Phytochemistry, Vol. 28, No. 2, pp. 575–577, 1989.
Printed in Great Britain.

0031-9422/89 $3.00+0.00

TRITERPENOID SAPONINS FROM *RANDIA ULIGINOSA* FRUITS

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(*Received* 13 *May* 1988)

Key Word Index—*Randia uliginosa*; Rubiaceae; triterpenoidal saponins.

Abstract—Three saponins from the ethanolic extract of the fruits of *Randia uliginosa* were characterized on the basis of chemical and spectral evidences as [α-L-arabinopyranosyl (1→3)-β-D-galactopyranosyl (1→3)]-3β-hydroxyolean-12-en-28-methyloate, [{α-L-arabinopyranosyl (1→3)} {β-D-galactopyranosyl (1→6)}-β-D-galactopyranosyl (1→3)]-3β-hydroxyolean-12-en-28-methyl-oate and [{α-L-arabinopyranosyl (1→3)} {β-D-galactopyranosyl (1→4)}-β-D-glucuro-nopyranosyl (1→3)]-3β-hydroxyolean-12-en-28-oic acid.

INTRODUCTION

Randia uliginosa DC is a well known plant distributed throughout India and used for the cure of various ailments such as diarrhoea, dysentery and a fish poison [1]. The root of this plant is aphrodisiac, haemitinic and good for the heart [1]. The fruits have been reported to contain oleanolic acid, cyanidin chloride and D-mannitol [2] but saponins have not been reported so far. The present paper describes the isolation and structure elucidation of three novel triterpenoidal saponins from the fruits of the title plant.

RESULTS AND DISCUSSION

The ethanolic extract of the fruits on repeated column chromatography afforded compounds **1**–**3**, which were positive for the characteristic tests for triterpenoids. On acid hydrolysis **1** and **2** furnished oleanolic acid methyl ester and two monosaccharides (L-arabinose and D-galactose in the ratio 1:1 and 1:2, respectively), while **3** gave oleanolic acid and L-arabinose, D-galactose and D-glucuronic acid in the ratio 1:1:1. The identities of oleanolic acid and its methyl ester were confirmed by direct comparison with authentic samples, co-TLC, mmp and co-IR. The sugars were identified by PC. The molecular mass and sugar sequence were established by FABMS in the negative ion mode. Compound **2** showed peaks at *m/z* 925 $[M-H]^-$, 763 $[(M-H)-(162)]^-$, 631 $[(M-H)-(162+132)]^-$, 469 $[(M-H)-(162+132+162)]^-$, corresponding to the subsequent loss of hexose, pentosyl hexose and dihexosyl pentose units. The permethylates (**1a** and **2a**) of compounds **1** and **2** prepared by the method of ref. [3], on acid hydrolysis afforded 2,3,4-tri-*O*-methyl-L-arabinose and 2,4,6-tri-*O*-methyl-D-galactose from **1a**, whereas 2,3,4,6-tetra-*O*-methyl-D-galactose, 2,3,4-tri-*O*-methyl-L-arabinose and 2,4-di-*O*-methyl-D-galactose were obtained from **2a**. The identities of permethylated sugars were confirmed by comparison with available authentic samples [4, 5]. The partial hydrolysis of **2** afforded PS_1 and PS_2, in addition to oleanolic acid methyl ester. Interglycoside linkages were determined by acid hydrolysis of the premethylethers of PS_1 and PS_2. On acid hydrolysis PS_1 permethylate gave 2,3,4,6-tetra-*O*-methyl-D-galactose while PS_2 permethylate gave 2,3,4-tri-*O*-methyl-L-arabinose and 2,4,6-tri-*O*-methyl-D-galactose. PS_2 was found identical with **1** (mmp, co-TLC and co-IR). The type of linkage at the glycosidic points and the position of linkages of sugars were further confirmed by ^1H and ^{13}C NMR spectra. ^{13}C NMR spectra showed all the signals recognized for Me, CH_2, CH and quaternary carbon atoms. The C-3 atom of the inner galactose in **1** appeared at δ 86.6 revealing deshielding of ~10 ppm for this carbon resonance in comparison to the reported values for methylpyranoside [6], as a result of glycosidation in this position. The anomeric protons were observed at δ5.25 (*d*, $J=7$ Hz) and 4.41 (*d*, $J=6.5$ Hz). Thus, compound **1** was characterized as [α-L-arabinopyranosyl (1→3)-β-D-galactopyranosyl (1→3)]-3β-hydroxyolean-12-en-28-methyl-oate.

In the ^{13}C NMR spectra of **2**, the C-3 and C-6 atom of the inner hexose appeared at δ86.7 and 71.1 revealing deshielding of ~10 ppm and ~7 ppm of these carbon resonances in comparison to the reported values for methyl-*O*-β-D-galactoside [6], as a result of glycosidation of these points. The anomeric signals of sugars were observed at δ5.32 (*d*, $J=8$ *Hz*), 4.52 (*d*, $J=8$ Hz) and 4.38 (*d*, $J=7$ Hz). Thus, compound **2** was characterized as [{α-L-arabinopyranosyl (1→3)} {β-D-galactopyranosyl (1→6)}-β-D-galactopyranosyl (1→3)]-3β-hydroxyolean-12-en-28-methyl-oate.

Compound **3** on methylation with diazomethane afforded its methylester **3a** which on acid hydrolysis gave the oleanolic acid methyl ester. This confirmed that the carboxy group was free and glycosidation was at C-3. The sodium borohydride reduction of **3a** afforded **3b**, resulting in the conversion of the –COOMe group to CH_2OH, and **3b** on acid hydrolysis gave D-galactose, L-arabinose and D-glucose. Compound **3b** was permethylated by the method of ref. [3]. Completion of the reaction was checked by IR. Acid hydrolysis of **3b** permethyl ether afforded 2,3,4,6-tetra-*O*-methyl-D-galactose, 2,3,4-tri-*O*-

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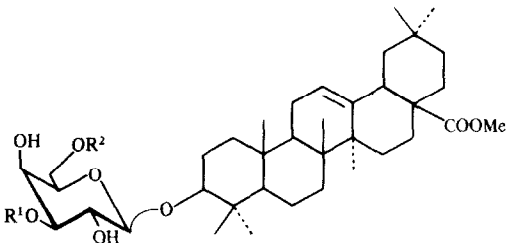
methyl-L-arabinose and 2,6-di-*O*-methyl-D-glucose. Compound **3b** was partially hydrolysed to yield prosapogenins PS_3 and PS_4. PS_3 and PS_4 on acid hydrolysis gave D-glucose and PS_4 also afforded L-arabinose. Permethyl ethers PS_3 and PS_4 afforded 2,3,4,6-tetra-*O*-methyl-D-glucose; and 2,3,4-tri-*O*-methyl-L-arabinose and 2,4,6-tri-*O*-methyl-D-glucose, respectively. In the ^{13}C NMR spectrum of **3**, the C-3 and C-4 of glucuronic acid were observed at δ 87.6 and 78.4, respectively, revealing deshielding of ~10 ppm and ~5 ppm of these carbon resonances in comparison to the reported values, as a result of glycosidation at C-3 and C-4. The anomeric signals of sugars were observed at δ 5.31 (*d*, $J = 8$ Hz), 4.43 (*d*, $J = 8$ Hz), and 4.38 (*d*, $J = 6.5$ Hz). Thus, compound **3** was characterized as [{α-L-arabinopyranosyl (1→3)} {β-D-galactopyranosyl (1→4)}-β-D-glucuronopyranosyl (1→3)]-3β-hydroxyolean-12-en-28-oic acid.

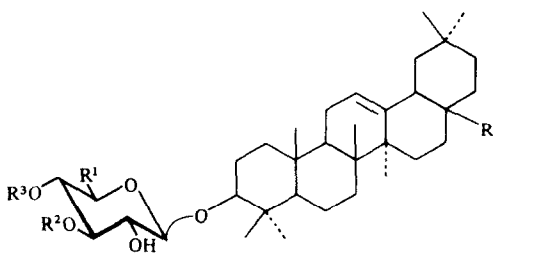
EXPERIMENTAL

Mps: uncorr. FABMS were obtained in the negative ion mode. ^1H and ^{13}C NMR were recorded at 400 MHz and 100 MHz respectively in CD_3OD using TMS as an internal standard. CC on silica gel (Merck, 60–120 mesh). TLC on Kieselgel 60 G (Merck), spots on TLC were visualized by spraying 10% H_2SO_4 followed by heating.

Extraction and isolation. Randia uliginosa fruits were supplied by Tamil Nadu Medicinal Plant, Farms and Herbal Medicine Corporation Ltd, Arumbakkam, Madras, India. The air-dried and coarsely powdered fruits were defatted with petrol. The solvent free material was exhaustively extracted with 90% EtOH until the extractives became colourless. The ethanolic extract was evapd to dryness and partitioned between *n*-BuOH and H_2O (1:1, ×4). The *n*-BuOH layer was concd under red. pres. and chromatographed over silica gel using $CHCl_3$–MeOH as eluant. Repeated CC afforded three compounds **1**–**3**.



	R^1	R^2
1	α-L-arabinopyranosyl	H
2	α-L-arabinopyranosyl	β-D-galactopyranosyl



	R^1	R^2	R^3
3	COOH	α-L-arabinopyranosyl	β-D-galactopyranosyl
3a	COOMe	α-L-arabinopyranosyl	β-D-galactopyranosyl
3b	CH_2OH	α-L-arabinopyranosyl	β-D-galactopyranosyl

Compound **1**. Crystallized as colourless needles from MeOH, mp 180–185°, ^1H NMR: δ 0.74, 0.80, 0.86, 0.91, 1.0, 1.1, 1.18 (each Me, 7 × tert-Me), 5.25 (1H, *d*, $J = 7$ Hz, C-1-H of galactose), 4.41 (1H, *d*, $J = 6.5$ Hz, C-1-H of arabinose), 5.11 (1H, *br s*, 12-H). ^{13}C NMR, C-1 to C-30 of aglycone: δ 39.7, 28.53, 91.04, 40.1, 56.9, 19.2, 33.5, 40.5, 48.1, 37.8, 23.9, 123.6, 145.09, 42.8, 28.6, 23.9, 47.2, 42.6, 47.6, 31.5, 34.8, 33.7, 28.8, 16.9, 15.9, 17.7, 26.4, 181.7, 33.9, 24.4, 51.5 (–OMe), carbon of sugar moiety: arabinopyranosyl: 104.5 (C-1), 71.5 (C-2), 75.0 (C-3), 70.1 (C-4), 67.5 (C-5); galactopyranosyl; 106.4 (C-1), 74.7 (C-2), 86.8 (C-3), 71.1 (C-4), 78.1 (C-5), 62.5 (C-6). FABMS (negative ion) *m/z*: 763, 631, 469, 455, 437, 365, 297.

Compound **2**. Crystallized from MeOH, mp 210–215°. ^1H NMR: δ 0.68, 0.72, 0.80, 0.83, 0.91, 1.02, 1.12 (each Me, 7 × tert-Me), 5.32 (1H, *d*, $J = 8$ Hz, C-1-H of galactose), 4.38 (1H, *d*, $J = 7$ Hz, C-1-H of arabinose), 4.52 (1H, *d*, $J = 8$ Hz, C-1-H of galactose), 5.19 (1H, *br s*, 12-H). ^{13}C NMR, C-1 to C-30 of aglycone: δ 39.8, 28.6, 91.1, 40.1, 57.0, 19.2, 33.5, 40.7, 48.3, 37.8, 24.0, 123.7, 144.7, 42.9, 28.8, 24.5, 47.9, 42.5, 47.2, 31.4, 34.8, 33.9, 28.6, 17.0, 16.0, 17.7, 26.4, 178.08, 33.0, 24.02, 51.6 (–OMe), carbon of sugar moiety: arabinopyranosyl; 105.6 (C-1), 73.8 (C-2), 74.6 (C-3), 71.1 (C-4), 67.0 (C-5); galactopyranosyl; 106.3 (C-1), 71.6 (C-2), 75.0 (C-3), 70.8 (C-4), 78.4 (C-5), 62.5 (C-6); inner galactopyranosyl: 95.6 (C-1), 71.1 (C-2), 86.6 (C-3), 70.8 (C-4), 78.2 (C-5), 71.6 (C-6). FABMS: 925, 763, 631, 469, 455, 437, 365, 255.

Acid hydrolysis. Compounds **1** and **2** (50 mg each) were separately hydrolysed in MeOH with 6% H_2SO_4 for 4 hr to afford aglycone (oleanolic acid methyl ester), colourless needles (MeOH), mp 198–200°, identified by co-TLC, mmp with authentic sample and co-IR. The neutralized and concd hydrolysate showed the presence of D-galactose and L-arabinose in the ratio 1:1 and 2:1, respectively, identified by PC (solvent *n*-BuOH–AcOH–H_2O, 4:1:5; R_f values 0.20 and 0.25, respectively).

Permethylation. Compounds **1** and **2** (each 5 mg) separately permethylated with dry DMSO (0.4 ml), dry *t*-BuONa (40 mg), finely powdered dry NaOH (10 mg) and MeI (0.3 ml). The mixture was stirred at room temp. for 1 hr. The soln was poured into ice water and extracted with Et_2O. The Et_2O layer was washed with a satd NaCl soln, dried and evapd. Complete methylation was checked by IR. The permethyl ethers, **1a** and **2a** of **1** and **2** were hydrolysed with 6% H_2SO_4 in the usual manner and the methylated sugars obtained were identified as 2,3,4-tri-*O*-methyl-L-arabinose and 2,4,6-tri-*O*-methyl-D-galactose from **1a** and 2,3,4-tri-*O*-methyl-L-arabinose, 2,3,4,6-tetra-*O*-methyl-D-galactose and 2,4-di-*O*-methyl-D-galactose from **2a**. These sugars were also confirmed by co-PC with aunthetic samples (*n*-BuOH–EtOH–H_2O, 5:1:4).

Partial hydrolysis. Compound **2** in 1 M HCl–BuOH (1:1, 50 ml) was heated at 70° for 3 hr. The BuOH layer was washed with H_2O and evapd *in vacuo*. The residue on CC yielded oleanolic acid methyl ester, PS_1 and PS_2. PS_2 was found to be identical to **1** (comparing with mmp, co-TLC and co-IR).

Permethylation of PS_1 and PS_2. PS_1 and PS_2 on permethylation followed by acid hydrolysis afforded 2,3,4,6-tetra-*O*-methyl-D-galactose from PS_1, 2,3,4-tri-*O*-methyl-L-arabinose and 2,4,6-tri-*O*-methyl-D-galactose from PS_2.

Compound **3**. Crystallized from MeOH, mp 240–245°. ^1H NMR: δ 0.71, 0.76, 0.84, 0.88, 0.90, 1.08, 1.20 (each Me, 7 × *tert*-Me), 5.31 (1H, *d*, $J = 8$ Hz, C-1-H of glucuronic acid), 4.43 (1H, *d*, $J = 8$ Hz, C-1-H of galactose), 4.38 (1H, *d*, $J = 6.5$ Hz, C-1-H of arabinose), 5.19 (1H, *br s*, 12-H). ^{13}C NMR, C-1 to C-30 of aglycone: δ 39.7, 28.5, 91.1, 40.05, 56.9, 19.2, 33.05, 40.06, 48.3,

37.8, 23.9, 123.6, 144.7, 42.8, 28.7, 23.9, 47.9, 42.5, 47.2, 31.4, 34.8, 33.9, 30.5, 16.9, 15.9, 17.7, 26.3, 177.9, 33.4, 24.4. Sugar moiety; arabinopyranosyl: 105.7 (C-1), 73.8 (C-2), 76.1 (C-3), 71.1 (C-4), 66.9 (C-5); galactopyranosyl: 106.4 (C-1), 75.0 (C-2), 77.4 (C-3), 70.9 (C-4), 77.8 (C-5), 62.4 (C-6); glucuronopyranosyl; 95.6 (C-1), 74.4 (C-2), 86.8 (C-3), 78.4 (C-4), 78.2 (C-5), 170.9 (C-6).

FABMS (Negative ion) *m/z*: 925, 763, 631, 455, 439, 394, 325, 297, 208.

Acid hydrolysis. Compound **3** was hydrolysed in MeOH with 6% H_2SO_4 for 4 hrs to afford aglycone oleanolic acid, colourless needles (MeOH), mp 300–310°, identified by co-TLC, mmp, co-IR and EIMS. The sugars were identified as D-galactose, L-arabinose and D-glucoronic acid (PC, *n*-BuOH–AcOH–H_2O, 4:1:5; R_f 0.20, 0.25 and 0.15, respectiely).

Methylation. A soln of **3** (300 mg) in MeOH was treated with excess ethereal CH_2N_2 and the whole reaction mixture allowed to stand for 4 hr at 5°. Removal of solvent under red. pres. gave the methyl derivative (**3a**) of glycoside **3**.

$NaBH_4$ *reduction of* **3a**. To a soln of **3a** (250 mg) in dry ether $NaBH_4$ (500 mg) was added under ice cooling and the reaction mixture was stirred for 20 hr at room temp. Me_2CO (2 ml) and H_2O were added to the mixture and evapd *in vacuo*. The residue was diluted with excess of H_2O and extracted with *n*-BuOH. The *n*-BuOH layer was washed, dried and concd. It afforded reduced glycoside **3b** (200 mg).

Permethylation of **3b**. Compound **3b** (50 mg) was permethylated as above, complete methylation was checked by IR. A permethyl ether of **3b** so obtained was hydrolysed with 3% H_2SO_4 in the usual manner and the methylated sugars obtained were identified as 2,3,4,6-tetra-*O*-methyl-D-galactose, 2,3,4-tri-*O*-methyl-L-arabinose and 2,6-di-*O*-methyl-D-glucose. Identities of sugars were confirmed by co-PC with authentic samples (solvent, *n*-BuOH–EtOH–H_2O, 5:1:4 and $NaIO_4$ oxidation result).

Partial hydrolysis of **3b**. Compound **3b** (150 mg) in 1M HCl–BuOH (1:1, 50 ml) was heated at 70° for 4 hr. The BuOH layer was washed with H_2O and evapd *in vacuo*. The residue on CC yielded prosapogenins PS_3 (30 mg) and PS_4 (45 mg). On hydrolysis these afforded D-galactose, L-arabinose and D-glucose.

Permethylation of PS_3 *and* PS_4. PS_3 and PS_4 (15 mg each) on permethylation as above, followed by acid hydrolysis gave 2,3,4,6-tetra-*O*-methyl-D-glucose from PS_3, 2,3,4-tri-*O*-methyl-L-arabinose and 2,4,6-tri-*O*-methyl-D-glucose from PS_4.

Acknowledgements—We thank Prof. T. Kawasaki (Setsunan, Japan) for FABMS, also RSIC, CDRI, Lucknow for spectra and University Grants Commission, New Delhi for financial assistance.

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