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Saponins From the Stem Bark of Guaiacum officinale

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Summary: Two saponins obtained from the stem bark of Guaiacum officinale were characterised on the basis of chemical and spectral evidence as 3-O-[α-L-rhamnopyranosyl(1→2)-α-L-arabinopyranosyl]-30-norolean-12,20(29)-dien-28-O--D-glucopyranosyl ester (1) and 3-O-[α-L-arabinopyranosyl]-oleanolic acid-28-O-β-D-glucopyranosyl ester (2). The former saponin was new and named guaianin A_2 whereas the latter has been reported previously from Panax japonicus [1].

Introduction

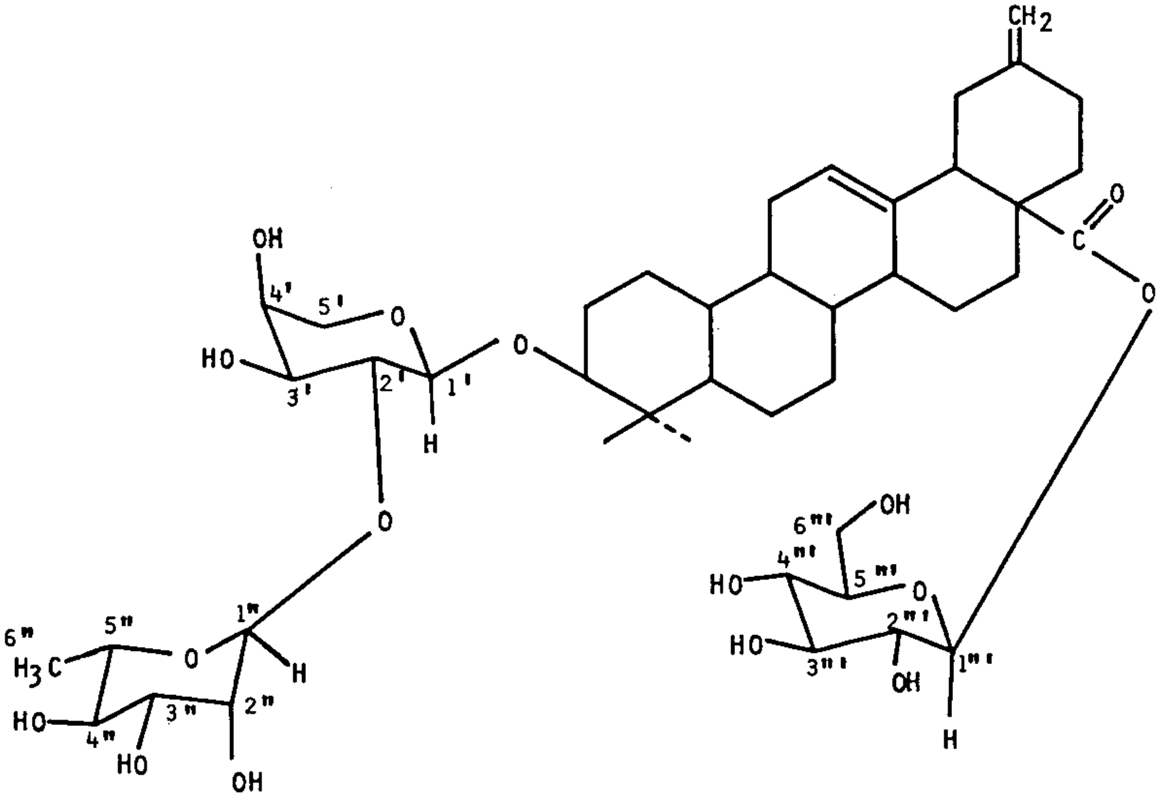
In the course of our continuing search for saponins from the stem bark of Guaiacum officinale we have reported several new saponins [2-4]. The reinvestigation of saponins from the same source further yielded a new saponin, named guaianin A_2, together with the known saponin 3-O-[α-L-arabinopyranosyl]-oleanolic acid-28-O-β-D-glucopyranosyl ester. The structure of guaianin A_2 has been established with the help of spectroscopic studies.

Results and Discussion

The crude saponin mixture obtained from the methanolic extract of the stem bark of Guaiacum officinale was chromatographed on a silica gel column. Compound 1 was eluted with chloroform-methanol (80:20) and was purified by semipreparative hplc using an RP-18 column.

The ^{13}C-nmr spectrum of 1 exhibited the anomeric signals at 104.67, 102.06 and 95.83 showing the presence of three sugar moieties in the molecule. The last signal revealed the presence of sugar residues attached to the aglycone by an ester linkage, which was established as glucose with the help of alkaline hydrolysis, while the former two signals were due to arabinose and rhamnose, respectively. The olefinic carbons due to the aglycone appeared at 124.35 (C-12), 144.25 (C-13), 107.35 (C-29) and 149.48 (C-20). The other peaks of the aglycone were similar to the previously reported aglycone 3 β hydroxy, 30-norolean 12,20(29) dien-28-oic acid [2-4].

The sequence of sugars was established through the FAB mass spectrum of the saponin, which showed a molecular ion peak at m/z 879 $(M-H)^-$ consistent with the molecular formula $C_{46}H_{72}O_{16}$. The other fragments appeared at m/z 717 (M-glucose), 571 (M-glucose+rhamnose) 439 (M-glucose -rhamnose-arabinose). The FAB mass spectrum showed that arabinose is directly attached to the aglycone, while rhamnose is the terminal sugar. The linkages of these two sugars were determined by ^{13}C-nmr spectroscopy. The downfield shifted signal of C-2



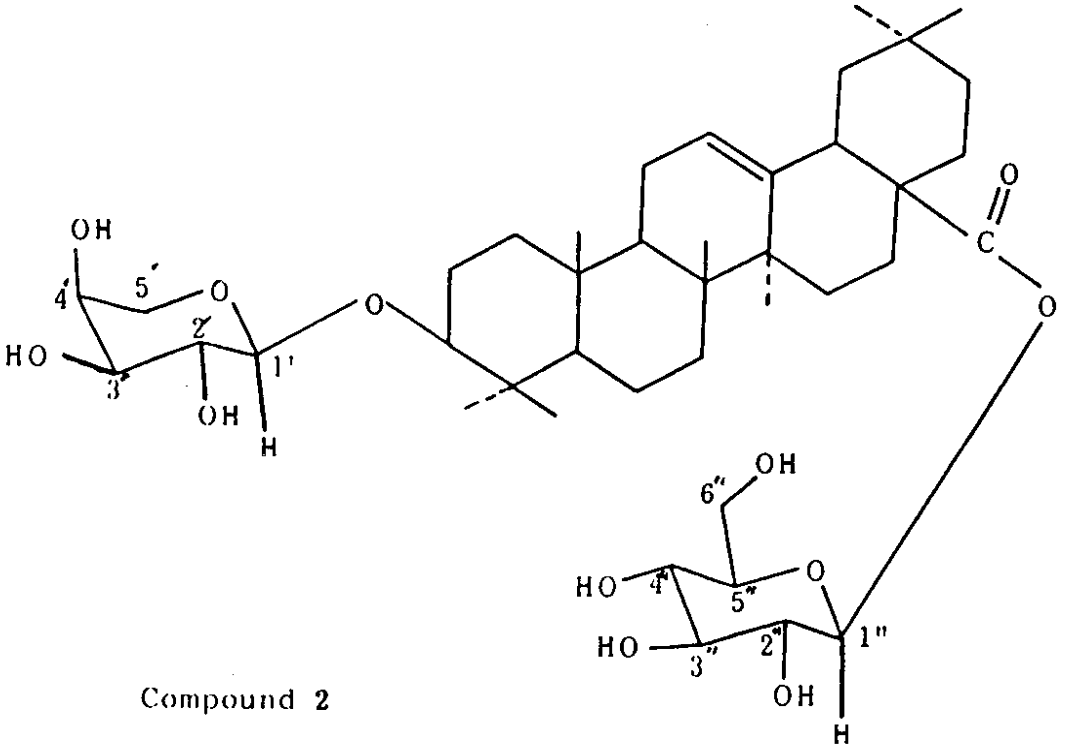
Compound I

of arabinose showed that the C-2 of arabinose is attached to rhamnose. The configuration of these sugars were determined by its ^{1}H-nmr spectrum which exhibited signals at 4.57 (d, J = 6.6Hz), 5.09 (d, J = 1.5Hz) and 5.37 (d, J = 7.9Hz) consistent with α-L-arabinose,α-L-rhamnose and β-D-glucose.

From all of these spectroscopic data the structure of Compound **1** has been established as 3-O [α-L-rhamnopyranosyl (1→2)-[α-L-arabinopyranosyl]-30-norolean-12, 20(29) dien-28-O-β-D-glucopyranosyl ester (**1**).

Compound **2** was eluted with chloroform-methanol (85:15) and further purified by semipreparative hplc using RP-18 column. The positive ion FAB/MS spectrum exhibited a molecular ion peak at m/z 773 $(M+Na)^+$ corresponding to molecular formula $C_{41}H_{66}O_{12}$ indicated that only two sugar moieties were present in compound **2**. On acid hydrolysis with methanolic HCl compound **2** furnished an amorphous material, identified as oleanolic acid, m.p. 302-4°, mass m/z 456. While the sugars were identified as L-arabinose and D-glucose in aqueous layer by comparison with authentic sample on thin layer chromatography.

The ^{1}H-nmr spectrum of **2** showed the presence of seven tertiary methyl signals in the regions δ 0.79-1.03 and olefinic proton at δ 5.01 (H-12). The signals of anomeric protons were



Compound 2

appeared at δ 4.27 (d, J = 6.5 Hz, H-1') and 5.36 (d, J = 7.9Hz, H-1") indicated that both sugars have 1,2-diequitorial relationship. The ^{13}C-nmr spectrum showed two olefinic carbon in its aglycone at 123.8 (CH), and 144.8 (quaternary), assigned to C-12 and C-13 respectively. It was concluded that only one double bond present in its aglycone, the aglycone in (2) was found to be oleanolic acid [5].

The ^{13}C-NMR spectrum exhibited only two anomeric carbon signals at 107.03, 95.07. The later signal showed that one sugar residue was attached by ester linkage at C-28, which was identified as D-glucose by alkaline hydrolysis. The ^{13}C-nmr spectrum also showed that C-3 signal appeared at a low field (90.21 ppm) which indicated that L-arabinose is attached at C-3 position.

From all of these accumulated evidences, compound **2** was formulated as 3-O-(α-L-arabinopyranosyl)-oleanolic acid 28, O-β-D-glucopyranosyl ester has been isolated from Japanese ginesng (Panax japonicus) [1].

Experimental

Melting points were recorded in glass capillary tubes on a Gallenkamp apparatus and a reuncorrected. Optical rotation was measured on a Schmidt & Haensch polartonic meter. ^{1}H-NMR (300 MHz) and ^{13}C-NMR (75.43MHz) spectra were recorded on a Bruker AM-300 Spectrometer in CD_3OD. Assignment was made using the DEPT Technique. HPLC was performed with a LDC Constametric pump, Model III instrument using an RP-18 column and refractor monitor III. TLC was performed on hptlc (Silica gel 60F_{254} Merck) and cellulose plates using the following solvent systems (a) n-BuOH-AcOH-H_2O (12:3:5), and (b) EtOAc-H_2O-MeOH-AcOH (65:15:15:20). Compounds were detected by spraying with ceric sulphate solution in 10% H_2SO_4 and aniline phthalate. U.V were recorded in MeOH on Shimadzu, optical rotations were measured as solutions in methanol. IR spectra on JASCO A-302 Spectrometer.

Extraction and Separation

The stem bark (40 kg) of G.officinale was collected from trees growing on the Karachi University Campus. The powdered bark was extracted three time with cold MeOH and the combined methanolic extract was evaporated at red. pressure to afford a gummy residue. The crude methanolic extract (200 gm) was chromatographed on a silica gel column using a gradient of MeOH in $CHCl_3$ as eluent.

Guaianin A_2 (1)

This was eluted from a silica gel column with $CHCl_3$-MeOH (4:1). The compound was purified by semipreparative HPLC using an RP-18 column and the eluent MeOH:H_2O (9:1), m.p. 220-30 (dec), $[\alpha]_D$ + 40.8° (c, 0.049 in MeOH). UV λ_{max} 212 nm. IR ν_{max} cm^{-1}, 3350-3400 (OH), 2925 (C-H), 1740, 1150, 1070. ^{1}H-NMR (CD_3OD, 300MHz): 0.80 (s, Me), 0.84 (s, Me), 0.95 (s, Me), 1.01 (s, Me), 1.19 (s,Me), 1.22 (d, J = 6.2Hz, H-6"), 4.57 (d, J = 6.6Hz, H-1'), 4.74 (br, s, H-29), 5.09 (d, J = 1.5 Hz), 5.31 (distorted t, H-12), 5.35 (d, J = 7.8Hz, H-1"').

^{13}C-NMR (CD_3OD, 75.43MHz): 38.45 (C-1), 26.99 (C-2), 90.67 (C-3), 39.94 (C-4), 57.87 (C-5), 19.38 (C-6), 33.94 (C-7), 40.83 (C-8), 48.15 (C-9), 37.45 (C-10), 24.20 (C-11), 124.35 (C-12), 144.25 (C-13), 42.61 (C-14), 28.99 (C-15), 24.55 (C-16), 48.75 (C-17), 48.15 (C-18), 42.61 (C-19), 149.48 (C-20), 37.95 (C-21), 30.89 (C-22), 28.69 (C-23), 17.06 (C-24), 16.04 (C-25), 17.79 (C-26), 26.34 (C-27), 177.35 (C-28), 107.35 (C-29), 104.67 (C-1'), 76.91 (C-2'), 70.25 (C-3'), 68.24 (C-4') 63.55 (C-5'), 102.06 (C-1"), 72.25 (C-2"), 72.25 (C-3"), 73.95 (C-4"), 70.25 (C-5"), 17.97 (C6"), 95.83 (C-1"'), 73.95 (C-2"'), 78.66 (C-3"'), 71.25 (C-4"'), 78.32 (C-5"'), 62.54 (C-6"').

FAB-Mass (Negative mode, Highfield): m/z 879 (M-H), 717 (M-glucose), 571 (M-glucose+rhamnose) 439 (M-glucose +rhamnose+arabinose).

Alkaline hydrolysis of 1

A solution of **1** (10 mg) in MeOH (5 ml) was treated with 2% NaOH (5 ml) and the whole mixture heated under reflux for 2 hr on a water bath and neutralized with Dowex 50. It was extracted with n-BuOH. The aqueous layer obtained was concentrated under red.press. and the residue was identified as D-glucose by TLC on cellulose plate developed in EtOAc: H_2O:MeOH:AcOH (65:15:15:20). The compound was detected by spraying with aniline phthalate.

Acid Hydrolysis of 1

5 mg of **1** was refluxed with methanolic HCl (9 ml MeOH, 1 ml H_2O, 1.5 ml HCl) for 3 hours. The reaction mixture was concentrated under red. pressure to remove MeOH, diluted with water and extracted with EtOAc. The aqueous layer was concentrated under reduced pressure and the residue obtained compared with standard sugars on TLC (solvent system b). Spots were detected by spraying with aniline phthalate which showed that the sugars were L-arabinose, L-rhamnose and D-glucose.

Compound (**2**) was eluted from the silica gel column with ($CHCl_3$:MeOH) (85:10), and further purified by HPLC, the eluent used being MeOH: H_2O (95:5), m.p. 220° (dec.).

^1H-NMR 0.76 (s, Me), 0.79 (s, Me), 0.94 (s, Me), 0.91 (s, Me), 0.93 (s, 6H,2xMe), 1.03 (s, Me), 3.20 (m, H-3), 5.24 (H-12), 5.36 (d, J = 7.9 Hz, H-1"), 4.27 (d, J = 6.5Hz, H-1').

^{13}C-NMR (CD_3OD, 75.43MHz): 39.87 (C-1), 27.02 (C-2), 90.73 (C-3), 40.18 (C-14), 57.09 (C-5), 19.38 (C-6), 34.94 (C-7), 39.96 (C-8), 48.96 (C-9), 37.96 (C-10), 24.56 (C-11), 123.88 (C-12), 144.87 (C-13), 47.28 (C-14), 28.93 (C-15), 24.56 (C-16), 48.15 (C-17), 42.25 (C-18), 42.97 (C-19), 30.94 (C-20), 33.19 (C-21), 34.94 (C-22), 28.62 (C-23), 17.79 (C-24), 16.01 (C-25), 16.98 (C-26), 26.31 (C-27), 178.10 (C-28), 33.49 (C-29), 23.97 (C-30), 107.03 (C-1'), 72.85 (C-2'), 73.38 (C-3'), 69.43 (C-4'), 66.25 (C-5'), 95.77 (C-1'), 78.38 (C-2"), 74.34 (C-3"), 71.23 (C-4"), 78.70 (C-5"), 63.53 (C-6").

Acid hydrolysis of ***2***

10 mg of **2** was hydrolysed with HCl in the same manner as for **1**. The aglycone was precipitated by adding H_2O (30 ml), the precipitate was filtrated and washed with water. The aglycone (4 mg) obtained, furnished an amorphous material which after crystallization, yielded white crystals of oleanolic acid m.p. 302-4°. It was treated with diazomethane to form the methyl ester m.p. 196-198°.

The aqueous layer evaporated under red.press. The sugars were identified as L-arabinose and D-glucose by comparison with authentic sample on TLC.

*Alkaline hydrolysis of (****2****)*

A solution of **2** (8 mg) in MeOH (5 ml) was hydrolyzed and worked up as described for **1**. The sugar was identified as β-D-glucose.

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