), 201[260 −$COOCH_3$]$^+$(100).

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