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Triterpene saponins from *Randia formosa*

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Abstract

Seven new triterpenoid saponins, randiasaponins I (**1**), II (**2**), III (**3**), IV (**4**), V (**5**), VI (**6**) and VII (**7**) as well as two known ones, ilexoside XXVII (**8**) and ilexoside XXXVII (**9**), were isolated from the methanolic extract of the leaves of *Randia formosa*. The structures of the new saponins were established as 3-*O*-α-L-arabinopyranosyl-3β,19α,23-trihydroxyursa-12,20(30)-dien-28-oic acid 28-β-D-glucopyranosyl ester (**1**), 3-*O*-β-D-glucopyranosyl-(1 → 3)-α-L-arabinopyranosyl rotundic acid (**2**), 3-*O*-β-D-glucopyranosyl-(1 → 3)-α-L-arabinopyranosyl pomolic acid 28-β-D-glucopyranosyl ester (**3**), 3-*O*-α-L-rhamnopyranosyl-(1 → 2)-α-L-arabinopyranosyl pomolic acid 28-β-D-glucopyranosyl ester (**4**), 3-*O*-α-L-rhamnopyranosyl-(1 → 2)-α-L-arabinopyranosyl siaresinolic acid 28-β-D-glucopyranosyl ester (**5**), 3-*O*-α-L-arabinopyranosyl ilexosapogenin A 28-β-D-glucopyranosyl ester (**6**), and 3-*O*-β-D-glucopyranosyl ilexosapogenin A 28-β-D-glucopyranosyl ester (**7**), based on spectral and chemical evidence. Besides the saponins, two common flavonoids kaempferol 3-*O*-rutinoside and rutin were also isolated.

Keywords: *Randia formosa*; Rubiaceae; Triterpenoid saponins; 3β,19α,23-Trihydroxy-ursa-12,20(30)-dien-28-oic acid; Rotundic acid; Pomolic acid; Siaresinolic acid; Ilexosapogenin A

1. Introduction

In the course of our study of plants from the flora of Panama, the constituents of *Randia formosa* Schum. (Rubiaceae) were investigated. Earlier phytochemical investigation revealed that the stem bark of the plant contains iridoids (Sainty et al., 1982). Saponins have already been found in the genus *Randia* (Murty et al., 1989; Dubois et al., 1990). However, no phytochemical study on the leaves of *R. formosa* has been reported in the literature so far.

This paper describes the isolation and structure elucidation of a total of nine urs-12-ene and olean-12-ene type triterpenoid saponins from the methanolic extract of the leaves of *R. formosa*.

2. Results and discussion

Leaves of *R. formosa* were extracted successively with CH_2Cl_2 and MeOH. The methanolic extract of the dried and powdered leaves afforded a mixture of glycosides which were separated by repeated column chromatography on normal and reversed phase silica gel, affording seven new triterpene mono- and bidesmosidic saponins, randiasaponins I–VII together with ilexoside XXVII (Yano et al., 1993) and ilexoside XXXVII (Amimoto et al., 1993).

Compound **1** was obtained as an amorphous white powder. The flow injection analysis (FIA) by liquid chromatography/electrospray-mass spectrometry (LC/ES-MS) in the positive ion mode of **1** showed a quasi molecular $[M + Na]^+$ peak at m/z 803 corresponding to a molecular formula of $C_{41}H_{64}O_{14}$. Compound **1** was suggested to be an ester glycoside as its IR spectrum showed a band at 1740 cm^{-1}. An alkaline hydrolysis of **1** yielded a prosapogenin and glucose,

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confirming this hypothesis. On acid hydrolysis, **1** afforded a mixture of compounds in the triterpenoid fraction. It has been previously reported that this type of saponin, bearing an exocyclic methylene functionality, gave some artifactual aglycones after acid hydrolysis (Ahmad et al., 1984, 1986). The sugar components were identified by TLC as arabinose and glucose (1:1) by comparison with authentic samples. A detailed analysis of the NMR spectral data of **1** revealed the features of a 19 oxygenated urs-12-ene type triterpene

Table 1
^{1}H-NMR spectral data for randiasaponins I–VII (**1**–**7**), (500 MHz, CD_3OD)[a]

H	**1**	**2**	**3**	**4**	**5**	**6**	**7**
	δ (ppm), J (Hz)	δ (ppm), J (Hz)	δ (ppm), J (Hz)	δ (ppm), J (Hz)	δ (ppm), J (Hz)	δ (ppm), J (Hz)	δ (ppm), J (Hz)
1	0.99[b], 1.65[b]	1.01[b], 1.61[b]	0.99[b], 1.64[b]	0.99[b], 1.62[b]	0.99[b], 1.62[b]	0.98[b], 1.61[b]	0.99[b], 1.61[b]
2	1.76[b], 1.87[b]	1.73[b], 1.84[b]	1.73[b], 1.84[b]	1.72[b], 1.82[b]	1.72[b], 1.82[b]	1.75[b], 1.85[b]	1.76[b], 1.95[b]
3	3.61[b]	3.61[b]	3.15 *dd* (11.5, 4.0)	3.11 (11.2, 3.9)	3.11 *dd* (11.2, 3.9)	3.62[b]	3.63[b]
5	1.27[b]	1.24[b]	0.79[b]	0.81b	0.81[b]	1.24[b]	1.26[b]
6	1.46[b]	1.50[b]	1.53[b]	1.52[b]	1.41[b]	1.39[b]	1.47[b]
7	1.32[b], 1.68[b]	1.28[b]	1.33[b], 1.54[b]	1.33[b], 1.52	1.33[b], 1.47[b]	1.26[b]	1.26[b], 1.60[b]
9	1.76[b]	1.73[b]	1.67[b]	1.72[b]	1.67[b]	1.70[b]	1.72[b]
11	1.97[b]	1.97[b]	1.97[b]	1.95[b]	1.95[b]	1.95[b]	1.96[b]
12	5.33[b]	5.28 *t* (3.4)	5.33 *br s*	5.30 *t* (3.9)	5.32[b]	5.30[b]	5.32[b]
15	1.06[b], 1.82[b]	1.01[b], 1.84[b]	1.02[b], 1.83[b]	0.99[b], 1.77[b]	1.03[b], 1.67[b]	1.02[b], 1.83[b]	1.02[b], 1.82[b]
16	1.76[b]	1.50[b]	1.63[b]	1.62[b]	1.72[b]	1.72[b]	1.72[b]
	2.74 *td* (13.5, 4.5)	2.50 *td* (13, 4.0)	2.61 *td* (13.5, 4.0)	2.61 *td* (13.2, 4.4)	2.32 *td* (13.2, 4.4)	2.32 *td* (13.0, 4.0)	2.32 *td* (13.0, 4.0)
18	2.65 *s*	2.50 *s*	2.51 *s*	2.51 *s*	3.05 *s*	3.04 *s*	3.04 *s*
19	–	–	–	–	3.27 *d* (4.0)	3.27 *d* (3.5)	3.27[b]
20	–	1.30[b]	1.36[b]	1.36[b]	–	–	–
21	2.10 *dt* (13.7, 4.9), 2.77 *td* (13.5, 5.0)	1.71[b]	1.71[b]	1.72[b]	1.70[b]	1.69[b]	1.67[b]
22	1.71[b], 1.95[b]	1.64[b], 1.73[b]	1.63[b], 1.77[b]	1.62[b], 1.77[b]	1.65[b], 1.77[b]	1.64[b], 1.77[b]	1.70[b], 1.79[b]
23	3.29 *d* (3.4), 3.61[b]	3.30[b], 3.63[b]	1.05 *s*	1.01 *s*	1.01 *s*	3.30[b], 3.60[b]	3.29 *d* (3.4), 3.64[b]
24	0.71 *s*	0.72 *s*	0.85 *s*	0.84 *s*	0.84 *s*	0.71 *s*	0.71 *s*
25	0.99 *s*	0.98 *s*	0.95 *s*	0.95 *s*	0.95 *s*	0.98 *s*	0.99 *s*
26	0.79 *s*	0.80 *s*	0.77 *s*	0.74 *s*	0.78 *s*	0.77 *s*	0.75 *s*
27	1.34 *s*	1.34 *s*	1.33 *s*	1.33 *s*	1.29 *s*	1.29 *s*	1.30 *s*
29	1.37 *s*	1.19 *s*	1.20 *s*	1.20 *s*	0.95 *s*	0.93 *s*	0.94 *s*
30	4.70 *br s*, 4.89 *br s*	0.93 *d* (6.8)	0.93 *d* (6.8)	0.92 *d* (6.8)	0.95 *s*	0.94 *d* (6.8)	0.93 *d* (6.8)
3-*O*-	Arabinose	Arabinose	Arabinose	Arabinose	Arabinose	Arabinose	Glucose
1′	4.32 *d* (6.8)	4.35 *d* (7.3)	4.29 *d* (7.0)	4.55 *d* (4.4)	4.55 *d* (4.4)	4.32 *d* (6.8)	4.40 *d* (7.5)
2′	3.54[b]	3.69[b]	3.71[b]	3.77[b]	3.77[b]	3.53[b]	3.17 *t* (8.5)
3′	3.50	3.61[b]	3.65[b]	3.74[b]	3.74[b]	3.50[b]	3.34[b]
4′	3.79[b]	4.03 *br s*	4.03 *br s*	3.79[b]	3.79[b]	3.79[b]	3.29[b]
5′	3.54[b]	3.57 *d* (11.7)	3.55 *d* (12.5)	3.48 *dd* (11.8, 3.0)	3.48 *dd* (11.8, 3.0)	3.55 *d* (12.5)	3.27[b]
	3.84 *dd* (12.2, 2.9)	3.86 *dd* (12.7, 2.0)	3.86 *dd* (12.5, 2.0)	3.85[b]	3.85[b]	3.84 *dd* (12.5, 3.0)	
6′							3.69[b], 3.84[b]
Terminal		Glucose	Glucose	Rhamnose	Rhamnose		
1″		4.54 *d* (7.3)	4.56 *d* (7.3)	5.09 d (0.9)	5.09 *d* (0.9)		
2″		3.29[b]	3.31[b]	3.88 *dd* (3.4, 1.5)	3.88 *dd* (3.4, 1.5)		
3″		3.31[b]	3.31[b]	3.68[b]	3.68[b]		
4″		3.34 *t* (8.8)	3.37[b]	3.38[b]	3.38[b]		
5″		3.37 *t* (8.8)	3.39[b]	3.81[b]	3.81[b]		
6″		3.69[b]	3.70[b]	1.23 *d* (5.9)	1.23 *d* (5.9)		
		3.83 *dd* (12.3, 2)	3.82 *dd* (11.2, 2.0)				
28-*O*-	Glucose		Glucose	Glucose	Glucose	Glucose	Glucose
1‴	5.34 *d* (8.3)		5.33 *d* (8.3)	5.37 *d* (8.3)	5.32 *d* (8.3)	5.32 *d* (8.3)	5.37 *d* (8.5)
2‴	3.31[b]		3.33[b]	3.38[b]	3.32[b]	3.32[b]	3.31[b]
3‴	3.33[b]		3.35[b]	3.34[b]	3.33[b]	3.34[b]	3.34[b]
4‴	3.35		3.37[b]	3.38[b]	3.34[b]	3.36[b]	3.35[b]
5‴	3.40 *t* (8.8)		3.42[b]	3.40[b]	3.40[b]	3.41[b]	3.40[b]
6‴	3.68[b], 3.80[b]		3.70[b]	3.70[b], 3.82[b]	3.70[b], 3.82[b]	3.68[b], 3.80[b]	3.69[b], 3.81[b]
			3.82 *dd* (11.2, 2.0)				

[a] Assignments were based on COSY, HMQC and HSQC experiments.
[b] Signal patterns are unclear due to overlapping.

saponin whose hydroxyl group at C-3 and carboxyl group at C-28 are glycosylated (Kakuna et al., 1992). Assignments for all proton and carbon resonances (see Tables 1 and 2) were achieved by COSY, HSQC and HMBC experiments. The ^{13}C-NMR spectrum revealed 41 carbon signals of which 11 were assigned to pentosyl and hexosyl units and the remaining 30 signals to a triterpenoid skeleton. The Δ^{12} functionality of the triterpenoid aglycone was deduced from the resonance of the sp^2 carbons C-12 (tertiary carbon) at δ 129.82 and C-13 (quaternary carbon) at δ 139.37. The ^{1}H-NMR spectrum of **1** exhibited resonances for the anomeric protons of the sugar moiety at δ 4.32 (*d*, J = 6.8 Hz), 5.34 (*d*, J = 8.3 Hz) which were assigned to the

Table 2
^{13}C-NMR spectral data for randiasaponins I–VII (**1**–**7**), (125 MHz, CD_3OD)

C	1	2	3	4	5	6	7
1	39.62	39.51	39.90	39.97	39.77	39.35	39.40
2	26.37	26.32	27.05	27.04	6.99	26.29	27.09
3	83.44	83.64	90.56	90.71	90.71	83.43	83.55
4	43.89	43.89	40.20	40.27	40.26	43.85	43.88
5	48.24	48.35	57.03	57.15	57.03	48.29	48.34
6	18.97	19.02	19.44	19.51	19.50	19.00	19.03
7	33.85	33.70	34.14	34.13	33.87	33.34	33.38
8	41.03	41.07	41.23	41.26	40.89	40.83	40.87
9	48.62	48.58	48.59	49.04	48.49	48.55	48.60
10	37.68	37.71	37.84	38.06	37.87	37.82	37.83
11	24.74	24.71	24.73	24.83	24.72	24.75	24.73
12	129.82	129.40	129.66	129.68	124.96	124.96	124.99
13	139.37	140.09	139.50	139.56	144.29	144.31	144.36
14	42.87	42.68	42.59	42.63	42.61	42.67	42.71
15	29.55	29.64	29.63	29.53	29.45	29.44	29.52
16	26.99	26.67	26.51	26.53	28.45	28.45	28.45
17	49.46	49.46	49.41	49.46	47.12	47.10	47.13
18	55.61	55.14	54.88	54.95	45.06	45.04	45.09
19	73.86	73.64	73.63	73.64	82.45	82.43	82.47
20	156.23	43.09	42.87	42.92	35.95	35.92	35.95
21	29.04	27.32	27.19	27.21	29.67	29.51	29.48
22	38.81	39.06	38.24	38.29	33.27	33.27	33.29
23	64.94	65.26	28.59	28.62	28.61	64.91	64.95
24	13.43	13.34	17.06	17.09	17.01	13.36	13.34
25	16.58	16.42	16.06	16.06	15.94	16.38	16.36
26	17.75	17.59	17.61	17.77	17.61	17.83	17.83
27	24.08	24.88	24.73	24.68	25.01	25.06	25.03
28	177.95	180.81	178.46	178.54	178.54	178.55	178.58
29	27.71	27.10	27.14	27.10	28.67	28.62	28.61
30	106.33	16.61	16.63	16.61	25.19	25.20	25.19
3-*O*-	Arabinose	Arabinose	Arabinose	Arabinose	Arabinose	Arabinose	Glucose
1′	106.28	106.14	107.01	104.74	104.74	106.27	105.72
2′	72.96	72.12	72.04	76.81	76.81	72.92	75.63
3′	74.53	84.27	83.80	72.99	72.99	74.48	78.72
4′	69.75	69.56	69.46	68.32	68.32	69.72	71.57
5′	66.81	66.87	66.62	63.64	63.64	66.79	77.71
6′							62.75
Terminal		Glucose	Glucose	Rhamnose	Rhamnose		
1″		105.52	105.30	102.01	102.01		
2″		75.33	75.25	72.15	72.15		
3″		77.94	77.80	72.15	72.15		
4″		71.17	71.13	73.87	73.87		
5″		77.69	77.56	70.21	70.21		
6″		62.35	62.34	17.99	17.99		
28-*O*-	Glucose		Glucose	Glucose	Glucose	Glucose	Glucose
1‴	95.83		95.70	95.79	95.76	95.77	95.82
2‴	73.62		73.78	73.91	73.86	73.88	73.86
3‴	78.61		78.45	78.69	78.54	78.64	78.57
4‴	71.11		71.05	71.12	71.07	71.04	71.10
5‴	78.30		78.20	78.31	78.28	78.26	78.34
6‴	62.40		62.39	62.43	62.38	62.36	62.40

anomeric protons of L-arabinose and D-glucose, respectively. Chemical shifts, multiplicities, coupling constants and magnitude in the ^{1}H-NMR spectrum, as well as ^{13}C-NMR, data indicated the α-configuration at the anomeric position for the arabinose unit, and the β-configuration for the glucose (Piacente et al., 1995). The signal of C-28 at δ 177.95 further confirmed the IR absorption at 1740 cm^{-1}, indicating the presence of an ester group rather than a free acid group (Durham et al., 1994). In addition, the shift observed for the anomeric carbon of β-D-glucose at δ 95.83 was in agreement with a site of glycosylation at the 28-carboxyl group. The ^{13}C-NMR spectrum of **1** also showed significant glycosylation shifts for C-3 (δ 83.44) of the aglycone. These results supported the presence of a bidesmosidic structure. In the HMBC experiment, correlations between C-3 (δ 83.44) of the aglycone and the anomeric proton (H-1′: δ 4.32 *d*, J = 6.8 Hz) of α-L-arabinose and C-28 (δ 177.95) of the aglycone and the anomeric proton (H-1″: 5.34 *d*, J = 8.3 Hz) of β-D-glucose were observed showing the interglycosidic connectivities (see Fig. 1). After the assignment of the ^{13}C-NMR signals of the sugar moiety, the resonances remaining for the aglycone of **1** were five methyls, eleven methylenes, five methines and nine quaternary carbons. The carbon and proton resonances for the aglycone moiety of **1** indicated an elemental formula of $C_{30}H_{46}O_5$, implying eight degrees of unsaturation, of which two were attributed to a Δ^{12} and a carbonyl functionality, five were attributed to a pentacyclic system, and one remained to be assigned. The ^{13}C-NMR spectrum of **1** showed the presence of two olefinic bonds, one of which is the Δ^{12} functionality. The other olefinic carbon signals appeared at δ 156.23 (quaternary carbon) and 106.33 (CH_2) indicating the presence of a $>C{=}CH_2$ moiety. The HSQC experiment of **1** showed correlations between the exocyclic methylene functionality and two resonances on the ^{1}H-NMR spectrum at δ 4.70 (*br s*) and δ 4.89 (*br s*). The presence of only five methyls in the ^{1}H-NMR (CH_3-24: δ 0.71, CH_3-25: δ 0.99, CH_3-26: δ 0.79, CH_3-27: δ 1.34, CH_3-29: δ 1.37) and ^{13}C-NMR spectra (CH_3-24: δ 13.43, CH_3-25: δ 16.58, CH_3-26: δ 17.75, CH_3-27: δ 24.08, CH_3-29: δ 27.71) suggested that the other double bond was present between C-20 and C-30. The

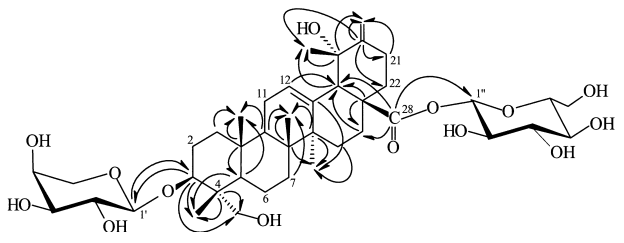


Fig. 1. Heteronuclear multiple bond correlations (HMBC) for **1**. Arrows point from carbon to proton.

HMBC experiment exhibited correlations particularly between C-20/H_3-29, C-20/H_2-30, C-19/H_2-30 and C-21/H_2-30. Further correlations are shown in Fig. 1. It is therefore concluded that the other double bond is between C-20 and C-30. Furthermore, carbon signals at δ 83.44 (C-3), 73.86 (C-19), and 64.94 (C-23) were consistent with assignments to hydroxylated carbons. The orientation of the hydroxyl group at C-19 was shown to be α by means of a consideration of the data in the literature (Akira et al., 1987; Takashi et al., 1991). Finally, a singlet proton signal at δ 2.65 and the corresponding carbon resonances at δ 55.61 were assigned to H-18 and C-18, respectively. These results clearly supported the identity of the aglycone moiety to be 3β,19α,23-trihydroxyursa-12,20(30)-dien-28-oic acid which has not been reported previously. Thus, the structure of saponin **1** was established as 3-*O*-α-L-arabinopyranosyl-3β,19α,23-trihydroxyursa-12,20(30)-dien-28-oic acid 28-β-D-glucopyranosyl ester, for which the trivial name randiasaponin I is proposed.

Compound **2** was obtained as an amorphous white powder, and exhibited a quasimolecular peak [M + Na]$^+$ at m/z 805 in the LC/ES-MS, corresponding to a molecular formula of $C_{41}H_{66}O_{14}$. The ^{1}H- and ^{13}C-NMR spectra of **2** displayed many similarities with those of **1** for the aglycone moiety, except for the C-20(30) olefinic bond. Instead of this, the ^{13}C-NMR spectrum of **2** showed an additional secondary methyl resonance at δ 16.61 which was correlated to a signal in the ^{1}H-NMR spectrum at δ 0.93 (*d*, J = 6.8 Hz) in HSQC experiments. In addition, the presence of a quaternary carbon at δ 43.09 for position 20 was characteristic of an urs-12-ene type structure (Kakuna et al., 1992). Compound **2** gave glucose and arabinose (1:1) on acid hydrolysis as in saponin **1**. But the absence of a glycosylation shift for the 28-carboxyl group (δ 180.81) suggested that **2** had a free 28-carboxyl group which was further confirmed by the IR absorption at 1700 cm^{-1}, indicating the presence of a free acid group rather than an ester group. A comparison of the ^{13}C-NMR spectrum of **2** with that of **1** showed that the arabinosyl unit was also attached to the C-3 position, and varied structurally from **1** by signals of a terminal β-D-glucopyranosyl unit which was deduced to be attached at C-3 of the α-L-arabinosyl unit of **2**. An obvious chemical shift in the ^{13}C-NMR spectrum at δ 84.27 assigned to C-3′ of the α-L-arabinosyl unit supported this suggestion. Furthermore, all sites of glycosylation were also established by HMBC experiments showing long-range correlations between C-1′ of the α-L-arabinose (δ 106.14) and H-3 (δ 3.61, *m*) of the aglycone, C-3′ of the α-L-arabinosyl moiety (δ 84.27) and H-1″ of the terminal β-D-glucopyranosyl unit (δ 5.34, *d*, J = 8.3 Hz). Comparison of spectral data with those reported for known aglycone moieties indicated that the genin of **2** was rotundic acid (Nakatani et al.,

1989). Therefore, the structure of **2** was established as 3-*O*-β-D-glucopyranosyl-(1 → 3)-α-L-arabinopyranosyl rotundic acid for which the trivial name randiasaponin II is proposed.

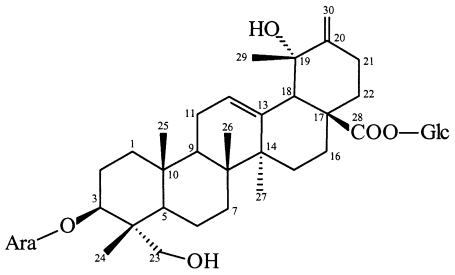
Compound **3** was obtained as an amorphous white powder. The molecular formula was deduced from the peak at *m/z* 951 $[M + Na]^+$ in the LC/ES-MS as $C_{47}H_{76}O_{18}$. Acid hydrolysis of **3** afforded glucose and arabinose (2:1). The ^{1}H- and ^{13}C-NMR spectra of **3** indicated the presence of one α-L-arabinopyranosyl unit (H-1′: δ 4.29, *d*, *J* = 7 Hz; C-1′: δ 107.01) and two β-D-glucopyranosyl units (H-1′: δ 4.56, *d*, *J* = 7.3 Hz; C-1′: δ 107.01 and H-1′: δ 5.33, *d*, *J* = 8.3 Hz; C-1′: δ 95.70). The carbon signals due to C-3 sugar moieties were almost superimposable with those of **2**, and the aglycone signals were very similar in both compounds, with the exception of the C-23 and C-28 moieties. In the ^{13}C-NMR of **3**, the presence of an additional tertiary methyl at δ 28.59 for C-23 indicated that the aglycone of **3** was pomolic acid. All ^{1}H- and ^{13}C-NMR spectral data assigned to the sapogenol moiety were also in agreement with those reported for pomolic acid (Wenjuan et al., 1986; Inada et al., 1987). On the other hand, the HMBC performed on **3** showed clearly the connectivity between C-28 (δ 178.46) of the aglycone and H-1′ of the β-D-glucose (δ 5.33, *d*, *J* = 8.3 Hz) indicating **3** to possess one additional glucose at C-28 when compared with **2**. Hence, **3** was formulated as 3-*O*-β-D-glucopyranosyl-(1 → 3)-α-L-arabinopyranosyl pomolic acid 28-β-D-glucopyranosyl ester for which the trivial name randiasaponin III is proposed.

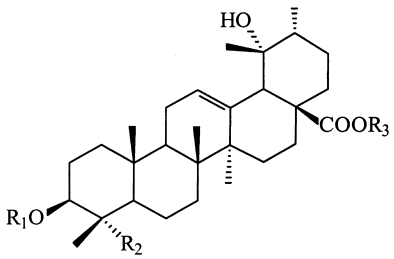
Compound **4** was obtained as an amorphous white powder. The molecular formula was deduced from the peak at *m/z* 935 $[M + Na]^+$ in the LC/ES-MS as $C_{47}H_{76}O_{17}$. Compound **4** afforded rhamnose, arabinose and glucose (1:1:1) on acid hydrolysis. All proton and carbon assignements based on 2D-NMR experiments (COSY, HMQC and HMBC) made clear that the set of carbon signals (C-1′: δ 102.01, C-2′/3′: δ 72.15, C-4′: δ 73.87, C-5′: δ 70.21, C-6′: δ 17.99) corresponding to a sugar moiety which is different from that of compounds **1**–**3** belongs to a terminal α-L-rhamnosyl unit. Comparison of the ^{13}C-NMR spectrum of **4** with that of **3** confirmed that **4** possesses a terminal α-L-rhamnosyl unit, instead of a terminal β-D-glucosyl unit as in **3**. An obvious chemical shift at 76.81 ppm assigned to C-2′ of the α-L-arabinosyl unit showed the site of glycosylation of the terminal α-L-rhamnopyranosyl unit to be at C-2′ of the α-L-arabinose. HMBC experiments performed with **4** confirmed this hypothesis, since the carbon signal at 76.81 ppm showed correlations to H-1′ and H-2′ of the L-rhamnosyl unit. Consequently, the structure of **4** was established as 3-*O*-α-L-rhamnopyranosyl-(1 → 2)-α-L-arabinopyranosyl pomolic acid 28-β-D-glucopyranosyl ester for which the trivial name randiasaponin IV is proposed.

Compound **5** was obtained as an amorphous white powder. The molecular formula was deduced from the peak at *m/z* 935 $[M + Na]^+$ in the LC/ES-MS as $C_{47}H_{76}O_{17}$. It afforded rhamnose, arabinose and glucose (1:1:1) on acid hydrolysis as in **4**. A comparison of the ^{13}C-NMR spectrum of **5** with that of **4** showed that the sugar moieties were identical in the two compounds. Compound **5** differed structurally from **4** in its aglycone moiety which showed a pair of signals at δ 124.96 (C-12) and 144.29 (C-13) in the ^{13}C-NMR spectrum, characteristic for the double bond of an olean-12-ene type structure (see Tables 1 and 2) (Doddrell et al., 1974). In addition, C-29 (δ 28.67) and C-30 (δ 25.19) tertiary methyl resonances, C-19 (δ 82.45) hydroxylated methine and C-20 (δ 35.95) quaternary carbon resonances supported the presence of an olean-12-ene type structure. All remaining proton and carbon resonances for the aglycone moiety were in agreement with those reported in literature for siaresinolic acid (Yaguchi et al., 1995; Bilia et al., 1994). Therefore, the structure of **5** was established as 3-*O*-α-L-rhamnopyranosyl-(1 → 2)-α-L-arabinopyranosyl siaresinolic acid 28-β-D-glucopyranosyl ester for which the trivial name randiasaponin V is proposed.

Compound **6** was obtained as an amorphous white powder. The molecular formula was deduced from the peak at *m/z* 805 $[M + Na]^+$ in the LC/ES-MS as $C_{41}H_{66}O_{14}$. It afforded arabinose and glucose (1:1) on acid hydrolysis. The aglycone of **6** varied structurally from that of **5** only in its C-4 substituent: a hydroxymethylene group in **6** instead of a methyl group in **5**. The ^{13}C-NMR spectrum showed C-4 quaternary carbon (δ 43.85), C-23 hydroxylated methylene (δ 64.91) and C-24 methyl resonances (δ 13.36) confirming the proposed structure. Therefore, the aglycone of **6** was identified as ilexosapogenin A by comparison of its spectral data with those reported for this aglycone in the literature (Amimoto et al., 1992). The ^{1}H- and ^{13}C-NMR spectra indicated the presence of one α-L-arabinosyl unit (H-1′: δ 4.32 *d*, *J* = 6.8 Hz; C-1′: δ 106.27) and one β-D-glucopyranosyl unit (H-1′: δ 5.32 *d*, *J* = 8.3 Hz; C-1′: δ 95.77) as in **1** and **2**. In the same way as **1**, the shifts observed on the carbons of the sugar moieties were in agreement with a site of glycosylation of an α-L-arabinosyl unit at the 3-hydroxyl group, and β-D-glucosyl unit at the 28-carboxyl group. Consequently, the structure of **6** was established as 3-*O*-α-L-arabinopyranosyl ilexosapogenin A 28-β-D-glucopyranosyl ester for which the trivial name randiasaponin VI is proposed.

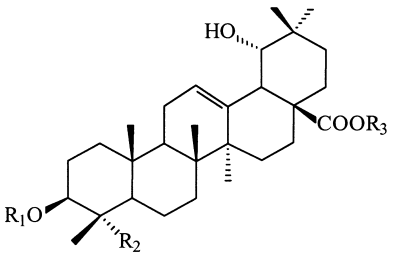
Compound **7** was obtained as an amorphous white powder. The molecular formula was deduced from the peak at *m/z* 835 $[M + Na]^+$ in the LC/ES-MS as $C_{42}H_{68}O_{15}$. Compound **7** afforded only glucose on acid





1 randiasaponin I

		R_1	R_2	R_3
2	randiasaponin II	-Ara(3→1)Glc	$-CH_2OH$	-H
3	randiasaponin III	-Ara(3→1)Glc	$-CH_3$	-Glc
4	randiasaponin IV	-Ara(2→1)Rha	$-CH_3$	-Glc
8	ilexoside XXVII	-Ara	$-CH_2OH$	-Glc
9	ilexoside XXXVII	-Glc	$-CH_2OH$	-Glc
10	rotundic acid	-H	$-CH_2OH$	-H



		R_1	R_2	R_3
5	randiasaponin V	-Ara(2→1)Rha	$-CH_3$	-Glc
6	randiasaponin VI	-Ara	$-CH_2OH$	-Glc
7	randiasaponin VII	-Glc	$-CH_2OH$	-Glc

hydrolysis. Comparison of the ^1H and ^{13}C-NMR spectra with those of **6** showed that both compounds had the same aglycone which is ilexosapogenin A. They differed only by a set of additional signals of a β-D-glucopyranosyl unit (H-1′: δ 4.40 *d*, J = 7.5 Hz; C-1′: δ 105.72) which was deduced to be attached to the C-3 position instead of an α-L-arabinosyl unit in **6**. 2D-NMR experiments of **7** confirmed this site of glycosylation (see Tables 1 and 2). Therefore, the structure of **7** was established as 3-*O*-β-D-glucopyranosyl ilexosapogenin A 28-β-D-glucopyranosyl ester for which the trivial name randiasaponin VII is proposed.

3. Experimental

3.1. General

IR spectra (cm^{-1}) were recorded on a Philips PU 9716 infrared spectrometer as pressed KBr disks. Optical rotations were mesured with a Perkin–Elmer 241 polarimeter using MeOH as solvent. ^1H- and ^{13}C-NMR spectra were recorded on a Varian UNITY INOVA 500 instrument at 500 and 125 MHz, respectively. The LC/ES-MS analyses were performed on a Finnigan MAT ion trap mass spectrometer equipped with a Finnigan electrospray. High-resolution measurements were made on a Bruker FTMS BioAPEX II instrument. Open column chromatography (CC): silica gel (15–40 and 63–200 μm, Merck). Medium-pressure liquid chromatography (MPLC): LiChroprep RP-18 (25–40 μm, 46 × 3.6 cm i.d., Merck); Büchi B-681 pump, Büchi B-683 detector 8210 (210 nm); Büchi 684 fraction collector; LKB Bromma 2210 recorder. TLC: silica gel F254, Merck: detection of saponins by spraying with Godin's reagent and of sugars by aniline phthalate reagent followed by heating at 100° for 5–10 min.

3.2. Plant material

Leaves of *R. formosa* Schum. were collected in Llano Carti, San Blas, Panama, in August 1994. A voucher specimen has been deposited at the National Herbarium of Panama, Panama City (FLORPAN 1675) and at the Institut de Pharmacognosie et Phytochimie, Lausanne, Switzerland (No. 94139).

3.3. Extraction and isolation

The air dried and powdered leaves of *R. formosa* (280 g) were extracted at room temperature succesively with CH_2Cl_2 (1500 ml × 3) and MeOH (1500 ml × 3) to afford 4 and 33 g of extracts, respectively. The MeOH extract was subjected to open CC on a normal phase silica gel (63–200 μm, column 75 × 6.5 cm i.d., step-gradient CH_2Cl_2/MeOH 95:5 → MeOH). Fifteen fractions were collected (I–XV). Fraction VI (6 g) rich in saponins according to the TLC control was submitted to MPLC (LiChroprep C-18, step-gradient H_2O → MeOH/H_2O 70:30 → MeOH) to give compounds **1** (117 mg), **2** (95 mg) and **3** (460 mg). The other fractions of this column, rich in saponins, were further subjected to CC on a normal phase silica gel using CH_3Cl_3/MeOH/H_2O mixtures of increasing polarity as eluent, to yield compounds **4** (102 mg), **5** (82 mg), **6** (538 mg), **7** (40 mg), **8** (401 mg) and **9** (55 mg).

3.4. Randiasaponin I (*1*)

White powder. $[\alpha]_D^{20}$ +45.9° (MeOH, *c* 0.17). IR ν_{max} cm^{-1} (KBr): 3420 (OH), 2935 (C–H), 1740 (ester CO), 1635 (C=C) and 1070 (C–O–C). ^{1}H- and ^{13}C-NMR (CD_3OD): see Tables 1 and 2. Positive HR ES-MS *m/z*: 803.4188 $[M + Na]^+$ (calcd. for $C_{41}H_{64}O_{14}Na$: 803.4188). LC/ES-MS: *m/z* 803 $[M + Na]^+$, 641 $[M + Na\text{-}Glu]^+$, 597 $[M + Na\text{-}Glu\text{-}COO^-]^+$, 509 $[M + Na\text{-}Glu\text{-}Ara]^+$, 491 $[M + Na\text{-}Glu\text{-}Ara\text{-}H_2O]^+$, 473 $[M + Na\text{-}Glu\text{-}Ara\text{-}2H_2O]^+$.

3.5. Randiasaponin II (*2*)

White powder. $[\alpha]_D^{20}$ +10.9° (MeOH, *c* 0.12). IR ν_{max} cm^{-1} (KBr): 3420 (OH), 2935 (C–H), 1700 (acid CO), 1635 (C=C) and 1070 (C–O–C). ^{1}H- and ^{13}C-NMR (CD_3OD): see Tables 1 and 2. Positive HR ES-MS *m/z*: 805.4343 $[M + Na]^+$ (calcd. for $C_{41}H_{66}O_{14}Na$: 803.4345). LC/ES-MS: *m/z* 805 $[M + Na]^+$, 761 $[M + Na\text{-}COO^-]^+$, 643 $[M + Na\text{-}Glu]^+$, 511 $[M + Na\text{-}Glu\text{-}Ara]^+$, 493 $[M + Na\text{-}Glu\text{-}Ara\text{-}H_2O]^+$.

3.6. Randiasaponin III (*3*)

White powder. $[\alpha]_D^{20}$ +6.67° (MeOH, *c* 0.33). IR ν_{max} cm^{-1} (KBr): 3420 (OH), 2935 (C–H), 1740 (ester CO), 1635 (C=C) and 1070 (C–O–C). ^{1}H- and ^{13}C-NMR (CD_3OD): see Tables 1 and 2. Positive HR ES-MS *m/z*: 951.4933 $[M + Na]^+$ (calcd. for $C_{47}H_{76}O_{18}Na$: 951.4924). LC/ES-MS: *m/z* 951 $[M + Na]^+$, 789 $[M + Na\text{-}Glu]^+$, 745 $[M + Na\text{-}Glu\text{-}COO^-]^+$, 627 $[M + Na\text{-}Glu\text{-}Glu]^+$, 495 $[M + Na\text{-}Glu\text{-}Glu\text{-}Ara]^+$, 477 $[M + Na\text{-}Glu\text{-}Glu\text{-}Ara\text{-}H_2O]^+$.

3.7. Randiasaponin IV (*4*)

White powder. $[\alpha]_D^{20}$ −65° (MeOH, *c* 0.24). IR ν_{max} cm^{-1} (KBr): 3420 (OH), 2935 (C–H), 1740 (ester CO), 1635 (C=C) and 1070 (C–O–C). ^{1}H- and ^{13}C-NMR (CD_3OD): see Tables 1 and 2. Positive HR ES-MS *m/z*: 935.4970 $[M + Na]^+$ (calcd. for $C_{47}H_{76}O_{17}Na$: 935.4975). LC/ES-MS: *m/z* 935 $[M + Na]^+$, 773 $[M + Na\text{-}Glu]^+$, 729 $[M + Na\text{-}Glu\text{-}COO^-]^+$, 627 $[M + Na\text{-}Glu\text{-}Rha]^+$, 495 $[M + Na\text{-}Glu\text{-}Rha\text{-}Ara]^+$, 477 $[M + Na\text{-}Glu\text{-}Rha\text{-}Ara\text{-}H_2O]^+$.

3.8. Randiasaponin V (*5*)

White powder. $[\alpha]_D^{20}$ −29.6° (MeOH, c 0.24). IR ν_{max} cm^{-1} (KBr): 3420 (OH), 2935 (C–H), 1740 (ester CO), 1635 (C=C) and 1070 (C–O–C). ^{1}H- and ^{13}C-NMR (CD_3OD): see Tables 1 and 2. Positive HR ES-MS *m/z*: 935.4966 $[M + Na]^+$ (calcd. for $C_{47}H_{76}O_{17}Na$: 935.4975). LC/ES-MS: *m/z* 935 $[M + Na]^+$, 773 $[M + Na\text{-}Glu]^+$, 729 $[M + Na\text{-}Glu\text{-}COO^-]^+$, 627 $[M + Na\text{-}Glu\text{-}Rha]^+$, 495 $[M + Na\text{-}Glu\text{-}Rha\text{-}Ara]^+$.

3.9. Randiasaponin VI (*6*)

White powder. $[\alpha]_D^{20}$ +7.1° (MeOH, *c* 0.14). IR ν_{max} cm^{-1} (KBr): 3420 (OH), 2940 (C–H), 1740 (ester CO), 1635 (C=C) and 1070 (C–O–C). ^{1}H- and ^{13}C-NMR (CD_3OD): see Tables 1 and 2. Positive HR ES-MS *m/z*: 805.4339 $[M + Na]^+$ (calcd. for $C_{41}H_{66}O_{14}Na$: 805.4345). LC/ES-MS: *m/z* 805 $[M + Na]^+$, 643 $[M + Na\text{-}Glu]^+$, 599 $[M + Na\text{-}Glu\text{-}COO^-]^+$, 511 $[M + Na\text{-}Glu\text{-}Ara]^+$.

3.10. Randiasaponin VII (*7*)

White powder. $[\alpha]_D^{20}$ +16.6° (MeOH, *c* 0.35). IR ν_{max} cm^{-1} (KBr): 3420 (OH), 2935 (C–H), 1740 (ester CO), 1635 (C=C) and 1070 (C–O–C). ^{1}H- and ^{13}C-NMR (CD_3OD): see Tables 1 and 2. Positive HR ES-MS *m/z*: 835.4450 $[M + Na]^+$ (calcd. for $C_{42}H_{68}O_{15}Na$: 835.4450). LC/ES-MS: *m/z* 835 $[M + Na]^+$, 673 $[M + Na\text{-}Glu]^+$, 629 $[M + Na\text{-}Glu\text{-}COO^-]^+$, 511 $[M + Na\text{-}Glu\text{-}Glu]^+$.

3.11. Alkaline hydrolysis of saponins

A sample of each saponin (5 mg) was refluxed with

3% KOH (5 ml) for 30 min, and worked-up in the usual way. The aqueous layer was neutralized by passing it through Amberlite MB-3 (mixed form) and lyophilized. The residue obtained was tested for sugar by TLC (EtOAc–MeOH–AcOH–H_2O, 13:3:4:3).

3.12. Acid hydrolysis of saponins

A sample of each saponin (5 mg) was refluxed with 5% HCl in 60% aqueous dioxane (5 ml) at 100°C for 2 h, cooled and filtered. The filtrate was neutralized by passing it through Amberlite MB-3 (mixed form) and lyophilized. The residues were examined for sugars by TLC (EtOAc–MeOH–AcOH–H_2O, 13:3:4:3).

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