A New Bidesmoside Saponin from the Bark of *Guaiacum officinale*

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Summary: A new bidesmosidic triterpene saponin, guaianin P was isolated from the stem bark of *Guaiacum officinale*. Its structure was established as oleanolic acid 3-*O*-{α-L-rhamnopyranosyl-(1→3)-α-L-rhamnopyranosyl-(1→2)-[β-D-glucopyranosyl-(1→3)]-α-L-arabinopyranoside}-28-*O*-β-D-glucopyranosyl ester by spectroscopic and chemical analyses

Key words: *Guaiacum officinale*, Zygophyllaceae, bidesmosidic saponin, guaianin P

Introduction

Saponins are common in a large number of plants and plant products that are important in human and animal nutrition. Several biological features such as membrane-permeabilising, immunostimulant and hypocholesterolaemic have been credited to saponins [1]. *Guaiacum officinale* L. (Zygophyllaceae) is a rich source of saponins. Tradionally its resin is used to cure angina, tonsillitis, rheumatoid arthritis, mucous membrane diseases and abnormalities of metabolic processes. We have reported mono- and bi-desmosidic saponins having akebonic acid and oleanolic acid as genins from this plant [2-4]. Re-investigation of the bark of *Guaiacum officinale* for further exploration led to the isolation and structure elucidation of guaianin P, a new bidesmosidic oleanolic acid type triterpene saponin. Structure elucidation was achieved by spectroscopic tools including 1D and 2D NMR and FAB–MS as well as by chemical means.

Results and Discussion

Guaianin P was purified from the *n*-BuOH soluble part of the methanolic extract of *G. officinale* by repeated column chromatography over normal and reversed phase silica gel. The FAB–MS (negative ion mode) displayed a pseudo-molecular ion $[M–H]^-$ at *m/z* 1203, which corresponded to the molecular formula of $C_{59}H_{96}O_{25}$. Other important ions were observed at *m/z* 1041, $[M–H–162 \text{ (glucose)}]^-$, *m/z* 895, $[M–H–162 \text{ (glucose)}–146 \text{ (rhamnose)}]^-$, 879 $[M–H–162 \text{ (glucose)}–162 \text{ (glucose)}]^-$, 749 $[M–H–162 \text{ (glucose)}–146 \text{ (rhamnose)}–146 \text{ (rhamnose)}]^-$, 733 $[M–H–162 \text{ (glucose)}–162 \text{ (glucose)}–146 \text{ (rhamnose)}]^-$, 587 $[M–H–162 \text{ (glucose)}–162 \text{ (glucose)}–146 \text{ (rhamnose)}–146 \text{ (rhamnose)}]^-$ and 455 $[M–H–162 \text{ (glucose)}–162 \text{ (glucose)}–146 \text{ (rhamnose)}–146 \text{ (rhamnose)}–132 \text{ (arabinose)}]^-$ (Fig. 1). The ^{1}H NMR spectrum displayed resonances for seven tertiary methyls [δ_H 0.79, 0.84, 0.90, 0.93, 0.94, 1.00 and 1.15], an olefin proton [δ_H 5.25 (t, J=3.7 Hz)] along with for five anomeric protons [δ_H 4.50 (d, H–1′), 5.13 (d, H–1″), 5.15 (d, H–1‴), 4.53 (d, H–1⁗) and 5.38 (d, H–1⁗′). The coupling constant values of anomeric protons [J=7.3 Hz, J=1.54 Hz, J=1.54 Hz, J=7.3 Hz and J=7.99 Hz] indicated the β-configurations for glucose units and α-configurations for arabinose and rhamnose moieties, respectively [2-5]. The methyl protons of two rhamnose units resonated at δ_H 1.22 (d, J=6.0 Hz, H–6″) and δ_H 1.20 (d, J=6.2 Hz, H–6‴). The ^{13}C NMR data of guaianin P (Table-1), showed 59 carbons, of which 30 were ascribed to the aglycone. The DEPT experiment was used to resolve the 30 carbon resonances of the aglycone into seven methyl, ten methylene, five methine and eight quaternary carbons. The ^{13}C NMR spectrum showed resonances for seven methyl groups [δ_C 28.7, 17.8, 16.2, 17.3, 26.5, 33.6 and 24.1], an olefin bond [δ_C 123.9 and 144.9], an oxygenated methine [δ_C 89.8] and for an acid carbonyl carbon [δ_C 178.1] related to aglycone moiety. The aglycone unit of the molecule was identified to be oleanolic acid on comparison of its NMR data with those reported in literature [2-5]. The remaining 29 carbon resonances including the anomeric carbons [δ_C 105.2 (C–1′), 101.8 (C–1″), 102.8 (C–1‴), 104.2 (C–1⁗) and 95.7 (C–1⁗′)] were assigned to five sugar moieties (α-arabinopyranose, two α-rhamnopyranoses and two β-glucopyranoses) [2-8]. The long range HMBC

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correlations among H–1′/C–3, H–1″/C–2′, H–1‴/C–3′, H–1″′/C–3″ and H–1⁗/C–28 revealed the position of arabinose at C–3, inner rhamnose at C–2′, terminal rhamnose at C–3″, glucose at C–3′ and other glucose at C–28. Alkaline hydrolysis followed by the FAB–MS analysis of the *n*-butanol layer of the reaction mixture exhibited ion at *m/z* 1041, corresponding to the molecule without ester linked sugar. The sugars obtained on acid hydrolysis were identified as glucopyaranose, rhamnopyranose and arabinopyranose by co-TLC with authentic samples. The locations of the functional groups and NMR data assignment of guaianin P were made by COSY, HMQC and HMBC spectra (Fig. 2) and were found in agreement with the resembled reported compounds [2-5]. Based on the above accumulated data the structure of guaianin P was determined as oleanolic acid 3-*O*-{α-L-rhamnopyranosyl-(1→3) -α-L-rhamnopyranosyl- (1→2)-[β-D-glucopyranosyl-(1→3)]-α-L-arabinopyranoside}-28-*O*-β-D-glucopyranosyl ester.

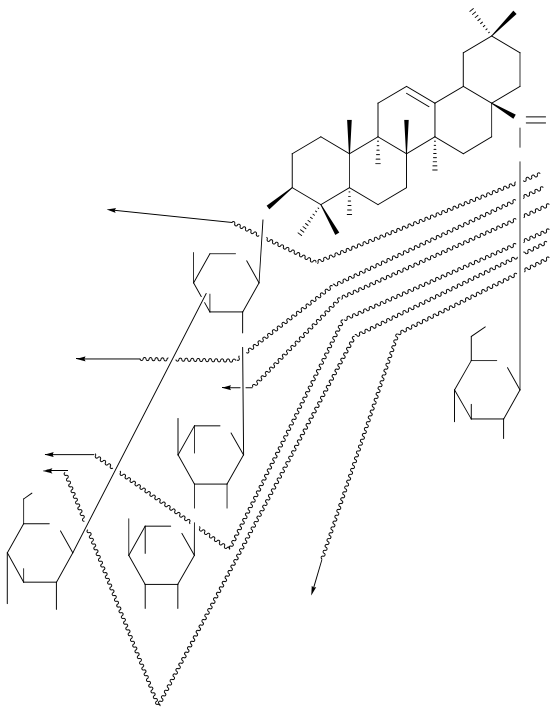

Fig. 1: Fragment ions observed in the (negative ion mode) FAB-MS of guaianin P.

Experimental Section

General

Column chromatography and flash chromatography were carried out with silica gel (70–230 μm mesh and 230–400 μm mesh). TLC was developed on the E. Merck silica gel plates (0.25 mm) and detected by cerium sulphate reagent followed by heating. Optical rotation was measured on a JASCO DIP–360 automatic digital polarimeter. IR and UV spectra were recorded on a JASCO A–100 and Shimadzu UV–254 spectrophotometers, respectively. NMR spectra were recorded on a Bruker AM–300 spectrometer. FAB–MS was performed on MAT–312 mass spectrometer. HPLC was performed on Shimadzu apparatus.

Plant Material

The bark of *G. officinale* L., was collected from the premises of Karachi University. The plant material was identified by Dr. M. Qaiser and a voucher specimen (No. 33 KUH) was deposited at the Herbarium of the University of Karachi, Pakistan.

Table-1: ^{13}C NMR data of guaianin P (CD_3OD, 75 MHz).

Aglycone	δ_C (Mult.)	Sugars	δ_C (Mult.)
1	40.2, CH_2	Ara. 1′	105.2, CH
2	27.2, CH_2	2′	75.1, CH
3	89.8, CH	3′	84.4, CH
4	40.8, C	4′	68.5, CH
5	57.3, CH	5′	64.7, CH_2
6	19.4, CH_2	Rha. 1″	101.8, CH
7	33.1, CH_2	2″	72.1, CH
8	40.4 C	3″	82.4, CH
9	49.1, CH	4″	72.1, CH
10	37.9 C	5″	69.8, CH
11	24.6, CH_2	6″	17.9, CH_3
12	123.9, CH	Rha. 1‴	102.8, CH
13	144.9, C	2‴	72.4, CH
14	43.0, C	3‴	72.3, CH
15	28.9, CH	4‴	73.8, CH
16	24.5, CH_2	5‴	70.2, CH
17	49.1, C	6‴	18.1, CH_3
18	42.6, CH	Glc. 1⁗	104.2, CH
19	47.3, CH	2⁗	75.4, CH
20	31.6, C	3⁗	78.3, CH
21	34.9, CH_2	4⁗	70.3, CH
22	34.0, CH_2	5⁗	78.7, CH
23	28.7, CH_3	6⁗	62.5, CH_2
24	17.8, CH_3	Glc. 1⁗′	95.7, CH
25	16.2, CH_3	2⁗′	73.9, CH
26	17.3, CH_3	3⁗′	78.0, CH
27	26.5, CH_3	4⁗′	71.1, CH
28	178.1, C	5⁗′	77.9, CH
29	33.6, CH_3	6⁗′	62.5, CH_2
30	24.1, CH_3		

Extraction and Isolation

The air-dried and chopped bark of *G. officinale*, (10 kg) was extracted with methanol (40 L x 3) at room temperature and then concentrated under reduced pressure. The resulting brown thick residue was dissolved in water, extracted successively with ethyl acetate and *n*-butanol. The *n*-butanol layer was concentrated to give a residue (250 g), which was subjected to repeated column chromatography and flash chromatography on silica gel with chloroform

and chloroform-methanol proportions. The fraction eluted with methanol-chloroform (1:3) was purified by HPLC with methanol-water (3:7) using RI detector and RP C_{18} semi preparative bondapak column to give guaianin P (35.4 mg).

Guaianin P

White powder. – $[\alpha]_D^{25}$ = + 1 (*c* = 0.27, MeOH). – IR (KBr): ν = 3430 (OH), 1735 (C=O) cm^{-1}. – ^{1}H NMR spectral data (CD_3OD, 300 MHz): δ_H = 5.38 (d, *J* = 8.0 Hz, H–1''''), 5.25 (t, *J* = 3.7 Hz, H–12), 5.15 (d, *J* = 1.5 Hz, H–1'''), 5.13 (d, *J* = 1.5 Hz, H–1''), 4.53 (d, *J* = 7.3 Hz, H–1''''), 4.50 (d, *J* = 7.3 Hz, H–1'), 3.98-3.13 (Oxygenated methines and methylenes), 1.22 (d, *J* = 6.0 Hz, H_3–6''), 1.20 (d, *J* = 6.2 Hz, H_3–6'''), 1.15 (s, H_3–27), 1.00 (s, H_3–23), 0.94 (s, H_3–25), 0.93 (s, H_3–30), 0.90 (s, H_3–29), 0.84 (s, H_3–26), 0.79 (s, H_3–24). – ^{13}C NMR spectral data (CD_3OD, 75 MHz): see Table-1. – FAB–MS (–ve ion mode): *m/z* = 1203 $[M–H]^-$, 1041 $[M–H–162]^-$, 895 $[M–H–162–146]^-$, 879 $[M–H–162–162]^-$, 733 $[M–H–162–146–162]^-$, 587 $[M–H–162–146–162–146]^-$, 455 $[M–H–162–146–162–146–132]^-$.

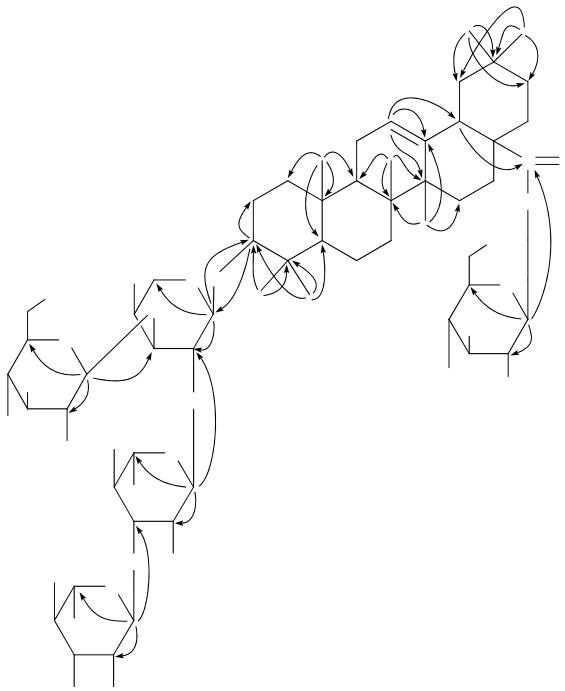

Fig. 2: Key HMBC interactions of guaianin P.

Acid Hydrolysis

Guaianin P (5 mg) was refluxed with 20% HCl for 2 hours. The residue was filtered after cooling the reaction mixture and identified as oleanolic acid by EIMS and co-TLC with authentic sample (isolated in our lab). The sugars in aqueous layer were identified as arabinose, rhamnose and glucose by co-TLC on cellulose with authentic samples [(ethyl acetate/water/methanol/acetic acid (13:3:3:4)]. Spots were detected by spraying it with aniline phthalate.

Alkaline Hydrolysis

Guaianin P (5 mg) was refluxed with 20% NaoH for two hours. After neutralization with Dowex 50, it was extracted with *n*-butanol. The sugar in aqueous layer was identified as glucose by co-TLC on cellulose with authentic sample. The *n*-butanol layer was applied to the (negative ion mode) FAB–MS, which exhibited ion at *m/z* 1041 $[M–glucose–H]^-$. [1]

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