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TWO SAPONINS FROM FRUITS OF *GUAIACUM OFFICINALE*

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Key Word Index—*Guaiacum officinale*; Zygophyllaceae; triterpene saponins; guaianin F and G; structural determination.

Abstract—From the fruit of *Guaiacum officinale*, two new saponins were isolated and characterized by chemical reactions and spectroscopic means as 3-*O*-[α-L-rhamnopyranosyl(1→3)α-L-rhamnopyranosyl(1→2)α-L-arabinopyranosyl(3→1)β-D-glucopyranosyl]-30-*nor*-olean-12,20(29)-dien-28-oic acid-28-*O*-[β-D-glucopyranosyl(1→6)β-D-glucopyranosyl]ester and 3-*O*-[α-L-rhamnopyranosyl(1→3)α-L-rhamnopyranosyl(1→2)α-L-arabinopyranosyl(3→1)β-D-glucopyranosyl]oleanolic acid-28-*O*-[β-D-glucopyranosyl(1→6)β-D-glucopyranosyl] ester.

INTRODUCTION

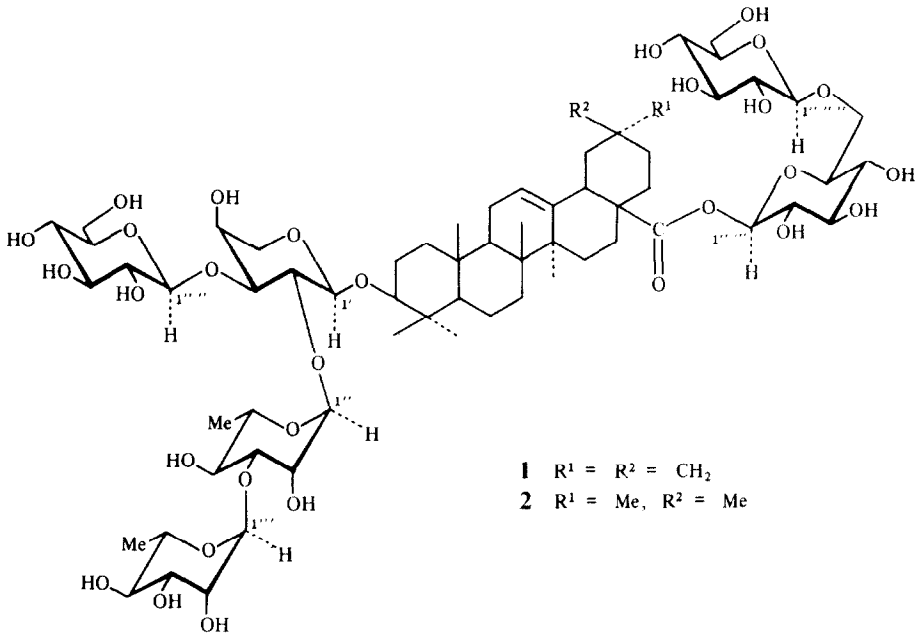
Previously we reported four new saponins from the bark of *Guaiacum officinale* [1–3]. In this paper we wish to report two new saponins named as guaianin F(**1**) and guaianin G(**2**) from fruits of this plant. Comparison of ^{13}C NMR spectra of **1** and **2** demonstrated that these two saponins are composed of identical sugars but different sapogenins. The sugar moieties in both saponins were identified as [α-L-rhamnopyranosyl(1→3)α-L-rhamnopyranosyl(1→2)α-L-arabinopyranosyl(3→1)β-D-glucopyranosyl] linked to C-3 and [β-D-glucopyranosyl (1→6)β-D-glucopyranosyl] ester linked to C-28 of 30-norolean-12, 20(29)-dien-28-oic and oleanolic acid in compounds **1** and **2**, respectively. The former aglycone was first isolated from callus tissue of *Akebia quinata* [4]. The sugar linkages and sequencing have been determined by ^{13}C NMR and negative FAB mass spectrometry, respectively.

RESULTS AND DISCUSSION

Compound **1** was purified by semipreparative HPLC (RP-18 column, MeOH–H_2O, 7:3); it afforded (40 mg) guaianin F. ^{13}C NMR chemical shifts in the intact saponin suggested that the aglycone of **1** is a noroleanane type triterpene, Carbon signals observed at 149.39 (C=) and 107.52 (=CH_2) showed the presence of one *exo*-methylene group in the aglycone. The aglycone of **1** was identified as 3β-hydroxy-30-norolean-12,20(29)-dien-28-oic acid by comparison with reported data [3, 4]. Acid hydrolysis of **1** liberated arabinose, rhamnose and glucose. The ^{13}C NMR spectrum exhibited six anomeric signals at δ 105.04, 104.71, 104.03, 102.76, 101.84 and 95.85 indicating the presence of six sugar moieties. The last signal showed that one sugar residue was attached to the aglycone by an ester bond; alkaline hydrolysis of **1** afforded prosaponin **1a**. The ^{13}C NMR spectrum of **1a** exhibited four anomeric signals at δ 105.06, 104.09, 102.71 and 101.79 indicating the presence of four sugar moieties. The disappearance of two anomeric signals at δ 104.71 and 95.85 in the ^{13}C NMR spectrum of **1a** showed that these two sugar moieties were attached to C-28 of the aglycone by an ester bond in compound **1**. The ^{13}C NMR spectrum of **1** shows that these sugars are two glucose, having (1→6) linkage [5]. The structure of prosaponin **1a** has been established as 3-*O*-[α-L-rhamnopyranosyl (1→3)α-L-rhamnopyranosyl(1→2)α-L-arabinopyranosyl (3→1)β-D-glucopyranosyl]-30-norolean 12,20(29)-dien-28-oic acid, by direct comparison with ^{1}H and ^{13}C NMR spectral data of guaianin D [3], isolated from the bark of *G. officinale*.

The negative FAB mass spectrum of **1** exhibited a $[M-H]^-$ peak at *m/z* 1349. The loss of two hexose units gives rise to the peak at *m/z* 1025 supporting the assumption that both of these sugars are in the form of ester linkage while the remaining monosaccharides were attached to the aglycone by glycosidic bonds. The fragments at *m/z* 879, 717, 571 and 439 show the loss of 2 × glucose + rhamnose, 3 × glucose + rhamnose, 3 × glucose + 2 rhamnose and 3 × glucose + 2 × rhamnose + arabinose, respectively. This sequence suggest that one glucose and one rhamnose are in the terminal position and that arabinose was directly attached to the aglycone.

The anomeric configuration of the sugars was determined from the ^{1}H NMR spectrum. The signals of the anomeric protons appeared at δ 4.32 (*d*, *J* = 7.71 Hz, H-1‴ and H-1⁗), 4.51 (*d*, *J* = 7.53 Hz, H-1′) and 5.31 (*d*, *J* = 7.90 Hz, H-1‴′) showing the 1,2-diequitorial relationship while the anomeric protons appearing at δ 5.14 (*d*, *J* = 1.56 Hz, H-1″) and 5.19 (*d*, *J* = 1.40 Hz, H-1‴) showed a 1,2-diaxial relationship which is consistent with β-glucose and α-rhamnose moieties. As, on the basis of the coupling constant of the anomeric signal in rhamnose, an α or β nature could not be deduced but α-rhamnose could easily be identified from its chemical shift in the ^{13}C NMR, where C-5 of α and β-rhamnose appeared at δ 69.4 and 73.5, respectively [6]. The low chemical shift of C-2 and C-3 of arabinose in the ^{13}C NMR spectrum of **1** shows the glycosidic linkage at these carbons. There were two structural possibilities, one was that both rhamnose molecules were linked to C-2 and C-3 of arabinose while the glucose was attached to one rhamnose. The other possibility was that glucose was attached to C-3 and the



1 $R^1 = R^2 = CH_2$
2 $R^1 = Me$, $R^2 = Me$

two rhamnose molecules were linked to C-2 of arabinose. The former case would give an upfield chemical shift of C-2 and C-3 in the ^{13}C NMR of arabinose while the latter would give a downfield chemical shift in the ^{13}C NMR spectrum of arabinose as in the case of C-2 in the saponin reported by Kimura *et al.* [7]. The ^{13}C NMR spectrum of **1** showed that glucose was attached to C-3 and that the two rhamnoses were attached to C-2 of arabinose. The above mentioned linkages were similar to those in guaianin D [3].

In view of the above spectral evidence the structure of guaianin F, (**1**), was concluded to be 3-*O*-[α-L-rhamnopyranosyl(1→3)α-L-rhamnopyranosyl(1→2)α-L-arabinopyranosyl(3→1)β-D-glucopyranosyl]-30-norolean-12,20(29)-dien-28-oic acid-28-*O*-[β-D-glucopyranosyl (1→6)β-D-glucopyranosyl] ester.

Compound **2** was purified from the same fraction as compound **1** by HPLC. The ^{13}C NMR spectrum of **2** showed that the aglycone was similar to oleanolic acid [8]. The anomeric signals of sugar moieties appeared at δ 105.02, 104.65, 104.01, 102.75, 101.83 and 95.75; these were identical to the sugars of **1**. Therefore, the interglycosidic linkages of these sugars were assigned by direct comparison with the ^{13}C NMR spectrum of **1**. The chemical shifts in ^{13}C NMR spectrum for the C-3 linked sugar moieties of **1** and **2** were similar as reported earlier for related saponins [3], while those of the C-28 linked sugar moieties of both **1** and **2** are given in Table 1.

The anomeric configuration of the sugars in **2** were determined from the ^1H NMR spectrum. The anomeric protons appearing at δ 4.32 (*d*, $J = 7.74$ Hz, H-1‴ and H-1⁗), 4.51 (*d*, $J = 7.68$ Hz, H-1′) and 5.35 (*d*, $J = 7.82$ Hz, H-1⁗) showed 1,2-axial–axial coupling and the signals at δ 5.13 (*d*, $J = 1.5$ Hz, H-1″) and 5.19 (*d*, $J = 1.42$ Hz, H-1″) showed 1,2-equatorial–equatorial coupling. The sequence of the sugars was established from the negative FAB mass spectrum which exhibited a $[M-H]^-$ at *m/z* 1365. The fragments at *m/z* 1041, 895, 733, 587 and 455 indicated the loss of 2 × glucose, 2 × glucose + rhamnose, 3 × glucose + rhamnose, 3 × glucose + 2 × rhamnose and 3 × glucose + 2 × rhanmose + arabinose, respectively, from the $[M-H]^-$. This sequence clearly indicates the attachment of arabinose with glucose on one side and with the rhamnose on the other side while arabinose was directly attached to the aglycone.

Table 1. ^{13}C NMR spectral data for 28-*O*-sugar moieties (CD_3OD)

C	1	2
	Glucose (inner)	
1	95.85	95.75
2	75.10	75.13
3	78.10	78.00
4	71.56	71.58
5	77.98	77.93
6	69.55	69.54
	Glucose (terminal)	
1	104.71	104.65
2	75.41	75.41
3	78.10	78.19
4	70.91	70.98
5	77.72	77.80
6	62.75	62.77

The above evidence led to the structure of **2** as 3-*O*-[α-L-rhamnopyranosyl(1→3)α-L-rhamnopyranosyl(1→2)α-L-arabinopyranosyl(3→1)β-D-glucopyranosyl]-oleanolic acid-28-*O*-[β-D-glucopyranosyl(1→6)β-D-glucopyranosyl]ester.

EXPERIMENTAL

^1H and ^{13}C NMR spectra were recorded at 300 and 75 MHz, respectively. HPLC was performed using a differential refractometer detector.

Extraction and isolation of saponins. Fruits of *G. officinale* were collected during July and August, 1986 from the campus of the University of Karachi. The air-dried powdered fruits were extracted with MeOH. The MeOH extract was evapd at red. pres. to afford a gummy residue. This residue was partitioned between EtOAc and H_2O. Both layers were sepd and the H_2O layer (containing saponins) was extracted with *n*-BuOH. The *n*-BuOH layer was sepd and evapd under red. pres. to afford 85 g of crude saponins. This saponin mixt. was subjected to silica gel CC using a gradient of MeOH in $CHCl_3$ as eluent. The fr. eluted with $CHCl_3$–MeOH (1:1) yielded 100 mg of a mixt. of two saponins which mixt. was purified by semipreparative HPLC using a RP-18 column with MeOH–H_2O (7:3). It afforded guaianin F, **1**, (40 mg) in pure form. Further elution with the same solvent furnished guaianin G, **2**, (22 mg) in pure form.

Guaianin F. ^{1}H NMR; δ 0.79 (*s*, Me), 0.85 (*s*, Me), 0.95 (*s*, Me), 1.01 (*s*, Me), 1.18 (*s*, Me), 1.22 (*d*, J = 6.21 Hz, H-6″), 1.25 (*d*, J = 6.24 Hz, H-6‴), 4.32 (*d*, J = 7.71 Hz, H-1‴″ and H-1‴″″), 4.51 *d*, J = 7.53 Hz, H-1′), 4.61 (*br s*, H-29), 5.14 (*d*, J = 1.56 Hz, H-1″), 5.19 (*d*, J = 1.40 Hz, H-1‴), 5.34 (*d*, J = 7.90 Hz, H-1‴″).

Acid hydrolysis of **1**. Compound **1** (10 mg) was hydrolysed with 2 M HCl in aq. MeOH (3.5 ml) at 100° for 3 hr. The MeOH was evapd under red. pres. and the mixt. dild with H_2O, extracted with EtOAc, the aq. layer neutralized with alkali and concd at red. pres. The residue obtained was compared with standard sugars by silica gel TLC. The analysis showed that the sugars were arabinose, rhamnose and glucose in guaianin F.

Alkaline hydrolysis of **1**. Compound **1** (15 mg) was refluxed with 2% NaOH in MeOH for 1 hr. After work-up prosaponin **1a** was obtained. The aq. layer showed the presence of glucose by silica gel TLC.

Prosaponin **1a**. ^{1}H NMR; δ 0.84 (*s*, Me), 0.88 (*s*, Me), 0.94 (*s*, Me), 1.02 (*s*, Me), 1.18 (*s*, Me), 1.23 (*d*, J = 6.24 Hz, H-6″), 1.25 (*d*, J = 6.20 Hz, H-6‴), 4.30 (*d*, J = 7.4 Hz, H-1‴″), 4.52 (*d*, J = 7.6 Hz, H-1′), 4.55 (*br s*, H-29), 5.14 (*d*, J = 1.48 Hz, H-1″), 5.18 (*d*, J = 1.52 Hz, H-1‴).

Guaianin G. ^{1}H NMR; δ 0.79 (*s*, Me), 0.84 (*s*, Me), 0.90 (*s*, Me), 0.93 (*s*, Me), 0.94 (*s*, Me), 1.01 (*s*, Me), 1.15 (*s*, Me), 1.22 (*d*, J = 6.27 Hz, H-6″), 1.25 (*d*, J = 6.27 Hz, H-6‴), 4.32 (*d*, J = 7.74 Hz, H-1‴″ and H-1‴″″), 4.51 (*d*, J = 7.68 Hz, H-1′), 5.13 (*d*, J = 1.5 Hz, H-1″), 5.19 (*d*, J = 1.42 Hz, H-1‴), 5.24 (*distorted t*, H-12), 5.35 (*d*, J = 7.82 Hz, H-1‴″).

Acid hydrolysis of **2**. Compound **2** (10 mg) was hydrolysed with 2 M HCl in aq. MeOH (3.5 ml) at 100° for 3 hr. The reaction mixt. was worked-up as described for **1**. The aq. layer showed the presence of arabinose, rhamnose and glucose by silica gel TLC.

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