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A TRITERPENOID SAPONIN FROM *ZYGOPHYLLUM PROPINQUUM*

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Key Word Index—*Zygophyllum propinquum*; Zygophyllaceae; triterpenoid saponin.

Abstract—A new triterpenoid saponin, 3-*O*-[α-L-arabinopyranosyl(1→2)-β-D-quinovopyranosyl]-quinovic acid-27-*O*-[β-D-glucopyranosyl]ester, along with two known compounds, 3-*O*-[β-D-glucopyranosyl]-β-sitosterol and erythrodiol-3-caffeate have been isolated from *Zygophyllum propinquum* and identified on the basis of spectroscopic and chemical evidence.

INTRODUCTION

About 100 species of *Zygophyllum* are known throughout the world [1]. *Zygophyllum propinquum* Decne (syn. *Z. coccineum*) is found in Sindh and Baluchistan provinces of Pakistan and the pharmacological properties of *Z. propinquum*, reported by Saad *et al.* [2], prompted us to work on its chemical constituents. The isolation of a new saponin zygophyloside C (**1**) and two known compounds, 3-*O*-[β-D-glucopyranosyl]-sitosterol (**2**) and erythrodiol-3-caffeate (**3**), are reported herein. Compounds **2** and **3** have not been reported from this source previously. All of these compounds were identified by spectral and chemical methods.

RESULTS AND DISCUSSION

Compound **1** was purified on a LH-20 column. The ^{13}C NMR chemical shifts in the intact saponin suggested that the aglycone of **1** had a urs-12-ene skeleton with a carboxylic group at C-27 [3]. The aglycone of **1** was identified as quinovic acid [3–5]. The ^{13}C NMR spectrum also exhibited three anomeric signals at δ106.6, 105.2 and 95.6 indicating the presence of three sugar moieties. The last signal showed that one sugar residue was attached to the aglycone by an ester bond [6]. Alkaline hydrolysis of **1** afforded a prosapogenin **1a**. The sugar liberated was glucose, identified by comparing with a standard sample on silica gel TLC. The ^{13}C NMR spectrum of **1a** exhibited two anomeric signals at δ106.5 and 105.1 indicating the presence of two sugar moieties. The disappearance of five methine signals at δ95.6, 73.9, 78.6, 71.3, 78.3 and one methylene signal at δ62.6 in the ^{13}C NMR spectrum of **1a** showed that one β-D-glucopyranosyl moiety was attached to the aglycone by an ester bond in **1** [4, 6]. The position of this glucose moiety was proposed to be at C-27 of quinovic acid on the basis of ^{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 ^{1}H NMR spectrum, which showed the deshielded signals of H-26 and H-12 [4]. The upfield ^{13}C chemical shift of C-27 also confirmed that the glucose moiety was attached at C-27 by an ester bond [4].

The sugar sequence of **1a** was determined by its negative ion FAB mass spectrum, the $[M-H]^-$ ion peak appeared at *m/z* 763. Other fragments were observed at *m/z* 719, 587 and 441 indicating the loss of $[M-H-CO_2]^-$, $[M-H-CO_2-\text{pentose}]^-$ and $[M-H-CO_2-\text{pentose}-\text{deoxy hexose}]^-$, respectively. This sequence showed that the pentose is the terminal moiety. This fact was confirmed by partial acid hydrolysis of **1a** which yielded another prosapogenin **1b**. The sugar obtained from the hydrolysate was identified as arabinose. The ^{13}C NMR spectrum of **1b** exhibited one anomeric signal indicating the presence of one sugar moiety. The methine and methyl signals showed the presence of β-D-quinovopyranosyl moiety [7]. The downfield C-3 signal of the aglycone indicated that the β-D-quinovose moiety is attached at this carbon [4]. Compound **1b** was previously isolated from cinchona bark [8]. The glycosidic linkage between arabinose and quinovose in **1** was determined by the downfield C-2 signal of β-D-quinovose at δ84.1.

The anomeric configuration of the sugar moieties was determined from the ^{1}H NMR spectrum [9]. The ^{1}H NMR spectrum of **1** showed three anomeric proton signals at δ4.37 (*d*, *J* = 7.44 Hz), 5.37 (*d*, *J* = 7.95 Hz) and 4.45 (*d*, *J* = 6.76 Hz) indicating the β-configuration for D-quinovose and D-glucose and the α-configuration for L-arabinose, respectively.

The structure of **1** was further supported by its negative ion FAB mass spectrum which showed a $[M-H]^-$ ion peak at *m/z* 925. The other fragments observed at *m/z* 763, 719, 587 and 441 were due to the loss of glucose, glucose $+CO_2$, glucose $+CO_2$ + arabinose and glucose $+CO_2$ + arabinose + quinovose, respectively, from the $[M-H]^-$ ion peak. Thus zygophyloside C is 3-*O*-[α-L-arabinopyranosyl(1→2)β-D-quinovopyranosyl] quinovic acid-27-*O*-[β-D-glucopyranosyl] ester.

Compound **2** showed a $[M-H]^-$ ion peak at *m/z* 575 in the negative ion FAB mass spectrum. The fragment which appeared at *m/z* 413 showed the loss of a hexose moiety. The acid hydrolysis of **2** yielded a sapogenin **2a** which was identified as a sitosterol by comparing with spectral data reported in the literature [10]. The sugar obtained from the hydrolysate was identified as glucose on silica gel TLC by comparing with an authentic sample. The ^{13}C NMR data (Table 1) revealed the β-D-pyranosyl

Table 1. ^{13}C NMR spectral data of compounds 1–3 and their derivatives

C	1	1a	1b	2	3	C	1	1a	1b	2	3
1	40.0	39.9	39.9	37.6	37.8	25	16.7	16.7	16.9	29.5	15.4
2	27.3	27.2	27.1	30.3	25.5	26	18.1	18.1	18.2	19.5	16.6
3	90.7	90.3	90.6	78.4	80.6	27	178.0	178.9	178.7	20.0	25.7
4	40.2	40.1	40.1	40.0	38.1	28	179.2	181.4	181.1	23.5	69.2
5	57.0	56.9	56.9	140.9	55.2	29	16.9	16.9	17.0	12.2	32.9
6	19.3	19.2	19.3	121.9	18.2	30	21.5	21.5	21.5	—	23.3
7	37.0	37.5	37.8	32.2	32.3	1′	105.2	104.9	106.5	102.6	147.2 (C-1′)
8	40.8	40.6	40.7	32.1	39.6	2′	84.1	84.0	75 9	75.3	143.7 (C-2′)
9	48.1	47.9	48.0	50.4	47.3	3′	76.9	76.8	78.0	78.2	115.0 (C-3′)
10	37.8	37.7	37.8	36.9	36.7	4′	77.8	77.6	77.1	71.7	144.3 (C-4′)
11	23.9	23.8	23.8	21.4	23.5	5′	73.7	73.7	73.0	77.3	121.8 (C-5′)
12	130.9	130.4	130.1	39.4	122.1	6′	18.1	18.1	18.2	62.9	115.2 (C-6′)
13	133.3	133.7	134.2	42.6	144.3	1″	106.5	106.4			144.3 (C-7′)
14	57.3	57.2	57.4	56.9	41.5	2″	72.7	72.6			115.2 (C-8′)
15	25.8	25.9	25.8	24.6	25.6	3″	74.1	74.0			167.0 (C-9′)
16	26.4	26.4	26.6	28.6	23.5	4″	69.6	69.5			
17	—	—	—	56.3	36.7	5″	67.4	67.2			
18	55.3	55.4	55.6	12.1	42.1	1‴	95.6				
19	40.4	40.2	40.4	19.3	46.3	2‴	73.9				
20	38.3	38.2	38.4	36.4	30.9	3‴	78.6				
21	31.2	31.5	31.3	19.1	33.9	4‴	71.3				
22	38.0	37.9	38.0	34.3	32.3	5‴	78.2				
23	19.2	19.0	19.1	26.5	27.8	6‴	62.6				
24	28.3	28.3	28.5	46.1	16.4						

configuration for glucose [11]. The acetylation of **2** yielded a tetraacetate compound **2b** which was identified as the acetylated glucoside of sitosterol. The anomeric configuration of glucose in **2b** was confirmed from the ^{1}H NMR spectrum which showed a doublet at δ4.57 (J = 7.96 Hz). Hence, on the basis of above evidence **2** is 3-*O*-[β-D-glucopyranosyl]-sitosterol.

Compound **3** showed UV absorptions at 211, 240, 295 and 330 nm, the last peak undergoing a strong bathochromic shift upon addition of aqueous alkali. This indicated the presence of a phenolic group in **3**, which was confirmed by a positive $FeCl_3$ test. The FD mass spectrum showed the molecular ion peak at m/z 604 corresponding to the molecular formula $C_{39}H_{56}O_5$. The EI mass spectrum of **3** exhibited the highest peak at m/z 234 corresponding to the ion resulting from the RDA fragmentation characteristic of Δ^{12}-amyrine derivatives [12]. It also gave rise to a strong peak at m/z 203 due to the loss of a methoxyl group from the fragment (*a*).

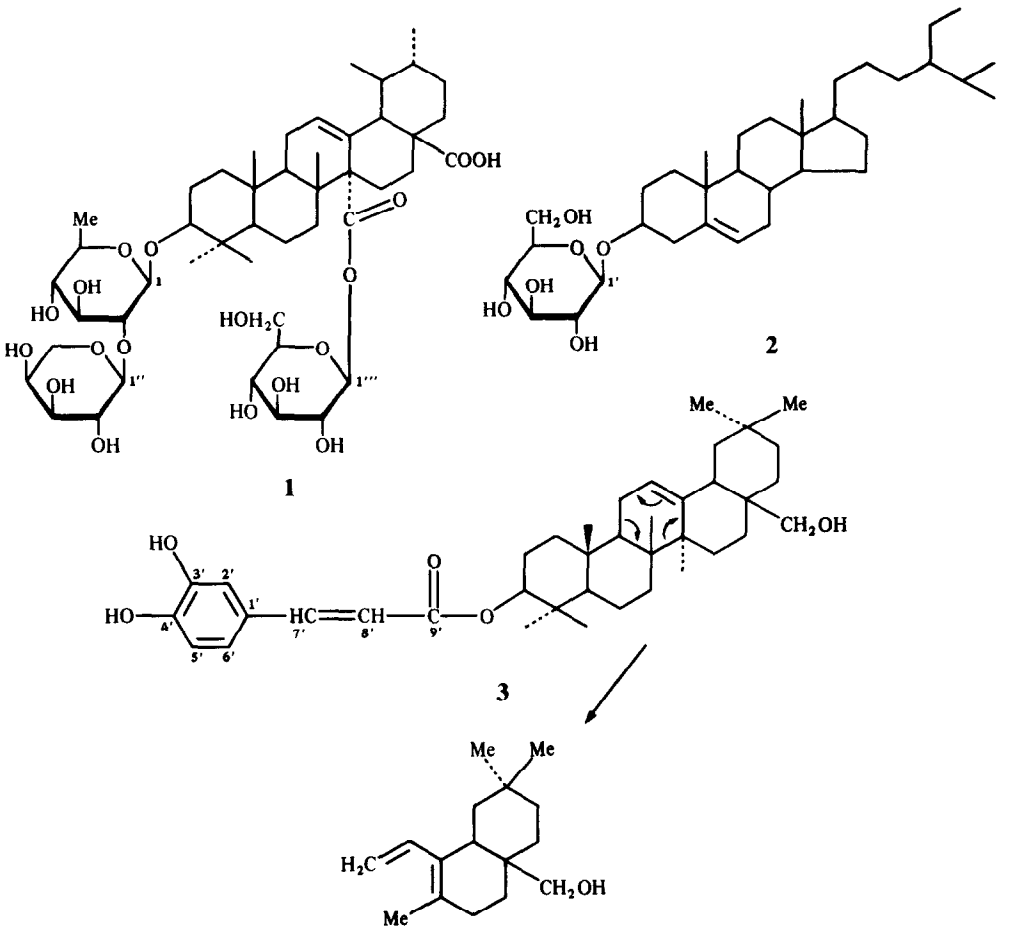
The ^{1}H NMR spectrum exhibited seven methyl signals at δ0.82 (*s*, H-24), 0.83 (*s*, H-29), 0.85 (*s*, H-30), 0.88 (*s*, H-25), 0.90 (*s*, H-26), 0.92 (*s*, H-23), 1.11 (*s*, H-27). The absence of secondary methyls excludes the ursane skeleton in compound **3** [13]. The ^{13}C NMR spectrum exhibited olefinic signals at δ122.1 and 144.3 which indicated the presence of an oleanane skeleton because in an ursane the C-12 is deshielded by 2 ppm and C-13 shielded by 5 ppm [13]. The ^{13}C NMR chemical shifts of all signals due to the aglycone were found identical to those of erythrodiol [14]. This correlation was further confirmed through the alkaline hydrolysis followed by the isolation of aglycone, mp 230° (ref. mp 235°) [15], the ^{1}H NMR spectral data of the aglycone were also very close to those reported for erythrodiol.

The ^{1}H NMR spectrum of **3** exhibited an ABq at δ3.22 and 3.55 (J = 10.0 Hz) due to a methoxyl (H-28) group and a triplet at δ4.06 (J = 8.1 Hz) due to H-3. The other resonance for **3** appeared at δ6.25 (*d*, J = 16 Hz, H-7′), 7.52 (*d*, J = 16 Hz, H-8′), 6.85 (*d*, J = 8.2 Hz, H-5′), 7.0 (*dd*, J = 8.0, 1.9 Hz, H-6′) and 7.06 (*br s*, H-2′). All these signals confirmed the presence of 3′,4′-dihydroxy-cinnamoyloxy moiety linked at C-3 of the aglycone which was confirmed through the upfield shift of the signal suggested the equatorial position of a caffeate moiety at C-3. All signals in the ^{13}C NMR spectrum due to the caffeate moiety were found identical to the reported chemical shifts [16]. Thus **3** is erythrodiol-3-caffeate. This compound was previously isolated from *Larrea tridentata* [17].

EXPERIMENTAL

CC was performed on Merck silica gel (70–230 mesh and LH-20). The purity of the samples was checked on DC-Microcards SIF 37341 (size 5 × 10 cm, layer thickness = 0.2 mm). ^{1}H and ^{13}C NMR spectra: 300 and 75 MHz, 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. Zygophyllum propinquum (20 kg) was collected from the Sindh province of Pakistan. It was crushed in an Ultraturrex homogenizer and extracted ×3 with MeOH. The combined MeOH extract was evapd at red. pres. to afford a gummy residue (800 g), which was partitioned between EtOAc and H_2O. The EtOAc fraction, after evapn of solvent under red. pres. afforded 200 g of a gummy extract which was subjected to CC on silica gel using the solvent systems *n*-hexane, *n*-hexane–Et_2O, Et_2O, $CHCl_3$, $CHCl_3$–MeOH and finally MeOH. The fraction obtained from *n*-hexane–Et_2O (11:9) was further chromatographed on a silica gel column using C_6H_6, C_6H_6–EtOAc, EtOAc and finally with MeOH. The fraction obtained from C_6H_6–EtOAc (9:1) afforded **3** (23.59 mg)



which was recrystallized with MeOH and yielded a powder, mp 255–256°.

From the same column, compound **2** (26.31 mg) was eluted with $CHCl_3$–MeOH (17:3) in the pure form. The fraction eluted with $CHCl_3$–MeOH (13:7) was further chromatographed over a LH-20 column to yield **1** (51.24 mg) in the pure state.

Zygophyloside C (**1**). ^1H NMR (CD_3OD, 300 MHz): δ5.62 (*m*, H-12), 0.81 (*s*, H-23), 1.01 (*s*, H-24), 0.96 (*s*, H-25), 0.87 (*s*, H-26), 0.91 (*d*, $J \approx 5.70$ Hz, H-29 and H-30), 4.37 (*d*, $J = 7.44$ Hz, H-1′), 1.24 (*d*, $J = 6.1$ Hz, H-6′), 4.45 (*d*, $J = 6.76$ Hz, H-1″) and 5.37 (*d*, $J = 7.95$ Hz, H-1‴); ^{13}C NMR (CD_3OD, 75 MHz): see Table 1; negative ion FAB-MS: *m/z* 925 $[M-H]^-$, 763 $[M-H-Glc]^-$, 719 $[M-H-Glc-CO_2]^-$, 587 $[M-H-Glc-CO_2-Ara]^-$ and 441 $[M-H-Glc-CO_2-Ara-quinovose]^-$.

Alkaline hydrolysis of compound **1**. Compound **1** (40 mg) was refluxed with 2% KOH in EtOH for 1 hr to give a prosapogenin **1a**. Negative ion FAB-MS: *m/z* 763 $[M-H]^-$, 719 $[M-H-CO_2]^-$, 587 $[M-H-CO_2-Ara]^-$ and 441 $[M-H-CO_2-Ara-quinovose]^-$; ^{13}C NMR (CD_3OD, 300 MHz): see Table 1. The sugar obtained as the result of alkaline hydrolysis was identified as glucose on TLC [EtOAc–HOAc–H_2O–MeOH (6:1:1:2)] followed by spraying with sugar reagent (orcinol, ferric chloride and sulphuric acid) [18] and heating.

Partial acid hydrolysis of compound **1a**. Compound **1a** (25.0 mg) was refluxed with 0.5 M HCl in aq. MeOH (10 ml) for 3 hr. The MeOH was evapd under red. pres. and the mixture was diluted with H_2O, neutralized with Ag_2CO_3 and extracted with *n*-BuOH. The *n*-BuOH extract was evapd under red. pres. which gave the prosapogenin **1b**; ^{13}C NMR (CD_3OD, 300 MHz) see Table 1. The liberated sugar was identified on silica gel TLC through comparison with standard sugar sample as arabinose.

3-O-[β-D-glucopyranosyl]-Sitosterol (**2**). Negative ion FAB-MS: *m/z* 575 $[M-H]^-$ and 413 $[M-H-Glc]^-$; ^{13}C NMR (pyridine-d_5, 75 MHz) see Table 1.

Acid hydrolysis of compound **2**. Compound **2** (10 mg) was hydrolysed with 2 M HCl in aq. MeOH (10 ml) at 100° for 3 hr. The MeOH was evapd under red. pres. and the mixture diluted with H_2O and extracted with EtOAc. The EtOAc extract was evapd under red. pres. to afford 6.32 mg of the aglycone, identified as sitosterol through comparison of its spectral data with those reported in ref. [10].

Acetylation of compound **2**. Compound **2** (10 mg) was acetylated with Ac_2O–pyridine (2:1) at room temp. for 24 hr. The reaction mixture was dried at high vacuum and purified through silica gel CC gave (11.53 mg) of **2b** in pure form; ^1H NMR ($CDCl_3$, 300 MHz): δ3.47 (*m*, H-3), 5.34 (distorted *t*, H-6), 0.64 (*s*, H-18), 0.97 (*s*, H-19), 0.90 (*d*, $J = 6.44$ Hz, H-21), 0.82 (*d*, $J = 7.12$ Hz, H-26), 0.80 (*d*, $J = 7.20$ Hz, H-27), 0.66 (*t*, $J = 7.24$ Hz, H-29), 4.57 (*d*, $J = 7.96$ Hz, H-1′), 1.98 (*s*, Ac), 2.00 (*s*, Ac), 2.03 (*s*, Ac), 2.05 (*s*, Ac), 3.66 (*m*, H-5′), 4.10 (*dd*, $J = 12.90$, 2.36 Hz, H-6′), 4.23 (*dd*, $J = 12.2$, 4.84 Hz, H-6′), 4.93 (*t*, $J = 8.04$ Hz), 5.05 (*t*, $J = 9.84$ Hz), 5.18 (*t*, $J = 9.48$ Hz) appeared due to H-2′, H-3′ and H-4′.

Erythrodiol-3-caffeate (**3**). FD-MS: *m/z* 604 ($[M]^+$, $C_{39}H_{56}O_5$), EIMS: *m/z* 234 (frag. *a*), 203, (frag. *a*-CH_2OH) and 162 (3′,4′-dihydroxyphenyl-2-propenoate); UV λ_{max}^{MeOH} nm: 211,

240, 295 and 330; $\lambda_{max}^{MeOH+NaOH}$ nm: 208, 265, 290 and 375; ^{1}H NMR ($CDCl_3$, 300 MHz): δ0.82 (*s*, H-24), 0.83 (*s*, H-29), 0.85 (*s*, H-30), 0.88 (*s*, H-25), 0.90 (*s*, H-26), 0.92 (*s*, H-23), 1.11 (*s*, H-27), 3.22 (1H, *d*, J = 10.64 Hz, H-28A), 3.55 (1H, *d*, J = 10.64 Hz, H-28B), 4.06 (*t*, J = 8.1 Hz, H-3), 5.20 (distorted *t*, H-12), 6.25 (*d*, J = 16.0 Hz, H-8'), 6.85 (*d*, J = 8.2 Hz, H-5'), 7.0 (*dd*, J = 8.0, 1.9 Hz, H-6'), 7.06 (*d*, J = 2.0 Hz, H-2'), 7.52 (*d*, J = 16.0 Hz, H-7'); ^{13}C NMR ($CDCl_3$, 75 MHZ) see Table 1.

Alkaline hydrolysis of compound **3**. Compound **3** (10 mg) was refluxed with 6% KOH in EtOH for 4 hr. The excess MeOH was evapd under red. pres. and the mixture diluted with H_2O and extracted with EtOAc. The EtOAc extract yielded a crystalline product which was identified as erythrodiol. Mp 230°, ^{1}H NMR ($CDCl_3$, 300 MHz): δ0.78 (*s*, H-24), 0.87 (*s*, H-29), 0.88 (*s*, H-30), 0.93 (*s*, H-25), 0.94 (*s*, H-26), 0.99 (*s*, H-23), 1.16 (*s*, H-27), 3.2 and 3.5 (AB*q*, J = 13.6 Hz, H-28), 5.28 (distorted *t*, H-12).

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5α-PORIFERAST-9(11)-EN-3β-OL FROM THE MARINE RED ALGA, *GRACILARIA EDULIS**

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Key Word Index—*Gracilaria edulis*; Gracilariaceae; red alga; sterol; 5α-poriferast-9(11)-en-3β-ol.

Abstract—A new sterol isolated from the marine red alga, *Gracilaria edulis*, has been characterized as 5α-poriferast-9(11)-en-3β-ol from its spectral data and chemical reactions.

INTRODUCTION

There has been continuing interest in the sterols of marine organisms including red algae, ever since the investigations of Henze [1] and Doree [2] showed them to be a source of new sterols. In connection with our interest in the isolation of biologically active compounds from marine organisms we examined the marine red alga, *Gracilaria edulis*, and report on the isolation of 5α-poriferast-9(11)-en-3β-ol (**1**) from the hexane extract of the alga. Cholesterol is by far the most common sterol of algae [7] and so far six species of *Gracilaria* have been examined for sterol content with each species containing cholesterol as the principal sterol [3–9]. This is the first isolation of the title compound from an alga and is the first instance of a C_{29} sterol as the principal sterol in any red alga.

RESULTS AND DISCUSSION

The sterol **1**, $C_{29}H_{50}O$ ([M]$^+$ 414.3862), was isolated as crystals. It gave a positive LB test, and the IR spec-

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