Triterpenoid Saponins from *Isertia pittieri*

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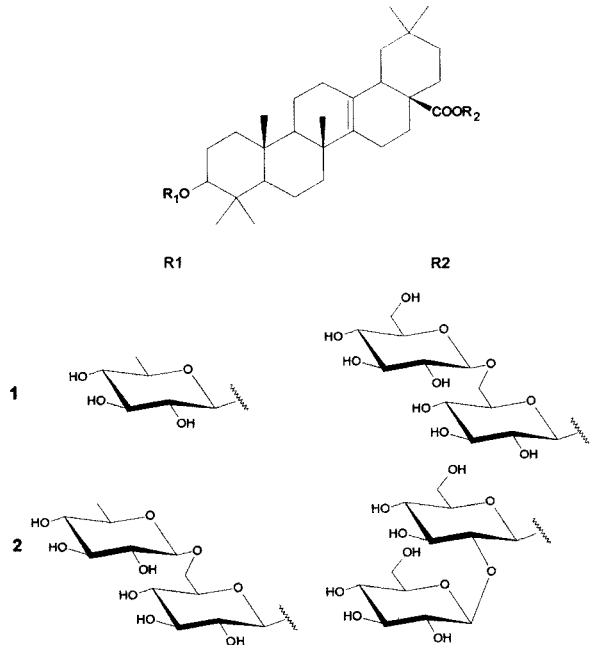
Two new 27-*nor*-triterpenoid saponins, pyrocincholic acid 3*β*-*O*-*β*-D-quinovopyranosyl-28-[*β*-D-glucopyranosyl(1→6)-*β*-D-glucopyranosyl] ester (**1**) and pyrocincholic acid 3*β*-*O*-*β*-D-quinovopyranosyl(1→6)-*β*-D-glucopyranosyl-28-[*β*-D-glucopyranosyl(1→2)-*β*-D-glucopyranosyl] ester (**2**) were isolated from the stem bark of *Isertia pittieri*, together with two known bidesmosidic quinovic acid glycosides. The structures of **1** and **2** were determined on the basis of spectroscopic studies.

Isertia pittieri (Standl.) Standl. (Rubiaceae) is an endemic tree used as a commercial source of timber on the Pacific coasts of both Colombia and Ecuador. Its aerial parts have been used as a detergent by local communities.[1] Previous phytochemical work on a related *Isertia* species, *I. haenkaena*, has led to the isolation of secoiridoids and triterpene glycosides.[2−4] As part of our chemical investigation on Latin American plants, we describe herein the isolation and structure determination of two new bidesmosidic 27-*nor*-triterpenoid glycosides, **1** and **2**, together with two known triterpene glycosides. 27-*nor*-triterpenoids have a C_{29} skeleton that derives from the normal C_{30} skeleton, and their occurrence and distribution is restricted to a few botanical families.[5]



The methanol extract of the stem bark of *I. pittieri* was successively extracted with *n*-hexane, CH_2Cl_2, EtOAc, and *n*-BuOH. The concentrated *n*-BuOH-soluble phase was fractionated by repeated preparative HPLC to give compounds **1** and **2**, along with two known triterpene glycosides which were identified as quinovic acid 3*β*-*O*-α-L-rhamnopyranosyl(28→1)-*β*-D-glucopyranosyl ester (**3**) and quinovic acid 3*β*-*O*-*β*-D-glucopyranosyl(1→4)-α-L-rhamnopyranosyl-(28→1)-*β*-D-glucopyranosyl ester (**4**). All the spectral data for compounds **3** and **4** were superimposable with those described in the literature.[6,7]

Compound **1** was obtained as an amorphous powder. Its molecular formula, $C_{47}H_{76}O_{17}$, was deduced from ^{13}C NMR, DEPT, and HRFABMS data giving a molecular ion peak at *m*/*z* 935.5004 $(M + Na)^+$. The ^{13}C NMR spectrum revealed 29 carbon signals for the aglycon, including two quaternary olefinic carbons at δ_C 130.3 and 137.0. No olefinic proton signal was observed in the ^1H NMR spectrum. Most of the signals of the aglycon could be assigned through ^2J and ^3J connectivities from the six angular methyl proton resonances in the HMBC spectrum. A comparison between the ^{13}C NMR spectra of **1** and pyrocincholic acid 3*β*-*O*-*β*-D-fucopyranoside[7] revealed that the carbon signals of the aglycons of the two molecules were almost identical, except for the chemical shift of the carboxylic carbon of **1** (δ_C 176.8 ppm rather than δ_C 180.2 ppm). These data indicated that compound **1** was a bidesmosidic glycoside of pyrocincholic acid, with sugar attachments at positions C-3 and C-28. In the HSQC spectrum three anomeric carbons at δ_C 106.8, 95.7, 105.3 gave correlations with three anomeric proton signals at δ_H 4.84 (d, $J = 7.9$ Hz, qui-1′), δ_H 6.24 (d, $J = 7.9$ Hz, glc-1″), and δ_H 5.01 (d, $J = 8.0$ Hz, glc-1‴). The HMBC spectrum of **1** displayed couplings between H-1′ (δ_H 4.84, qui) and C-3 (δ_C 89.0), H-1″ (δ_H 6.24, glc) and C-28 (δ_C 176.8), and H-1‴ (δ_H 5.01, glc) and C-6″ (δ_C 69.5), indicating 3-*O*-*β*-D-quinovopyranosyl and 28-*O*-*β*-D-glucopyranosyl (1→6)-*β*-D-glucopyranosyl linkages. Full assignments of the ^1H and ^{13}C NMR signals were secured from the COSY, HSQC, HMBC, ROESY, and TOCSY spectra (Table 1). From these data, the structure of compound **1** was established as pyrocincholic acid 3*β*-*O*-*β*-D-quinovopyranosyl-28-[*β*-D-glucopyranosyl(1→6)-*β*-D-glucopyranosyl] ester.

Compound **2** was obtained as an amorphous powder. Its molecular formula, $C_{53}H_{86}O_{22}$, was deduced from ^{13}C NMR, DEPT, and HRFABMS spectral data [*m*/*z* 1097.5571 $(M + Na)^+$]. The carbon signals of the aglycon moiety of **2** were almost identical to those of **1**, again suggesting the aglycon to be pyrocincholic acid, with the sugar attachments at positions C-3 and C-28. The ^{13}C NMR spectrum showed four anomeric peaks at δ_C 106.8 [δ_H 4.85 (d, $J = 7.68$ Hz), glc-1′], δ_C 105.3 [δ_H 4.99 (d, $J = 7.68$ Hz), qui-1″], δ_C 93.6 [δ_H 6.12 (d, $J = 8.0$ Hz), glc-1‴], and δ_C 104.9 [δ_H 5.61 (d,

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Table 1. ^{1}H and ^{13}C NMR Spectral Data in Pyridine-d_5 at 300/75 MHz for Compounds **1** and **2**

	1		**2**			**1**		**2**	
C	δ_H	δ_C	δ_H	δ_C	C	δ_H	δ_C	δ_H	δ_C
1	1.69, 0.96	38.5	1.63, 0.95	38.5		Qui(1→C3)		Glc(1→C3)	
2	2.27, 1.94	26.9	2.25, 1.94	26.9	1′	4.84	106.8	4.85	106.8
3	3.39	89.0	3.37	89.1	2′	4.04	76.0	4.02	76.1
4		39.6		39.7	3′	4.15	78.0	4.00	78.6
5	0.77	55.8	0.78	55.9	4′	3.73	77.0	4.08	72.6
6	1.50, 1.31	18.8	1.51, 1.30	18.8	5′	3.78	72.8	3.97	78.5
7	1.79, 1.15	39.7	1.80, 1.12	39.8	6′	1.67	18.9	4.62, 4.30	69.2
8		38.1		38.1		Glc(1→C28)		Qui(1→6glc)	
9	1.03	56.5	1.04	56.6	1″	6.24	95.7	4.99	105.3
10		37.2		37.2	2″	4.14	75.2	4.01	76.1
11	1.57, 1.51	18.1	1.55, 1.51	18.2	3″	3.89	78.4	4.13	77.8
12	2.32,1.99	32.1	2.31, 1.99	32.1	4″	4.32	71.6	3.72	77.0
13		130.3		130.0	5″	4.09	78.4	3.79	72.8
14		137.0		137.2	6″	4.34, 4.69	69.5	1.65	18.9
15	2.61, 2.13	21.0	2.61, 2.12	21.5		Glc(1→6glc)		Glc′(1→C28)	
16	2.03	24.8	2.03	23.9	1‴	5.01	105.3	6.12	93.6
17		45.7		45.8	2‴	4.01	74.1	4.35	79.1
18	2.72	39.5	2.71	39.5	3‴	4.19	78.8	4.22	78.3
19	1.65, 1.19	41.5	1.64, 1.17	41.6	4‴	3.88	71.0	4.12	70.5
20		30.9		30.6	5‴	4.35	78.5	4.04	78.5
21	1.33, 1.14	34.4	1.31, 1.15	34.4	6‴	4.46, 4.35	62.7	4.66, 4.44	63.7
22	1.95, 1.72	32.1	1.96, 1.70	31.0				Glc″(1→2glc′)	
23	1.31	28.2	1.31	28.3	1⁗			5.61	104.9
24	0.96	16.7	0.95	16.7	2⁗			4.09	75.3
25	0.80	16.7	0.80	16.7	3⁗			4.20	77.5
26	1.12	20.9	1.12	21.2	4⁗			4.19	71.6
28		176.8		176.8	5⁗			3.87	78.5
29	0.89	32.4	0.88	32.4	6⁗			4.48, 4.35	62.7
30	0.88	25.0	0.87	25.2					

J = 7.3 Hz), glc-1⁗]. In the HMBC spectrum, ^3J connectivities were observed between H-1′ (δ_H 4.85) and C-3 (δ_C 89.1), H-1″ (δ_H 4.99) and C-6′ (δ_C 69.2), H-1‴ (δ_H 6.12) and C-28 (δ_C 176.8), and H-1⁗ (δ_H 5.61) and C-2‴ (δ_C 79.1). These glycosidic linkages were confirmed by COSY, HSQC, ROESY, and TOCSY NMR spectral data analysis. Therefore, the structure of compound **2** was concluded to be pyrocincholic acid 3β-*O*-β-D-quinovopyranosyl(1→6)-β-D-glucopyranosyl-28-[β-D-glucopyranosyl(1→2)-β-D-glucopyranosyl] ester.

Compounds **1** and **2** are described here for the first time from nature. To our knowledge, pyrocincholic acid glycosides have been isolated only from the Rubiaceae family, four from *Adena rubella*,[8,9] and one from *I. haenkaena*.[4,10]

Experimental Section

General Experimental Procedures. Optical rotations were measured on a Perkin-Elmer 241 polarimeter. IR spectra were recorded on a Perkin-Elmer 1600 series FTIR. All NMR spectra were recorded on NMR Bruker DRX-300 at 300 MHz for ^{1}H NMR, ^{1}H−^{1}H COSY, HSQC, HMBC, ^{1}H−^{1}H ROESY, and ^{1}H−^{1}H TOCSY and 75 MHz for ^{13}C NMR and ^{13}C DEPT 135 in pyridine-d_5 using standard Bruker microprograms. Chemical shifts are reported in ppm relative to pyridine-d_5 (δ_H 8.71, δ_C 149.9 ppm). In the HMBC experiments, 7.14 Hz was used as the long-range coupling constant between protons and carbons. HRFABMS were recorded on a JEOL-MS700. Preparative HPLC (Gilson Co.) was performed using a Waters Symmetry Prep C_{18}, 19 × 300 mm, 7 μm column.

Plant Material. The stem bark of *Isertia pittieri* was collected in Bajo Calima, Colombia, in June 1997. A voucher specimen (BW118) was deposited at the Herbarium of the University del Valle, Cali, Colombia.

Extraction and Isolation. The methanol extract (80 g) of the stem bark (700 g) of *I. pittieri* was extracted successively with *n*-hexane, CH_2Cl_2, EtOAc, and *n*-BuOH. The concentrated *n*-BuOH-soluble fraction (20 g) was fractionated by preparative HPLC [CH_3CN-H_2O (0.07% TFA) (10% to 30% in 30 min), flow rate = 12 mL/min, detection at 205 nm] to give five fractions. Fraction 4 was purified again by preparative HPLC [CH_3CN-H_2O (26% to 30% in 30 min), flow rate = 12 mL/min, detection at 205 nm] to give compounds **1** (20 mg) and **2** (17 mg), along with compounds **3** (22 mg) and **4** (23 mg).

Compound 1: amorphous white powder; $[\alpha]_D^{20}$ −20.0° (*c* 0.011, MeOH); IR (KBr) ν_{max} 3400, 2930, 1720, 1640, 1460, 1380, 1050 cm^{-1}; ^{1}H NMR and ^{13}C NMR, see Table 1; HRFABMS *m/z* 935.5004 (calcd for $C_{47}H_{76}O_{17}Na$ [M + Na]$^+$).

Compound 2: amorphous white powder; $[\alpha]_D^{20}$ +2.2° (*c* 0.009, MeOH); IR (KBr) ν_{max} 3400, 2940, 1730, 1650, 1450, 1380, 1050 cm^{-1}; ^{1}H NMR and ^{13}C NMR, see Table 1; HRFABMS *m/z* 1097.5571 (calcd for $C_{53}H_{86}O_{22}Na$ [M + Na]$^+$).

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