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ISOLATION AND STRUCTURE ELUCIDATION OF SAPONINS FROM THE FRUIT OF *GUAIACUM OFFICINALE*

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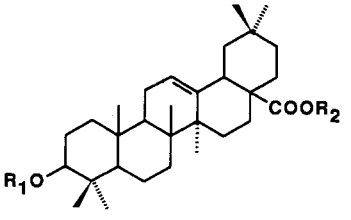
ABSTRACT.—Five new saponins, guaianins H, I, J, K, and L, have been isolated from the fruit of *Guaiacum officinale*. Their structures were elucidated as 3-*O*-[α-L-rhamnopyranosyl(1→3)β-D-glucopyranosyl(1→3)α-L-arabinopyranosyl]oleanolic acid [**1**], 3-*O*-[α-L-rhamnopyranosyl(1→2)α-L-rhamnopyranosyl(1→2)α-L-arabinopyranosyl(1→3)β-D-glucuronopyranosyl]oleanolic acid-28-*O*-[β-D-glucopyranosyl(1→6)β-D-glucopyranosyl] ester [**2**], 3-*O*-[α-L-arabinopyranosyl(1→3)β-D-glucuronopyranosyl]oleanolic acid-28-*O*-[α-L-rhamnopyranosyl(1→2)α-L-rhamnopyranosyl(1→4)β-D-glucopyranosyl(1→6)β-D-glucopyranosyl] ester [**3**], 3-*O*-[β-D-glucuronopyranosyl]oleanolic acid-28-*O*-[α-L-rhamnopyranosyl(1→2)α-L-rhamnopyranosyl(1→2)α-L-arabinopyranosyl[(3→1)β-D-glucopyranosyl](1→3)β-D-glucopyranosyl] ester [**4**], and 3-*O*-[β-D-glucopyranosyl(1→4)β-D-glucuronopyranosyl(3→1)α-L-arabinopyranosyl]oleanolic acid-28-*O*-[α-L-rhamnopyranosyl(1→6)β-D-glucopyranosyl(1→6)β-D-glucopyranosyl] ester [**5**], respectively, by negative ion fabms, ^{1}H- and ^{13}C-nmr spectroscopic studies, and chemical reactions.

Saponins constitute a pharmacodynamic group of natural products with a wide range of biological activities (1–4). The pharmacological importance attached to these compounds has prompted us to investigate their natural occurrence in *Guaiacum officinale* L. (Zygophyllaceae). As a result of preliminary work on the fruit of *G. officinale*, we have reported two new saponins, guaianins F and G, belonging to the noroleanane and oleanane series, respectively (5). The present investigation led to the isolation of five new saponins **1–5**, named as guaianins H, I, J, K, and L, respectively. All of these saponins were purified by reversed-phase semipreparative hplc (RP-18 column).

The ^{13}C-nmr spectra of the intact saponins suggested that the aglycone of **1–5** was oleanolic acid (6). The spectral data of guaianins I, J, and K indicated that these three saponins were isomers with respect to sugar moieties and had the same molecular formula ($C_{65}H_{104}O_{31}$) but differed in the sugar sequence, linkages, and retention times in hplc analysis. The sugar sequencing and linkages have been established by negative ion fabms and ^{13}C-nmr spectrometry, respectively.



1 R_1=Rha(1→3)Glc(1→3)Ara, R_2=H
2 R_1=Rha(1→2)Rha(1→2)Ara(1→3)GlcU, R_2=Glc(1→6)Glc
3 R_1=Ara(1→3)GlcU, R_2=Rha(1→2)Rha(1→4)Glc(1→6)Glc
4 R_1=GlcU, R_2=Rha(1→2)Rha(1→2)Ara(3→1)Glc(1→3)Glc
5 R_1=Glc(1→4)GlcU(3→1)Ara, R_2=Rha(1→6)Glc(1→6)Glc
6 R_1=Rha(1→2)Rha(1→2)Ara(1→3)GlcU, R_2=H
7 R_1=Glc(1→4)GlcU(3→1)Ara, R_2=H

Ara = α-L-arabinopyranosyl
Glc = β-D-glucopyranosyl
GlcU = β-D-glucuronopyranosyl
Rha = α-L-rhamnopyranosyl

The acid hydrolysis of **2–5** yielded the same sapogenin that was identified as oleanolic acid by comparing with spectral and physical data reported in the literature (6,7). The sugars obtained from the hydrolysates were identified as glucuronic acid, glucose, arabinose, and rhamnose on tlc by comparing with authentic samples. The ^{13}C-nmr spectral data (Table 1) deduced the β-D-pyranosyl configuration for glucose and glucuronic acid and the α-L-pyranosyl configuration for arabinose and rhamnose (8).

The ^{13}C-nmr spectrum of compound **1** showed anomeric carbon signals appearing at δ 107.11, 105.22, and 102.61, which indicated the presence of three sugar moieties. A comparison of the ^{13}C-nmr spectrum of **1** with that of guaianin isolated from the stem bark of *G. officinale* (9) demonstrated that compound **1** has the same sugar moieties but with a different sapogenin. Therefore, the interglycosidic linkages of these sugars were assigned by direct comparison with the ^{13}C-nmr spectrum of guaianin.

TABLE 1. ^{13}C-nmr Spectral Data for 3-*O*-Sugar Moieties and 28-*O*-Sugar Moieties.

Compound	Sugar	C-1	C-2	C-3	C-4	C-5	C-6
	3-*O*-sugar moieties						
1	Arabinose	107.11	72.37	83.70	69.78[a]	66.83	—
	Glucose	105.22	75.99	83.80	70.03	77.86	62.37
	Rhamnose	102.61	72.13	72.25	74.00	69.49[a]	17.89
2	Glucuronic Acid	106.04	74.26	85.89	71.81	77.65	176.50
	Arabinose	104.90	76.07	74.19	69.62[a]	63.14	—
	Rhamnose (inner)	98.31	77.34	71.30	73.59	69.91[a]	17.86
	Rhamnose (terminal)	100.89	71.92	72.00	73.59	70.76	17.86
6	Glucuronic Acid	106.08	74.90	85.74	71.68	77.24	176.57
	Arabinose	104.92	76.18	74.16	69.62	63.04	—
	Rhamnose (inner)	98.25	77.30	71.69	74.01	69.89	17.82
	Rhamnose (terminal)	100.88	71.78	71.94	74.01	69.89	17.82
3	Glucuronic Acid	106.10	74.28[a]	85.92	71.81	77.61	176.80
	Arabinose	105.00	72.03	74.23[a]	69.58[c]	63.15	—
4	Glucuronic Acid	106.07	75.28	78.31	73.45	77.60	176.49
5	Glucuronic Acid.	105.20	74.90	86.36	78.20	77.71[a]	176.30
	Glucose	104.23	75.83	78.01	70.77	77.83[a]	62.35
	Arabinose	104.44	72.21	74.90	69.60	63.23	—
7	Glucuronic Acid	105.25	75.00	86.24	78.24	77.70	176.43
	Glucose	104.21	75.82	77.99	70.78	77.81	62.30
	Arabinose	104.47	72.18	75.00	69.62	63.22	—
	28-*O*-sugar moieties						
2	Glucose (inner)	95.57	74.85	77.78	71.80	77.46[b]	69.44
	Glucose (terminal)	104.33	74.84	77.78	71.80	77.64[b]	62.45
3	Glucose (inner)	95.55	74.83	77.70	71.76	77.45[b]	69.28
	Glucose (outer)	104.30	74.83	76.30	77.68	77.59[b]	62.40
	Rhamnose (inner)	98.26	77.19	71.24	73.54	69.87[c]	17.89
	Rhamnose (terminal)	100.88	71.71	71.97	73.54	70.59	17.89
4	Glucose (inner)	95.51	74.75	85.75	71.70	77.49[b]	62.32
	Arabinose	104.98	76.14	83.12	69.62[a]	63.07	—
	Rhamnose (inner)	98.25	77.36	71.16	73.55	69.89[a]	17.82
	Rhamnose (outer)	100.89	71.72	71.93	73.55	70.51	17.82
	Glucose (terminal)	104.24	74.15	77.71	70.80	77.26[b]	62.14
5	Glucose (inner)	95.50	74.78	77.62	70.77	77.40[b]	69.27
	Glucose (outer)	104.23	74.78	78.01	70.77	77.53[b]	67.00
	Rhamnose	102.84	71.19	71.99	73.49	70.56	17.75

[a–c]Assignments may be reversed.

The structure of compound **1** was further suppoted by its negative ion fabms, which showed an $[M-H]^-$ ion peak at *m/z* 895. The other fragments observed at *m/z* 749, 587, and 455 were due to the loss of rhamnose, rhamnose + glucose, and rhamnose + glucose + arabinose, respectively, from the $[M-H]^-$ ion peak. This fragmentation showed that all the sugars of compound **1** were linked to each other by interglycosidic linkages as determined by ^{13}C-nmr data (Table 1).

From the above evidence, the structure of guaianin H [**1**] was determined to be 3-*O*-[α-L-rhamnopyranosyl(1↦3)β-D-glucopyranosyl(1↦3)α-L-arabinopyranosyl]oleanolic acid.

The ^{13}C-nmr spectrum of compound **2** indicated the presence of six sugar moieties. The anomeric ^{13}C chemical shifts appeared at δ 106.04, 104.90, 104.33, 100.89, 98.31, and 95.57. The appearance of a downfield methylene signal at δ 69.44, revealing the presence of a (1↦6) linkage between two glucose units and the last anomeric signal, suggested that the β-D-glucopyranosyl(1↦6)β-D-glucopyranosyl moiety was linked to C-28 of oleanolic acid by an ester bond (5,10).

The negative ion fabms of compound **2** showed an $[M-H]^-$ ion peak at *m/z* 1379. The fragments observed at *m/z* 1217 and 1055 indicated the sequential loss of two glucose units from the $[M-H]^-$ ion peak and thus authenticated the presence of both glucoses in the form of an ester linkage (5).

The alkaline hydrolysis of **2** yielded the prosaponin **6**. The ^{13}C-nmr spectrum of **6** exhibited the anomeric signals at δ 106.08, 104.92, 100.88, and 98.25, indicating the presence of four sugar moieties. The negative ion fabms of **6** showed an $[M-H]^-$ ion peak at *m/z* 1055. The other fragments observed at *m/z* 909, 763, 631, and 455 showed the loss of rhamnose, 2 × rhamnose, 2 × rhamnose + arabinose and 2 × rhamnose + arabinose + glucuronic acid, respectively, from the $[M-H]^-$ ion peak. This sequence showed the sequential loss of two rhamnose units and suggested that both rhamnose units were linked to each other by glycosidic linkage. This sequence also indicated that rhamnose moieties were linked to arabinose, which was linked to glucuronic acid; this, in turn, was attached to the C-3 of oleanolic acid.

The overlapped ^{13}C-nmr assignment of one α-L-rhamnose, on corresponding methyl glycosides (8), suggested that it was a terminal sugar. The points of attachment of sugar units were also determined by using the glycosidation rule (11,12). The downfield ^{13}C chemical shift of inner rhamnose at δ 77.30 showed (1↦2) linkage between two rhamnose units (13).

The glycosidic linkages of α-L-rhamnose to α-L-arabinose and α-L-arabinose to β-D-glucuronic acid were determined by the downfield ^{13}C-chemical shifts of methines at δ 76.18 and 85.74 and showed (1↦2) and (1↦3) linkages, respectively (10,14). The downfield C-3 signal of oleanolic acid at δ 91.39 concluded that the sugar moieties were attached at this carbon (9).

The anomeric signals in the ^{1}H-nmr spectrum of **6** appeared at δ 4.42 (d, J = 7.73 Hz, Ara) and 4.70 (d, J = 7.48 Hz, GlcU), which showed 1,2-axial-axial coupling, and at δ 4.87 (d, J = 1.57 Hz, Rha) and 5.51 (d, J = 1.70 Hz, Rha), showing 1,2-equatorial-equatorial coupling. Hence, on the basis of foregoing evidence, the structure of prosaponin **6** has been established as 3-*O*-[α-L-rhamnopyranosyl(1↦2)α-L-rhamnopyranosyl(1↦2)α-L-arabinopyranosyl(1↦3)β-D-glucuronopyranosyl]oleanolic acid.

The disappearance of two anomeric signals at δ 104.33 and 95.57 in the ^{13}C-nmr spectrum of **6** showed that these two sugar units were attached to C-28 of aglycone by an ester bond in compound **2**. The disappearance of the downfield methylene signal at δ 69.44 confirmed the presence of (1↦6) linkage between two glucose units.

The ^{1}H-nmr spectrum of compound **2** displayed the signals of anomeric protons at δ 4.38 (d, J = 7.71 Hz, Glc), 4.65 (d, J = 7.41 Hz, Ara) and 5.37 (d, J = 7.92 Hz,

Glc), which showed 1,2-axial-axial coupling, and at δ 5.15 (d, $J = 1.50$ Hz, Rha) and 5.50 (d, $J = 1.74$ Hz, Rha), which showed 1,2-equatorial-equatorial coupling. The anomeric signal of GlcU was overlapped by the solvent (CD_3OD and D_2O) signal at 4.69–4.99 ppm.

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

Compound **3** showed a $[M - H]^-$ ion peak at *m/z* 1379 in negative ion fabms. The fragments at *m/z* 1233, 1087, 925, 763, 631, and 455 expressed the sugars sequence, determined as $[M - H -$ rhamnose$]^-$, $[M - H - 2 \times$ rhamnose$]^-$, $[M - H - 2 \times$ rhamnose − glucose$]^-$, $[M - H - 2 \times$ rhamnose − 2 × glucose$]^-$, $[M - H - 2 \times$ rhamnose − 2 × glucose − arabinose$]^-$, and $[M - H - 2 \times$ rhamnose − 2 × gluose − arabinose − glucuronic acid$]^-$, respectively.

The ^{13}C nmr of compound **3** exhibited anomeric signals that appeared at δ 106.10, 105.00, 104.30, 100.88, 98.26, and 95.55 and suggested that guaianin J contains six sugar moieties. The signal at δ 95.55 showed that one glucose was attached to the aglycone by an ester bond, and the downfield signal of a methylene at δ 69.28 showed that two glucoses had (1↦6) linkage (5,10). The ^{13}C-nmr assignments also indicated that one rhamnose and arabinose were terminal sugars (8). The sequence of sugars, determined by negative ion fabms, suggested that two rhamnose units were linked to two glucose units. The ^{13}C nmr concluded that these sugar units were attached to C-28 of the aglycone in linear form. The glycosidic linkage between two rhamnose units was found to be the same as in compound **2**, while the downfield C-4 signal of the outer glucose showed (1↦4) linkage between inner rhamnose and outer glucose (10).

The alkaline hydrolysis of compound **3** afforded a prosaponin which had an $[M - H]^-$ ion peak at *m/z* 763 in negative ion fabms. The other peaks appeared at *m/z* 631 and 455 and showed the loss of arabinose and arabinose + glucuronic acid, respectively, from the $[M - H]^-$ ion peak, while the ^1H-nmr spectrum displayed the anomeric signals at δ 4.40 (d, $J = 7.0$ Hz, Ara) and 4.68 (d, $J = 7.32$ Hz, GlcU), which showed 1,2-axial-axial coupling. These results confirmed that in guaianin J, the four sugar moieties attached to C-28 by an ester bond were two rhamnose and two glucose units, and two sugar moieties linked to C-3 of oleanolic acid by glycosidic bond were arabinose and glucuronic acid.

The sequence of sugars, in negative ion fabms of **3**, also indicated that arabinose was attached to glucuronic acid which in turn was attached to the C-3 of the aglycone. The downfield C-3 chemical shift of glucuronic acid, in the ^{13}C-nmr spectrum of **3**, showed (1↦3) linkage between arabinose and glucuronic acid (14).

The ^1H-nmr spectrum of compound **3** showed only five anomeric proton signals at δ 4.37 (d, $J = 7.80$ Hz, Glc), 4.42 (d, $J = 7.10$ Hz, Ara), 5.15 (d, $J = 1.50$ Hz, Rha), 5.36 (d, $J = 8.08$ Hz, Glc) and 5.52 (d, $J = 1.49$ Hz, Rha). The first, second, and fourth signals showed 1,2-axial-axial coupling, while the third and last signals showed 1,2-equatorial-equatorial coupling. The anomeric signal of GlcU was overlapped by a broad signal that appeared at δ 4.60–4.95, due to the CD_3OD and D_2O.

The above spectral evidence led us to conclude that the structure of guaianin J [**3**] is 3-*O*-[α-L-arabinopyranosyl(1↦3)β-D-glucuronopyranosyl]oleanolic acid-28-*O*-[α-L-rhamnopyranosyl(1↦2)α-L-rhamnopyranosyl(1↦4)β-D-glucopyranosyl(1↦6)β-D-glucopyranosyl] ester.

The sugar sequence of compound **4** was determined by its negative ion fabms. The $[M - H]^-$ ion peak was similar to those of guaianins I and J, while the other fragments appearing at *m/z* 1217, 1071, 925, 793, 631, and 455 showed the loss of glucose, glu-

cose + rhamnose, glucose + 2 × rhamnose, glucose + 2 × rhamnose + arabinose, glucose + 2 × rhamnose + arabinose + glucose, and glucose + 2 × rhamnose + arabinose + glucose + glucuronic acid, respectively, from the $[M-H]^-$ ion peak. The alkaline hydrolysis of compound **4** yielded a prosaponin whose negative ion fabms showed an $[M-H]^-$ ion peak at *m/z* 631 and an ion at *m/z* 455 (oleanolic acid) resulting from the loss of glucuronic acid from the $[M-H]^-$ ion peak (15). The negative ion fabms of the prosaponin suggested that in compound **4** only glucuronic acid was attached to the C-3 and the remaining five sugar units were attached to the C-28 of oleanolic acid by interglycosidic bonds.

The ^{13}C-nmr spectrum of compound **4** confirmed the presence of six sugar units and showed that one glucose was linked to the aglycone by an ester bond (5,10). Because the ^{13}C-nmr assignments of one glucose, one rhamnose, and glucuronic acid were similar to those of methyl glycosides (8), they were assumed to be terminal sugars. The assignments of both rhamnose units indicated that they have the same glycosidic linkage as described for compounds **2** and **3**. The C-2 and C-3 downfield shifts of α-L-arabinose showed the glycosidic linkage at these carbons. There were two structural possibilities. One was that rhamnose units were linked to C-2 and the glucose was attached to the C-3 of arabinose. The other possibility was that glucose was attached to C-2 and rhamnose units were linked to the C-3 of arabinose. The ^{13}C-nmr studies of saponins demonstrated that the C-2 of arabinose appeared at upfield in the former and downfield in the latter case (10). The ^{13}C-nmr spectrum of compound **4** showed that glucose was attached to C-3 and rhamnose units were attached to the C-2 of arabinose (16). The sequence of sugars indicated that arabinose was attached to the C-28 glucose by a glycosidic bond. A comparison of ^{13}C chemical shifts of 28-*O*-β-D-glucopyranosyl with β-D-3-*O*-methyl glucopyranosyl (17) pointed out the (1↦3) linkage between arabinose and glucose.

The anomeric configuration of sugars in compound **4** was determined from the ^{1}H-nmr spectrum. The anomeric protons appeared at δ 4.38 (d, J = 7.74 Hz, Glc), 4.50 (d, J = 7.01 Hz, Ara) and 5.37 (d, J = 7.95 Hz, Glc) and showed 1,2-axial-axial coupling, and the signals at δ 5.14 (d, J = 1.41 Hz, Rha) and 5.50 (d, J = 1.56 Hz, Rha) showed 1,2-equatorial-equatorial coupling. The anomeric signal of GlcU was overlapped by the CD_3OD and D_2O signal which appeared at 4.60–4.90 ppm.

In view of the above spectral evidence, the structure of guaianin K [**4**] was concluded to be 3-*O*-[β-D-glucuronopyranosyl]oleanolic acid-28-*O*-[α-L-rhamnopyranosyl(1↦2)α-L-rhamnopyranosyl(1↦2)α-L-arabinopyranosyl[(3↦1)β-D-glucopyranosyl](1–3)β-D-glucopyranosyl] ester.

Compound **5** is the most polar saponin isolated, so far, from the fruit of *G. officinale*. The ^{1}H-nmr spectrum indicated the presence of six sugar moieties. The anomeric signals appeared at δ 4.38 (d, J = 7.70 Hz), 4.64 (d, J = 7.68 Hz), 4.95 (d, J = 7.80 Hz), and 5.36 (d, J = 8.08 Hz), and showed the β configuration for three D-glucose and one D-glucuronic acid moieties, while the anomeric signals appearing at δ 4.48 (d, J = 7.64 Hz) and 5.15 (d, J = 1.52 Hz) showed the α-configuration for L-arabinose and L-rhamnose moieties. This configuration was confirmed by ^{13}C-nmr assignments of sugar moieties (Table 1).

The ^{13}C-nmr signals appearing at δ 105.20 (CH), 104.44 (CH), 104.23 (2 × CH), 102.84 (CH), and 95.50 (CH) were due to the six sugar moieties. The sequence of sugars was established by negative ion fabms, which exhibited an $[M-H]^-$ ion peak at *m/z* 1395. The other ions appeared at *m/z* 1249, 1087, 925, 793, 631, and 455 and showed the loss of rhamnose, rhamnose + glucose, rhamnose + 2 × glucose, rhamnose + 2 × glucose + arabinose, rhamnose + 2 × glucose + arabinose + glucose, and rhamnose + 2 × glucose + arabinose + glucose + glucuronic acid, respectively.

The alkaline hydrolysis of compound **5** yielded a prosaponin **7**, which showed an $[M-H]^-$ ion peak at *m/z* 925. The important fragments were observed at *m/z* 793, 631, and 455 and were the same as those observed in the negative ion fabms of compound **5**. The ^{1}H-nmr spectrum of **7** exhibited three anomeric proton signals at δ 4.50 (d, $J=7.71$ Hz, Ara), 4.68 (d, $J=7.53$ Hz, Glc), and 4.96 (d, $J=7.80$ Hz, GlcU), which showed 1,2-diaxial coupling. The ^{13}C-nmr spectrum of **7** confirmed the presence of three sugar moieties. The anomeric signals that appeared at δ 105.25, 104.47, and 104.21 were due to the glucuronic acid, arabinose, and glucose, respectively. The ^{13}C-nmr assignments also showed that arabinose and glucose had the same chemical shifts as reported in the literature for their methyl glycosides (8). Therefore, they could be assigned as terminal sugars. The ^{13}C-nmr signal appearing at δ 86.24 suggested that the glycosidic linkage between arabinose and glucuronic acid was the same as in compounds **2** and **3**, while the remaining downfield signal at δ 78.24 indicated a (1↦4) linkage between glucose and glucuronic acid (18). The possibility of (1↦2) linkage between glucose and glucuronic acid was eliminated by comparing the ^{13}C-nmr data of saponins reported by Nie *et al.* (19). It was demonstrated by these authors that when glucose was attached at C-2 of glucuronic acid, the ^{13}C-chemical shift was observed at about 79–83 ppm. Hence, on the basis of foregoing evidence, the structure of prosaponin **7** was determined as 3-*O*-[β-D-glucopyranosyl(1↦4)β-D-glucuronopyranosyl(3↦1)α-L-arabinopyranosyl]oleanolic acid.

The disappearance of anomeric signals at δ 4.38, 5.15, and 5.36 in the ^{1}H nmr and at δ 104.23, 102.84, and 95.50 in the ^{13}C-nmr spectrum of **7** indicated that two glucose units and a rhamnose unit were attached to the C-28 of the aglycone by an ester bond in compound **5**. The disappearance of the downfield ^{13}C-nmr signals of methylene at δ 69.27 and 67.00 allowed us to place (1↦6) and (1↦6) linkages between two glucose units and rhamnose to glucose, respectively (5,20), while the anomeric signal at δ 95.50 showed the direct attachment of glucose to C-28 of the aglycone (5).

In the presence of the above evidence, the structure of guaianin L [**5**] was elucidated as 3-*O*-[β-D-glucopyranosyl(1↦4)β-D-glucuronopyranosyl(3↦1)α-L-arabinopyranosyl]oleanolic acid-28-*O*-[α-L-rhamnopyranosyl(1↦6)β-D-glucopyranosyl(1↦6)β-D-glucopyranosyl] ester.

EXPERIMENTAL

EXPERIMENTAL INSTRUMENTS.—Cc was performed on Merck Si gel 60 and LH-20. The purity of the samples was checked on DC-Micro-cards SIF 37341 (size 5 × 10 cm, layer thickness 0.2 mm); the hplc consisted of a Waters model 501 pump as a solvent delivery system, a Rheodyne sample injector with a 100 μl loop, a Supelco RP-18 column (25 cm × 10 mm i.d.), and a Waters model R-401 differential refractometer detector connected with a Kipp & Zonen BD-40 recorder. Eims was recorded on a Finnigan MAT-112 spectrometer coupled with a PDP 11/34 Computer system. The negative ion fab mass spectra were recorded on a Jeol JMS-HX110 spectrometer coupled with a PDP 11/73 computer system. The ^{1}H- and ^{13}C-nmr spectra were recorded on Bruker AM-300 and 75 MHz, respectively. The DEPT experiments were carried out with $\theta=45°$, 90°, and 135°; the quaternary carbons were determined by subtraction of these spectra from the broad band ^{13}C-nmr spectrum.

EXTRACTION AND ISOLATION OF SAPONINS.—The extraction was described previously (5). The Si gel cc of the saponin mixture (85 g) afforded a fraction eluted with $CHCl_3$-MeOH (11:9), which was further chromatographed over an LH-20 column. The elution of a mixture of two minor saponins (25 mg) was carried out with pure MeOH. The separation of these saponins was carried out on hplc using MeOH-H_2O (4:1) delivered to the semipreparative reversed-phase (RP-18) column at flow rate 3.5 ml/min. This separation led to the isolation of the known saponin, guaianin (8.52 mg) and a new saponin, guaianin H (13.50 mg). Another fraction (95 mg) eluted with $CHCl_3$-MeOH (9:11) yielded a mixture of three saponins (hplc), which was purified on semipreparative hplc under the above conditions using the solvent system MeOH-H_2O (13:7). It afforded guaianin I (12.50 mg), guaianin J (18.00 mg), and guaianin K (25.30 mg) in pure form. A more polar fraction eluted with $CHCl_3$-MeOH (2:3) yielded 65 mg of a mixture of two

saponins. This mixture was purified on semipreparative hplc, under the same conditions mentioned for guaianin H, using MeOH-H_2O (1:1) to afford guaianin I (30.5 mg) and guaianin L (24.53 mg). Another most polar fraction, eluted with $CHCl_3$-MeOH (7:13) yielded 32.96 mg of a mixture of two saponins. This mixture was purified on semipreparative hplc, using MeOH-H_2O (1:1) and yielded guaianin I (10.77 mg) and guaianin L (11.85 mg).

GUAIANIN H [**1**].—Negative ion fabms *m/z* $[M-H]^-$ 895, $[M-H-$rhamnose$]^-$ 749, $[M-H-$rhamnose − glucose$]^-$ 587, $[M-H-$rhamnose − glucose − arabinose$]^-$ 455; ^1H nmr (CD_3OD) δ 5.24 (distorted t, H-12), 1.05 (s, H-23), 0.84 (s, H-24), 0.94 (s, H-25), 0.79 (s, H-26), 1.15 (s, H-27), 0.89 (s, H-29), 0.95 (s, H-30), 4.56 (d, J = 7.80 Hz, H-1′), 4.28 (d, J = 7.00 Hz, H-1″), 5.18 (d, J = 1.53 Hz, H-1‴), 1.24 (d, J = 6.22 Hz, H-6‴); ^{13}C nmr (CD_3OD) see Table 1.

GUAIANIN I [**2**].—Negative ion fabms *m/z* $[M-H]^-$ 1379, $[M-H-$glucose$]^-$ 1217, $[M-H-2\times$glucose$]^-$ 1055, $[M-H-2\times$glucose − rhamnose$]^-$ 909, $[M-H-2\times$glucose − 2 × rhamnose$]^-$ 763, $[M-H-2\times$glucose − 2 × rhamnose − arabinose$]^-$ 631, $[M-H-2\times$glucose − 2 × rhamnose − arabinose − glucuronic acid$]^-$ 455; ^1H nmr ($CD_3OD + D_2O$) δ 5.25 (distorted t, H-12), 1.04 (s, H-23), 0.83 (s, H-24), 0.92 (s, H-25), 0.77 (s, H-26), 1.13 (s, H-27), 0.90 (s, H-29), 0.93 (s, H-30), 4.65 (d, J = 7.41 Hz, H-1″), 5.50 (d, J = 1.74 Hz, H-1‴), 1.31 (d, J = 6.12 Hz, H-6‴), 5.15 (d, J = 1.50 Hz, H-1⁗), 1.28 (d, J = 6.18 Hz, H-6⁗), 5.37 (d, J = 7.92 Hz, H-1⁗), 4.38 (d, J = 7.71 Hz, H-1⁗); ^{13}C nmr ($CD_3OD + D_2O$) see Table 1.

ACID HYDROLYSIS OF GUAIANIN I [**2**].—Compound **2** (25 mg) was hydrolyzed with 2 M HCl in aqueous MeOH (10 ml) in a boiling H_2O bath for 3 h. The MeOH was evaporated under reduced pressure, and the mixture was diluted with H_2O and extracted with EtOAc. The EtOAc extract was evaporated under reduced pressure to afforded 8 mg of aglycone, identified by eims and ^1H- and ^{13}C-nmr spectra as oleanolic acid: eims *m/z* $[M]^+$ 456 ($C_{30}H_{48}O_3$), [RDA − fragment a] 248 ($C_{16}H_{24}O_2$), [RDA − fragment b] 207 ($C_{14}H_{23}O$), [RDA fragment a − COOH group] 203; ^1H nmr ($CDCl_3$), δ 3.21 (m, $W½$ = 10.99 Hz, H-3α), 5.26 (t, J = 3.36 Hz, H-12), 2.81 (dd, J = 13.59, 3.99 Hz, H-18β), 0.97 (s, H-23), 0.76 (s, H-24), 0.94 (s, H-25), 0.74 (s, H-26), 1.12 (s, H-27), 0.89 (s, H-29), 0.92 (s, H-30); ^{13}C nmr ($CDCl_3$) see Tori *et al.* (6).

The H_2O layer was neutralized with dilute alkali solution and concentrated at reduced pressure. The residue obtained was compared with standard sugars on Si gel tlc [EtOAc-HOAc-H_2O-MeOH (6:1:1:2)], which showed that the sugars were arabinose, rhamnose, glucose, and glucuronic acid in guaianin I.

ALKALINE HYDROLYSIS OF GUAIANIN I [**2**].—Compound **2** (28.77 mg) was refluxed with 2% KOH in MeOH for 1 h and on usual workup gave the prosaponin **6**: negative ion fabms *m/z* $[M-H]^-$ 1055, $[M-H-$rhamnose$]^-$ 909, $[M-H-2\times$rhamnose$]^-$ 763, $[M-H-2\times$rhamnose − arabinose$]^-$ 631, $[M-H-2\times$rhamnose − arabinose − glucuronic acid$]^-$ 455; ^1H nmr ($CD_3OD + D_2O$) δ 5.23 (distorted t, H-12), 1.02 (s, H-23), 0.83 (s, H-24), 0.91 (s, H-25), 0.80 (s, H-26), 1.11 (s, H-27), 0.85 (s, H-29), 0.92 (s, H-30), 4.70 (d, J = 7.48 Hz, H-1′), 4.42 (d, J = 7.73 Hz, H-1″), 5.51 (d, J = 1.70 Hz, H-1‴), 1.32 (d, J = 6.83 Hz, H-6‴), 4.87 (d, J = 1.57 Hz, H-1⁗), 1.28 (d, J = 6.00 Hz, H-6⁗); ^{13}C nmr ($CD_3OD + D_2O$) see Table 1.

GUAIANIN J [**3**].—Negative ion fabms *m/z* $[M-H]^-$ 1379, $[M-H-$rhamnose$]^-$ 1233, $[M-H-2\times$rhamnose$]^-$ 1087, $[M-H-2\times$rhamnose − glucose$]^-$ 925, $[M-H-2\times$rhamnose − 2 × glucose$]^-$ 763, $[M-H-2\times$rhamnose − 2 × glucose − arabinose$]^-$ 631, $[M-H-2\times$rhamnose − 2 × glucose − arabinose − glucuronic acid$]^-$ 455; ^1H nmr (CD_3OD) + D_2O) δ 5.24 (distorted t, H-12), 1.03 (s, H-23), 0.82 (s, H-24), 0.92 (s, H-25), 0.77 (s, H-26), 1.13 (s, H-27), 0.89 (s, H-29), 0.93 (s, H-30), 4.42 (d, J = 7.10 Hz, H-1″), 5.36 (d, J = 8.08 Hz, H-1‴), 4.37 (d, J = 7.80 Hz, H-1⁗), 5.52 (d, J = 1.49 Hz, H-1⁗), 1.30 (d, J = 5.92 Hz, H-6⁗), 5.15 (d, J = 1.50 Hz, H-1⁗), 1.27 (d, J = 6.04 Hz, H-6⁗); ^{13}C-nmr ($CD_3OD + D_2O$) see Table 1.

ACID HYDROLYSIS OF GUAIANIN J [**3**].—Compound **3** (10 mg) was hydrolyzed as compound **2**. The aglycone was identified by comparing with the sapogenin of **2** on tlc as oleanolic acid, and the sugars obtained from the hydrolysate were identified on tlc [EtOAc-HOAc-H_2O-MeOH (6:1:1:2)] as arabinose, rhamnose, glucose, and glucuronic acid by comparing with authentic sugar samples.

ALKALINE HYDROLYSIS OF GUAIANIN J [**3**].—Compound **3** (8 mg) was refluxed with 2% KOH in MeOH for 1 h and on usual workup gave a prosaponin: negative ion fabms *m/z* $[M-H]^-$ 763, $[M-H-$arabinose$]^-$ 631, $[M-H-$arabinose − glucuronic acid$]^-$ 455; ^1H nmr (CD_3OD) δ 5.25 (distorted t, H-12), 1.03 (s, H-23), 0.82 (s, H-24), 0.91 (s, H-25), 1.12 (s, H-27), 0.88 (s, H-29), 0.92 (s, H-30), 4.68 (d, J = 7.32 Hz, H-1′), 4.40 (d, J = 7.0 Hz, H-1″).

GUAIANIN K [**4**].—Negative ion fabms *m/z* $[M-H]^-$ 1379, $[M-H-$glucose$]^-$ 1217, $[M-H-$

glucose − rhamnose]$^-$ 1071, [M − H − glucose − 2 × rhamnose]$^-$ 925, [M − H − glucose − 2 × rhamnose − arabinose]$^-$ 793, [M − H − glucose − 2 × rhamnose − arabinose − glucose]$^-$ 631, [M − H − glucose − 2 × rhamnose − arabinose − glucose − glucuronic acid]$^-$ 455; ^{1}H nmr ($CD_3OD + D_2O$) δ 5.24 (distorted t, H-12), 1.02 (s, H-23), 0.81 (s, H-24), 0.90 (s, H-25), 0.76 (s, H-26), 1.12 (s, H-27), 0.89 (s, H-29), 0.92 (s, H-30), 5.37 (d, J = 7.95 Hz, H-1″), 4.50 (d, J = 7.01 Hz, H-1‴), 5.50 (d, J = 1.56 Hz, H-1⁗), 1.30 (d, J = 6.21 Hz, H-6⁗), 5.14 (d, J = 1.41 Hz, H-1⁗′), 1.25 (d, J = 6.18 Hz, H-6⁗′), 4.38 (d, J = 7.74 Hz, H-1⁗″); ^{13}C nmr ($CD_3OD + D_2O$) see Table 1.

ACID HYDROLYSIS OF GUAIANIN K [4].—Compound **4** (10 mg) was hydrolyzed and worked up as with compound **2**. The EtOAc layer indicated the presence of oleanolic acid, while the H_2O layer showed the presence of arabinose, rhamnose, glucose, and glucuronic acid by using the same method as described for compounds **2** and **3**.

ALKALINE HYDROLYSIS OF GUAIANIN K [4].—Compound **4** (10 mg) was refluxed with 2% NaOH in MeOH for 1 h and on usual workup gave the prosaponin: negative ion fabms *m/z* [M − H]$^-$ 631, [M − H − glucuronic acid]$^-$ 455.

GUAIANIN L [5].—Negative ion fabms *m/z* [M − H]$^-$ 1395, [M − H − rhamnose]$^-$ 1249, [M − H − rhamnose − glucose]$^-$ 1087, [M − H − rhamnose − 2 × glucose]$^-$ 925, [M − H − rhamnose − 2 × glucose − arabinose]$^-$ 793, [M − H − rhamnose − 2 × glucose − arabinose − glucose]$^-$ 631, [M − H − rhamnose − 2 × glucose − arabinose − glucose − glucuronic acid]$^-$ 455; ^{1}H nmr ($CD_3OD + D_2O$) δ 5.24 (distorted t, H-12), 1.04 (s, H-23), 0.85 (s, H-24), 0.91 (s, H-25), 0.76 (s, H-26), 1.12 (s, H-27), 0.89 (s, H-29), 0.93 (s, H-30), 4.95 (d, J = 7.80 Hz, H-1′), 4.48 (d, J = 7.64 Hz, H-1″), 4.64 (d, J = 7.68 Hz, H-1‴), 5.36 (d, J = 8.08 Hz, H-1⁗), 4.38 (d, J = 7.70 Hz, H-1⁗′), 5.15 (d, J = 1.52 Hz, H-1⁗″), 1.25 (d, J = 6.28 Hz, H-6⁗″); ^{13}C nmr ($CD_3OD + D_2O$) see Table 1.

ACID HYDROLYSIS OF GUAIANIN L [5].—Compound **5** (10 mg) was hydrolyzed and worked up as with compound **2**. The EtOAc and H_2O layers showed the same results as described for compound **2**.

ALKALINE HYDROLYSIS OF GUAIANIN L [5].—Compound **5** (26.28 mg) was refluxed with 2% KOH in MeOH for 1 h and on usual workup gave the prosaponin **7**: negative ion fabms *m/z* [M − H]$^-$ 925, [M − H − arabinose]$^-$ 793, [M − H − arabinose − glucose]$^-$ 631, [M − H − arabinose − glucose − glucuronic acid]$^-$ 455; ^{1}H nmr ($CD_3OD + D_2O$) δ 5.18 (distorted t, H-12), 1.02 (s, H-23), 0.85 (s, H-24), 0.91 (s, H-25), 0.80 (s, H-26), 1.09 (s, H-27), 0.89 (s, H-29), 0.92 (s, H-30), 4.96 (d, J = 7.80 Hz, H-1′), 4.50 (d, J = 7.71 Hz, H-1″), 4.68 (d, J = 7.53 Hz, H-1‴); ^{13}C nmr ($CD_3OD + D_2O$) see Table 1.

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