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SAPONINS FROM *ZYGOPHYLLUM PROPINQUUM*

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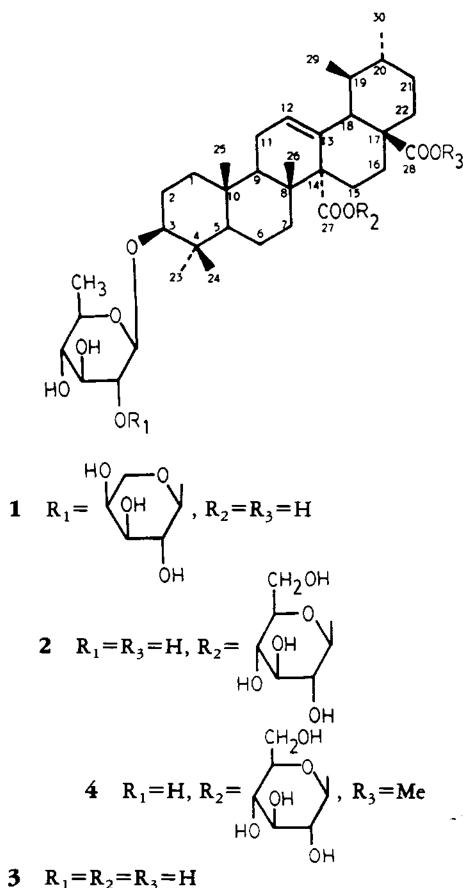
ABSTRACT.—Two new saponins, 3-*O*-[α-L-arabinopyranosyl (1→2) β-D-quinovopyranosyl] quinovic acid [**1**] and 3-*O*-[β-D-quinovopyranosyl] quinovic acid-27-*O*-[β-D-glucopyranosyl] ester [**2**], along with a known saponin, 3-*O*-[β-D-quinovopyranosyl] quinovic acid [**3**], have been isolated from *Zygophyllum propinquum* and identified on the basis of spectral and chemical evidence.

Zygophyllum propinquum Decne. (syn. *Zygophyllum coccineum* L.) (Zygophyllaceae) is found in the Sindh and Baluchistan provinces of Pakistan. It is known to cause lowering of blood pressure and is also used as a diuretic, antipyretic, and local anesthetic; it has antihistaminic activity and causes stimulation and then depression of isolated amphibian heart, relaxation of isolated intestine, and contraction of the uterus (1). These results of pharmacological studies of *Z. propinquum* induced us to work on its chemical constituents and led to the isolation and characterization of two new and one known saponin of quinovic acid; these are zygophyloside A [**1**], zygophyloside B [**2**], and 3-*O*-[β-D-quinovopyranosyl] quinovic acid [**3**], respectively. Compound **3** was previously isolated from cinchona bark in 1963, and its structure was determined by chemical



methods (2). Here, we report the structure elucidation of compounds **1–3** on the basis of their negative ion fabms, ^{1}H- and ^{13}C-nmr spectra, and chemical reactions.

Compound **3** was purified by repeated cc: $[\alpha]^{20}D$ 33.0 (c = 0.424, MeOH). The ir spectrum showed strong absorption at 3650–2400 cm^{-1} (OH of carboxylic group and sugars). The peak at 1700 cm^{-1} indicated the presence of a carboxylic group, while the sharp signal at 1069 cm^{-1} appeared to be due to the C-O group. The uv spectrum showed a broad peak at 203 nm in MeOH as end absorption. The ^{13}C-nmr spectrum, summarized in Table 1, showed 36 carbon resonances, indicating the presence of a single monosaccharide moiety from the one anomeric signal at δ 106.52. The methine and methyl resonances at δ 75.93, 78.01, 77.05, 72.99, and 18.23 were due to C-2′, C-3′, C-4′, C-5′, and C-6′, respectively, of the β-D-quinovopyranosyl moiety (3). The downfield ^{13}C chemical shift at δ 90.69 showed that the monosaccharide moiety was attached to C-3 of the aglycone (4). The olefinic resonances of the aglycone at δ 134.20 and 130.13, corresponding to quaternary and methine carbons, suggested the urs-12-ene skeleton with a carboxylic group at C-27 (5). The carbonyl carbons at δ 181.56 and 179.00 showed the presence of two unsubstituted carbonylic groups at C-17 and C-14, respectively, in the aglycone moiety (4,5). The ^{13}C-nmr spectral data of compound **3** were consistent with quinovic acid as the aglycone (4–6).

The structure of compound **3** was further supported by its ^{1}H-nmr and negative ion fab mass spectra. The ^{1}H-nmr spectrum indicated the presence of four tertiary methyl singlets for C-23, C-24, C-25, and C-26 at δ 0.82, 1.01, 0.97, and 0.89, respectively. A doublet at δ 0.91 (J = 5.16 Hz) represented two secondary methyl signals of C-29 and C-30, and a multiplet at δ 5.59 was due to H-12 of the aglycone (4,6). The anomeric signal of the β-D-quinovopyranosyl moiety, which appeared at δ 4.26 (d, J = 7.60 Hz), showed 1,2-diaxial coupling of the pyranose sugar. A doublet at δ 1.25

TABLE 1. ^{13}C-nmr Spectral Data of Saponins **1–3** and Derivative **4** in CD_3OD.

Carbon	Compound				Carbon	Compound			
	1	2	3	4		1	2	3	4
C-1	40.05	40.02	39.93	40.10	C-23	19.11	19.24	19.18	19.17
C-2	27.26	27.13	27.13	27.11	C-24	28.33	28.56	28.53	28.47
C-3	90.50	90.70	90.69	90.57	C-25	16.74	16.91	16.90	16.92
C-4	40.22	40.14	40.13	40.12	C-26	18.07	18.10	18.17	17.75
C-5	57.06	56.99	56.92	57.28	C-27	179.13	178.00	179.00	177.32
C-6	19.32	19.33	19.33	19.24	C-28	181.50	180.00	181.56	177.83
C-7	37.72	37.05	37.81	36.86	C-29	16.85	17.07	17.08	17.05
C-8	40.76	40.96	40.70	41.13	C-30	21.49	21.43	21.55	21.45
C-9	48.07	48.14	48.03	48.57	C-1′	105.14	106.49	106.52	106.58
C-10	37.88	37.91	37.88	37.88	C-2′	84.07	75.99	75.93	75.92
C-11	23.89	23.94	23.88	23.83	C-3′	77.00	78.11	78.01	78.29
C-12	130.40	131.01	130.13	131.28	C-4′	77.90	77.10	77.05	77.05
C-13	134.07	133.33	134.20	132.94	C-5′	73.70	73.03	72.99	72.98
C-14	57.50	57.40	57.50	57.42	C-6′	18.15	18.20	18.23	18.19
C-15	25.80	25.82	25.86	25.84	C-1″	106.46	95.71		95.66
C-16	26.58	26.52	26.63	26.27	C-2″	72.81	74.02		73.94
C-17[a]	—	—	—	—	C-3″	74.22	78.61		78.65
C-18	55.65	55.40	55.69	55.05	C-4″	69.59	71.46		71.26
C-19	40.42	40.27	40.46	40.23	C-5″	67.28	78.38		77.99
C-20	38.38	38.30	38.40	38.40	C-6″	—	62.77		62.58
C-21	31.30	31.19	31.36	31.02	$COOCH_3$	—	—		51.78
C-22	38.05	38.04	38.06	38.28					

[a]Signal masked by CD_3OD peaks.

($J = 6.12$ Hz) was due to the methyl signal of the quinovose moiety. The negative ion fab mass spectrum of compound **3** exhibited a $[M - H]^-$ ion peak at m/z 631 consistent with the molecular formula $C_{36}H_{56}O_9$. The other fragments observed at m/z 587 and 441 were due to the loss of $[M - H - COO]^-$ and $[M - H - COO - \text{quinovose}]^-$, respectively.

In view of the above spectral evidence the structure of compound **3** was concluded to be 3-*O*-[β-D-quinovopyranosyl] quinovic acid.

Compound **2** was purified by flash chromatography using the solvent system $CHCl_3$-MeOH (87:13), $[\alpha]^{20}D$ +34.09 ($c = 0.088$, MeOH). The ir spectrum showed strong absorption at 3650–2400 cm^{-1} (OH of carboxylic group and sugars), 1725 cm^{-1} (>C=O of ester group), 1700 cm^{-1} (>C=O of carboxylic acid group), and 1070 cm^{-1} (C-O group). The uv spectrum showed a broadened absorption at 201.2 nm in MeOH. The ^{13}C-nmr spectrum of the intact saponin showed that the aglycone was quinovic acid (4–6). Anomeric signals appeared at δ 106.49 and 95.71, indicating the presence of two sugar moieties. The latter signal showed that one sugar residue was attached to the aglycone by an ester bond (4,7). Alkaline hydrolysis of compound **2** afforded a prosaponin. The sugar liberated was glucose, identified by comparing with a standard sample on Si gel tlc. The ^1H- and ^{13}C-nmr spectra of the prosaponin exhibited only one anomeric signal at δ 4.27 (d, $J = 7.72$ Hz) and 106.47, respectively, and indicated the presence of only one sugar moiety. The disappearance of six signals at δ 95.71 (CH), 74.02 (CH), 78.61 (CH), 71.46 (CH), 78.38 (CH), and 62.77 (CH_2) from the ^{13}C-nmr spectrum of the prosaponin indicated that one β-D-glucopyranosyl moiety was attached to the aglycone by an ester bond (4). The structure of the prosaponin was established as **3** by direct comparison with ^1H- and ^{13}C-nmr spectral data. The position of the glucose moiety linked in ester form was proposed to be at C-27 of quinovic acid on the basis of the ^{13}C-resonances of C-12, C-13, C-14, and of the C-27 and C-28 carboxylic groups; all these values matched well with those found in the 27-*O*-β-D-glucopyranosyl ester of quinovic acid (4). The position of glucose in the ester linkage at C-27 was supported by its ^1H-nmr spectrum, which showed the deshielded signals of H-26 and H-12 at δ 0.90 (s, 3H) and 5.62 (m, 1H), respectively (4). Methylation of compound **2** yielded a methyl ester **4**. Comparison of ^{13}C-nmr data (Table 1) of **2** and **4** indicated that only the ^{13}C chemical shift of C-28 was moved upfield due to the formation of the methyl ester in **4** while the other chemical shifts were similar to those of compound **2**; this confirmed that the glucose moiety was attached at C-27 by an ester bond (4).

The negative ion fabms of compound **2** exhibited a $[M - H]^-$ ion peak at m/z 793. The sugar moieties attached to the aglycone by an ester bond were eliminated first (7). The fragment observed at m/z 631 indicated the loss of the 27-O-glucose from the $[M - H]^-$ ion, while the remaining fragments at m/z 587 and 441 showed the loss of $[M - H - \text{glucose} - COO]^-$ and $[M - H - \text{glucose} - COO - \text{quinovose}]^-$, respectively.

The anomeric configuration of the sugar moieties was determined from the ^1H-nmr spectrum (8). The anomeric proton signals appeared at δ 4.28 (d, $J = 7.72$ Hz) and 5.37 (d, $J = 8.04$ Hz), which showed 1,2-diaxial coupling. Differentiation between the anomeric signals of the β-D-quinovopyranosyl and β-D-glucopyranosyl moieties was achieved by comparison of the ^1H-nmr spectra of compounds **2** and **3**. The disappearance of the downfield signal at δ 5.37 in the ^1H-nmr spectrum of **3** indicated the position of the anomeric signal of the β-D-glucopyranosyl moiety, while the remaining anomeric signal was due to the β-D-quinovopyranosyl moiety.

The above evidence led to the structure of zygophyloside B [**2**] as 3-*O*-[β-D-quinovopyranosyl] quinovic acid-27-*O*-[β-D-glucopyranosyl] ester.

Compound **1** was purified by flash chromatography using the solvent system $CHCl_3$-MeOH (89:11): $[\alpha]^{20}D$ −20.83 (*c* = 0.048, MeOH). The ir spectrum showed a broad signal at 3650–2400 cm^{-1} (OH of carboxylic acid and sugar), 1700 cm^{-1} (>C=O of carboxylic acid), and 1065 cm^{-1} (C-O group). The uv spectrum showed end absorption at 201.8 nm in MeOH. The ^{13}C-nmr spectrum of the intact saponin showed that the aglycone was the same as compounds **2** and **3**. Anomeric signals appeared at δ 106.46 and 105.14 in the ^{13}C-nmr spectrum and at δ 4.45 (d, J = 7.00 Hz) and 4.38 (d, J = 7.47 Hz) in the ^{1}H-nmr spectrum, which indicated the presence of two sugar moieties. The sequence of sugars was established from negative ion fabms, which exhibited a $[M-H]^-$ ion peak at *m/z* 763. Other fragments appeared at *m/z* 719, 587, and 441 indicating the loss of $[M-H-COO]^-$, $[M-H-COO-\text{pentose}]^-$, and $[M-H-COO-\text{pentose}-\text{deoxyhexose}]^-$, respectively. This sequence clearly suggested that pentose was a terminal sugar and deoxyhexose was linked with the aglycone by an interglycosidic bond; this was confirmed by the downfield ^{13}C chemical shift of C-3 of the aglycone (4,6).

Compound **1** was partially hydrolyzed with 0.5 M HCl and yielded a prosaponin of **1** by elimination of the terminal pentose moiety. The structure of this prosaponin, determined by ^{13}C-nmr spectroscopy, was the same as that of compound **3**. The sugar obtained from the hydrolysate was identified as arabinose on tlc by comparing with an authentic sample. The assignments of the ^{13}C chemical shifts of α-L-arabinose were made by comparison with the ^{13}C-nmr data of methyl α-L-arabinose, reported in the literature (3) and confirmed by subtraction of the ^{13}C-nmr data of the prosaponin from **1**. The points of attachment of sugar units were determined through ^{13}C chemical shifts in which the upfield shifts of β carbons and the downfield shifts of α carbons were characteristic for the establishment of interglycosidic linkages (9). Comparison of the ^{13}C-nmr spectra of compounds **1** and **3** showed that the anomeric and C-3 signal of β-D-quinovose appeared upfield and the C-2 signal of β-D-quinovose appeared downfield, which allowed us to place a (1↦2) linkage between the arabinose and quinovose moieties.

The anomeric configuration of the sugar moieties in compound **1** was determined from the ^{1}H-nmr spectrum. The anomeric signals appearing at δ 4.38 (d, J = 7.47 Hz, H-1′) and 4.45 (d, J = 7.00 Hz, H-1″) showed 1,2-diaxial coupling which was consistent with β-quinovose and α-arabinose moieties. Hence, on the basis of the foregoing evidence, the structure of zygophyloside A [**1**] was elucidated as 3-*O*-[α-L-arabinopyranosyl(1↦2)β-D-quinovopyranosyl] quinovic acid.

EXPERIMENTAL

EXPERIMENTAL INSTRUMENTS.—Cc was performed on Merck Si gel (70–230 mesh). The purity of the samples was checked on DC-Micro-cards SIF 37341 (size 5 × 10 cm, layer thickness = 0.2 mm); flash chromatography (fc) was performed using an Eyela flash chromatograph EF-10 and Merck Si gel (230–400 mesh) column. Negative ion fab mass spectra were recorded on a Jeol JMS-Hx110 spectrometer coupled to a PDP 11/73 computer system. ^{1}H- and ^{13}C-nmr spectra were recorded on Bruker AM-300 and 75 MHz nmr spectrometers, respectively. The DEPT experiments were carried out with θ = 45°, 90°, and 135°; the quaternary carbons were determined by subtraction of these spectra from the broad band ^{13}C-nmr spectrum.

EXTRACTION AND ISOLATION.—*Z. propinquum* (20 kg) was collected from the Sindh province of Pakistan. It was crushed in an Ulturrex homogenizer and extracted three times with MeOH. The combined MeOH extract was evaporated at reduced pressure to afford a gummy residue (800 g), which was partitioned between EtOAc and H_2O. The EtOAc fraction, after evaporation of solvent under reduced pressure, afforded 200 g of a gummy extract which was subjected to cc on Si gel using the solvent system *n*-C_6H_{14}, *n*-C_6H_{14}/Et_2O, Et_2O, $CHCl_3$, $CHCl_3$/MeOH, and finally MeOH. The fraction eluted by $CHCl_3$-MeOH (4:1) was a mixture of one minor and two major saponins. This saponin mixture was further chromatographed on a Si gel column using $CHCl_3$ and $CHCl_3$/MeOH in order of increasing polarity. Com-

pound **3** (12.5 mg) was eluted in pure form with $CHCl_3$-MeOH (19:1), but the fraction eluted with $CHCl_3$-MeOH (17:3) was a mixture of two major saponins. This saponin mixture was purified by flash chromatography using a gradient of MeOH in $CHCl_3$ as eluent. Compound **1** (45.90 mg) and compound **2** (35.65 mg) were obtained in pure form from $CHCl_3$-MeOH (89:11) and $CHCl_3$-MeOH (87:13), respectively.

ZYGOPHYLOSIDE A [**1**].—Mp 258.4° (dec), $[\alpha]^{20}D$ −20.83 (c = 0.048, MeOH); ir (KBr) 3650–2400 cm^{-1} (OH of sugars and COOH group), 1700 cm^{-1} (>C=O of COOH group), 1065 cm^{-1} (C-O group); uv λ (MeOH) max 201.8 nm (end absorption); ^1H nmr (CD_3OD, 300 MHz) δ 5.60 (1H, m, H-12), 0.82 (3H, s, H-23), 1.02 (3H, s, H-24), 0.97 (3H, s, H-25), 0.89 (3H, s, H-26), 0.92 (6H, d, J = 6.00 Hz, H-29 and H-30), 4.38 (1H, d, J = 7.47 Hz, H-1′), 1.24 (3H, d, J = 6.13 Hz, H-6′), 4.45 (1H, d, J = 7.00 Hz, H-1″); ^{13}C nmr (CD_3OD, 75 MHz) see Table 1; negative ion fabms m/z $[M-H]^-$ 763, $[M-H-COO]^-$ 719, $[M-H-COO-\text{arabinose}]^-$ 587, $[M-H-COO-\text{arabinose}-\text{quinose}]^-$ 441.

PARTIAL ACID HYDROLYSIS OF COMPOUND **1**.—Compound **1** (24.10 mg) was refluxed with 0.5 M HCl in aqueous MeOH (10 ml) for 3 h. The MeOH was evaporated under reduced pressure, and the mixture was diluted with H_2O, neutralized with Ag_2CO_3, and extracted with n-BuOH. The n-BuOH extract was evaporated under reduced pressure and gave the prosaponin of **1** (17.54 mg). The structure of the prosaponin was found to be the same as compound **3**. The aqueous layer was filtered, and the filtrate was concentrated under reduced pressure. The residue obtained was identified as L-arabinose by comparison with an authentic sample on Si gel tlc developed in EtOAc-HOAc-H_2O-MeOH (6:1:1:2), followed by spraying with sugar reagent (10) and heating.

ZYGOPHYLOSIDE B [**2**].—Mp 200° (dec), $[\alpha]^{20}D$ +34.09 (c = 0.088, MeOH); ir (KBr) 3650–2400 cm^{-1} (OH of COOH group and sugars), 1725 (>C=O of ester group), 1700 cm^{-1} (>C=O of COOH group), 1070 cm^{-1} (C-O group); uv λ (MeOH) max 201.2 nm (end absorption); ^1H nmr (CD_3OD, 300 MHz) δ 5.62 (1H, m, H-12), 0.82 (3H, s, H-23), 1.01 (3H, s, H-24), 0.97 (3H, s, H-25), 0.90 (3H, s, H-26), 0.92 (6H, d, J = 5.68 Hz, H-29 and H-30), 4.28 (1H, d, J = 7.72 Hz, H-1′), 1.24 (3H, d, J = 6.12 Hz, H-6′), 5.37 (1H, d, J = 8.04 Hz, H-1″), ^{13}C nmr (CD_3OD, 75 MHz) see Table 1; negative ion fabms m/z $[M-H]^-$ 793, $[M-H-\text{glucose}]^-$ 631, $[M-H-\text{glucose}-COO]^-$ 587, $[M-H-\text{glucose}-COO-\text{quinovose}]^-$ 441.

ALKALINE HYDROLYSIS OF COMPOUND **2**.—Compound **2** (25 mg) was refluxed with 2% NaOH in EtOH for 1 h to give a prosaponin. The structure of the prosaponin was determined by spectroscopic means as **3**. The sugar was identified, by the procedure described for **1**, as D-glucose.

METHYLATION OF COMPOUND **2**.—Compound **2** (10.0 mg) in MeOH was treated with excess ethereal CH_2N_2, and the whole reaction mixture was allowed to stand for 12 h. Removal of the solvent under reduced pressure gave the methyl ester **4**.

3-*O*-[β-D-QUINOVOPYRANOSYL] QUINOVIC ACID [**3**].—Mp 235° (dec), $[\alpha]^{20}D$ +33.0 (c = 0.424, MeOH); ir (KBr) 3650–2400 cm^{-1} (OH of COOH group and sugar), 1700 cm^{-1} (>C=O of carboxylic acid group), 1069 cm^{-1} (C-O group); uv λ (MeOH) max 203 nm (end absorption); ^1H nmr (CD_3OD, 300 MHz) δ 5.59 (1H, m, H-12), 0.82 (3H, s, H-23), 1.01 (3H, s, H-24), 0.97 (3H, s, H-25), 0.89 (3H, s, H-26), 0.91 (6H, d, J = 5.16 Hz, H-29 and H-30), 4.26 (1H, d, J = 7.60 Hz, H-1′), 1.25 (d, J = 6.12 Hz, H-6′); ^{13}C nmr (CD_3OD, 75 MHz) see Table 1; negative ion fabms m/z $[M-H]^-$ 631, $[M-H-COO]^-$ 587, $[M-H-COO-\text{quinovose}]^-$ 441.

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