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Separation and identification of a new saponin from the flowers of *Guaiacum officinale* L.

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A triterpenoid saponin, guaianin *O* (**1**), oleanolic acid 3-*O*-{α-L-rhamnopyranosyl-(1 → 2)-[β-D-glucopyranosyl-(1 → 3)]-α-L-arabinopyranoside}-28- *O*-[β-D-glucopyranosyl]-ester, was isolated from the *n*-butanol extract of flowers of *Guaiacum officinale* L. The structural elucidation of **1** was accomplished by extensive studies of both one and two dimensional ^{1}H, ^{13}C-NMR spectra, the FAB mass spectrum, and alkaline and acid hydrolyses.

Keywords: guaianin *O*; triterpenoid bidesmosidic saponin; flowers; *Guaiacum officinale* L.; Zygophyllaceae

1. Introduction

Guaiacum officinale L. is both a medicinal and an ornamental plant and belongs to the family Zygophyllaceae (Ghafoor, Nasir, & Ali, 1974). The resin of *G. officinale* is used as medicine for a number of diseases, e.g. in the beginning stages of angina, tonsillitis, rheumatoid arthritis, mucous membrane diseases and in abnormalities of metabolic processes. It is diuretic, diaphoretic, sudorific and sialogogic. It is also a good antioxidant and antiinflammatory agent (Duwiejua, Zeitlin, Waterman, & Gray, 1994; Hoppe, 1975). A chemical literature survey revealed the presence of both mono and bidesmosidic saponins having akebonic and oleanolic acids as genins (Ahmed, Bano, Fatima, & Bano, 1988; Tori, Seo, Shimaoka, & Tomita, 1974). The compound **1** was isolated as glass.

2. Results and discussion

Guaianin *O* was isolated from the *n*-BuOH extract of the flowers of *G. officinale*. The *n*-BuOH extract was subjected to repeated column and flash chromatography. The fractions, eluted with 20% methanol in chloroform, were finally purified by HPLC using a reverse phase C_{18} column. The compound (**1**) was purified from

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among a number of reported compounds (Ahmed et al., 1988; Ahmed, Perveen, & Bano, 1990).

The FABMS of **1** indicated the loss of masses of 132, 146 and 162 due to the loss of arabinose, rhamnose and glucose from molecule, respectively (Figure 1). These sugars were identified by the co-TLC comparison of the water layer of the acid hydrolysate of **1** with the authentic sugar samples. The pseudomolecular ion peak at *m/z* 1057 $[M-H]^-$, molecular weight 1058, corresponding to the molecular formula $C_{53}H_{86}O_{21}$ showed the presence of 11 degrees of unsaturation in the molecule. The next ion peak at *m/z* 895, $[(M-H)\text{-}162]^-$ showed the removal of an ester glucose. The other ion peaks at *m/z* 749, $[(M-H)\text{-}162\text{-}146]^-$, 733 (small) $[(M-H)\text{-}162\text{-}162]^-$, indicated branching in the sugar chain, 587 $[(M-H)\text{-}162\text{-}146\text{-}162]^-$ and 455 $[(M-H)\text{-}162\text{-}146\text{-}162\text{-}132]^-$ or [oleanolic acid–H]$^-$ indicated the removal of terminal rhamnose and glucose as well as internal arabinose, respectively. The fragment *m/z* 455 indicated the presence of aglycon, oleanolic acid, molecular weight 456 and molecular formula $C_{30}H_{48}O_3$. The alkaline hydrolysis followed by FABMS of the butanol layer showed *m/z* 895, the absence of an ester bond sugar glucose.

In ^{1}H-NMR (500 MHz, C_5D_5N) spectra, the protons of each of the seven methyl groups of the aglycon appeared as singlets at δ 1.17, 1.09, 0.83, 1.06, 1.23, 0.88 and 0.86 of 3H for each of H-23, H-24, H-25, H-26, H-27, H-29 and H-30, respectively. The H-12 proton appeared as a triplet at δ 5.39 ($J = 3.45$ Hz) and the H-3 as an overlapped signal at δ 3.57. In the ^{1}H-NMR spectrum, the four-anomeric proton

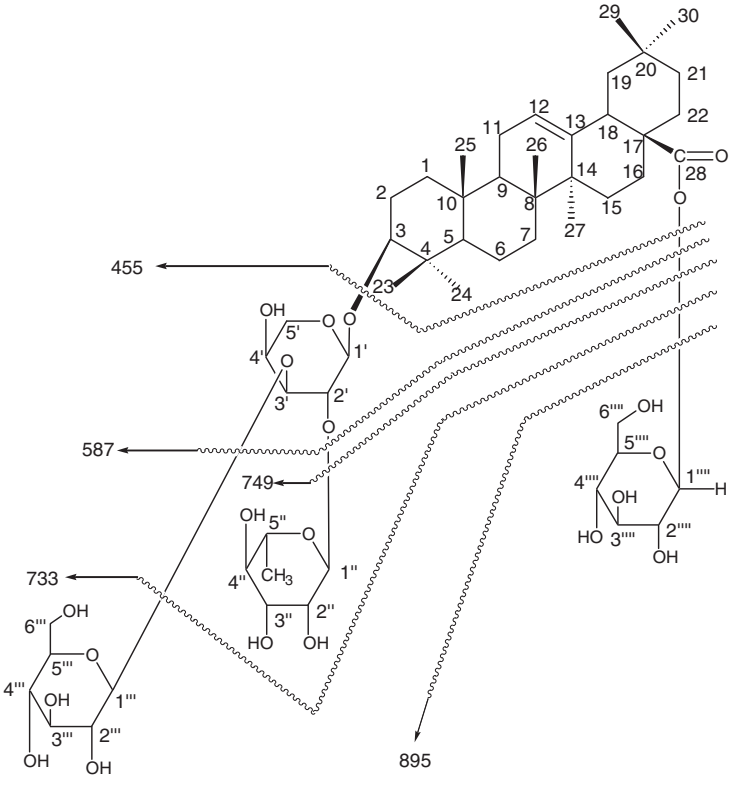

Figure 1. Positions and FAB negative *m/z* 1057 of **1**.

signals appeared at δ 4.84 (H-1′), 6.11 (H-1″), 5.07 (H-1‴) and 6.30 (H-1⁗). The first and last two signals appeared as doublets, while H-1″ appeared as broad singlets due to the overlapping of the two signals. The *J*-values were calculated as $J_{1'-2'} = 5.50$ Hz, $J_{1'''-2'''} = 6.8$ Hz and $J_{1''''-2''''} = 8.0$ Hz. These coupling constants indicated the β-D-pyranosyl configuration for glucose and α-L-pyranosyl for arabinose and rhamnose, respectively. The methyl protons of rhamnose resonated as a doublet at δ 1.60 ($J = 6.15$ Hz). The study and the comparison of all the above accumulated data proved that the aglycon of **1** was oleanolic acid. The ^{13}C-NMR (125 MHz, C_5D_5N) (BB) of **1** showed that 30 carbons out of 53 consisted of aglycon in the molecule. The DEPT pulse sequence showed the presence of 7 methyl, 10 methylene and 5 methyne groups. The eight quaternary carbon atoms were detected by the broadband ^{13}C-NMR spectrum. The peaks identified an endocyclic double bond between the C-12 and C-13 appeared at δ 122.8 (CH) and 144.0 (quaternary carbon atom), respectively (Ahmed et al., 1988; Tori et al., 1974). A slight low frequency shift of C-28, δ 176.40 and its IR absorption at 1740 cm^{-1} suggested a glycosidic linkage occurred at it. The attachment of polysaccharide chain at C-3 of aglycon was confirmed by the 10 ppm high frequency chemical shift δ 88.07 (Ahmed et al., 1988; Tori et al., 1974). Hence it was a 3,28-bidesmosidic compound (Ahmed et al., 1988, 1990). The signals for the seven-methyl groups of the aglycon appeared at δ 27.9, 16.95, 15.58, 17.39, 25.88, 32.93 and 23.57 for the C-23-C-27, C-29 and C-30, respectively, in the ^{13}C-NMR spectrum. The remaining 23 carbon signals out of 53 were assigned to the four sugar moieties attached to the C-3 and C-28 carbons of aglycon. The three-anomeric carbon signals of the sugars at the C-3 carbons of aglycon appeared at δ 104.48 (C-1′), 101.72 (C-1″) and 104.39 (C-1‴) for arabinose, rhamnose and glucose, respectively. A 10 ppm low frequency chemical shift δ 95.86 (C-1⁗) was observed for the anomeric carbon of the glucose moiety attached at the C-28 carbonyl carbon of aglycon as ester linkage. We found that four carbon signals around δ 78-79 appeared for the two C-3‴, C-3⁗ and two C-5‴, C-5⁗ of the two terminal glucose moieties. The high frequency chemical shifts of C-2′ and C-3′ of arabinose δ 74.74 and 81.78 were due to the attachment of C-1″ and C-1‴ of terminal rhamnose and glucose to those positions. The ^{13}C-NMR values of the three terminal sugars, two glucose and one rhamnose, were assigned with the help of one- and two-dimensional-NMR spectra and compared to reported data (Ahmad, Saba, Ali, Muhammad, & Alam, 2000; Ahmad & Saba, 1993; Ahmad, Saba, & Khan, 2004; Ahmed et al., 1988; Seo, Tomita, Tori, & Yoshimura, 1978). The points of attachment of the two terminal sugars, rhamnose and glucose, to the internal arabinose were confirmed via COSY-45° and HMBC. The sugar linkages and their ^{13}C-NMR data of **1** were in agreement with the reported compound except the aglycon part (Ahmed et al., 1990).

The interglycosidic linkages were confirmed by the study of COSY-45° and were verified by HMBC (Figure 2). The HMBC spectrum showed that the H-1′ signal at δ 4.84 showed a long range connectivity with the carbons C-3, C-2′, C-3′ and C-5′ confirmed that arabinose was directly attached to the C-3 of aglycon and its C-2′ and C-3′ had high frequency ^{13}C-NMR chemical shift. The H-1″ proton δ 6.11 showed long-range connectivity with the carbons C-2′, C-2″, C-3″ and C-5″, verifying the (C-1″-C-2′) glycosidic linkage between rhamnose and arabinose. The anomeric proton H-1‴ δ 5.07 showed connectivity with C-3′, C-2‴, C-3‴ and C-5‴, which confirmed a (C-1‴-C-3′) glycosidic linkage between glucose and arabinose. The anomeric proton

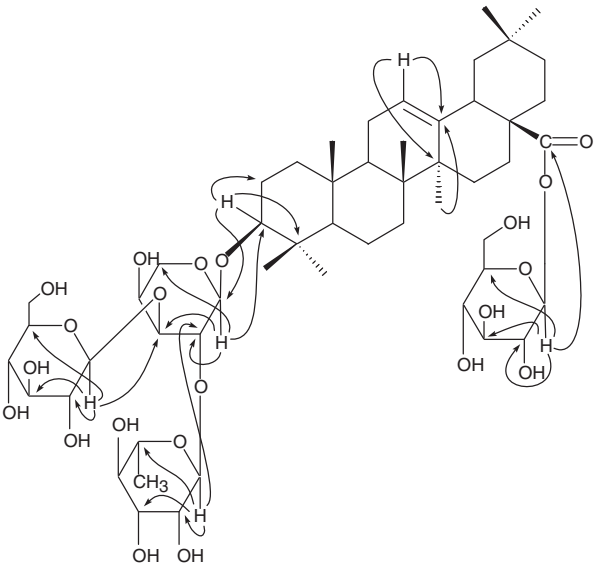

Figure 2. HMBC interactions of **1**.

H-1‴′ (δ 6.30) showed coupling activity with the carbons C-28, C-2‴′, C-3‴′ and C-5‴′. These linkages were also compared by the reported data (Ahmed et al., 1990). Based on all of the above accumulated data, the structure of compound **1** has been determined as oleanolic acid 3-*O*-{α-L-rhamnopyranosyl-(1 → 2)-[β-D-glucopyranosyl-(1 3)]-α-L-arabinopyranoside}-28- *O*-[β-D-glucopyranosyl]-ester.

3. Experimental

3.1. *General details*

Melting points were determined in glass capillary tubes using a Gallenkamp melting point apparatus. Optical rotation was measured using a Jasco DIP-360 automatic digital polarimeter. The IR spectrum was recorded on a Jasco A-100 spectrophotometer. The UV-spectrum was recorded in methanol on Shimadzu UV 254. The one- and two-dimensional ^{1}H and ^{13}C-NMR spectra were recorded on a Bruker AM-500 spectrometer at 500 and 125 MHz, respectively, on the same instrument. Mass spectra were recorded as fast atom bombardment (FAB) measurements, which were performed on a MAT 312 mass spectrometer. HPLC was performed on a Shimadzu apparatus.

3.2. *Plant material*

The flowers of *G. officinale* L., 5 kg (fresh), were collected from the premises of Karachi University in April and were identified by Dr M. Qaiser; a voucher specimen is deposited in the Herbarium of the Department of Karachi University, Pakistan (no. 33 KUH).

3.3. *Extraction and purification of 1*

The air-dried and ground material of the flowers of *G. officinale* (5 kg fresh) was extracted with distilled with methanol (5 L × 3) at room temperature and then concentrated. The resulting brown, thick residue was dissolved in water, extracted with ethyl acetate followed by extraction with *n*-butanol (1.5 L). The combined *n*-butanol layer was concentrated (9 g) and subjected to repeated column chromatography and flash chromatography on silica gel with chloroform, chloroform : methanol and methanol. The fraction eluted with methanol : chloroform (20 : 80, v/v) contained a mixture of compounds. They were separated and purified by HPLC having the solvent system methanol : water (30 : 70, v/v) using an RP C_{18} semi preparative bondapak column. Compound **1** was purified via HPLC using 29% water in methanol and obtained as glass, m.p. 360°–365°C (decomposed). $[\alpha]_D^{25}$ + 1° (c 0.85, CH_3OH).

^{1}H-NMR (500 MHz, C_5D_5N): δ 0.83, s, 3H, (C**H**$_3$); 0.86, s, 3H, (C**H**$_3$); 0.**88**, s, 3H, (C**H**$_3$); 1.06, s, 3H, (C**H**$_3$); 1.09, s, 3H, (C**H**$_3$); 1.17, s, 3H, (C**H**$_3$); 1.23, s, 3H, (C**H**$_3$); 3.57, overlapped, 1H, C3-H; 5.39, t, *J* 3.45 Hz, 1H, C12-H; 4.84, d, *J* = 5.5 Hz, 1H, C1′-H; 4.62, m, 1H, C2′-H; 4.30, m, 1H, C3′-H; 4.51, m, 1H, C4′-H; 4.22, m, 2H, C5′-H; 6.11, b.s., 1H, C1″-H; 4.69, m, 1H, C2″-H; 4.56, m, 1H, C3″-H; 4.25, m, 1H, C4″-H; 4.55, m, 1H, C5″-H; 1.60, d, *J* = 6.15, 3H, C6″-H; 5.07, d, *J* = 6.8 Hz, 1H, C1‴-H; 3.92, m, 1H, C2‴-H; 4.16, m, 1H, C3‴-H; 4.33, m, 1H, C4‴-H; 4.01, m, 1H, C5‴-H; 4.43, m, 2H, C6‴-H; 6.30, d, *J* = 8.0 Hz, 1H, C1⁗-H; 4.18, m, 1H, C2⁗-H; 3.88, m, 1H, C3⁗-H; 4.13, m, 1H, C4⁗-H; 4.27, m, 1H, C5⁗-H; 4.45, m, 2H, C6⁗-H.

^{13}C-NMR (125 MHz, C_5D_5N): δ (C 1) 38.82; (C-2) 26.40; (C-3) 88.07; (C-4) 46.92; (C-5) 55.79; (C-6) 18.43; (C-7) 33.30; (C-8) 39.8; (C-9) 47.86; (C-10) 39.49; (C-11) 23.32; (C-12) 122.80; (C-13) 144.00; (C-14) 42.05; (C-15) 28.16; (C-16) 23.71; (C-17) 49.97; (C-18) 41.53; (C-19) 45.57; (C-20) 30.68; (C-21) 38.82; (C-22) 33.80; (C-23) 16.95; (C-24) 27.90; (C-25) 15.58; (C-26) 17.39; (C-27) 25.88; (C-28) 176.40; (C-29) 32.93; (C-30) 23.57; (C-1′) 104.48; (C-2′) 74.74; (C-3′) 81.78; (C-4′) 67.91; (C-5′) 64.55; (C-1″) 101.72; (C-2″) 72.28; (C-3″) 72.44; (C-4′) 73.82; (C-5″) 69.96; (C-6″) 18.38; (C-1‴) 104.39; (C-2‴) 74.86; (C-3‴) 78.11; (C-4‴) 70.91; (C-5‴) 79.21; (C-6‴) 62.13; (C-1⁗) 95.86; (C-2⁗) 74.04; (C-3⁗) 78.46; (C-4⁗) 71.34; (C-5⁗) 78.80; (C-6″) 62.45.

FAB mass spectrum (negative mode): *m*/*z*, 1057 $[M-H]^-$, 895 $[M-H\text{-}162]^-$, 749 $[M-H\text{-}162\text{-}146]^-$, 733 (small) $[M-H\text{-}162\text{-}162]^-$ 587 $[M-H\text{-}162\text{-}146\text{-}162]^-$, 455 $[M-H\text{-}162\text{-}146\text{-}162\text{-}132]^-$or $[aglycon-H]^-$.

UV (MeOH) λ_{max}: 202 nm; IR ν_{max} (KBr): 3450 (OH), 2910 (C–H) 1740 (C=O), 1620 (C=C) and 1100–1000 cm^{-1} (C–O–C).

3.4. *Acid hydrolysis of 1*

About 10 mg of compound **1** was refluxed with dioxane : water : hydrochloric acid (1 : 1 : 1.5) for 3 h. The reaction mixture was concentrated under reduced pressure to remove methanol. Water was added. The residue of aglycon was filtered and washed with water. The aglycon, oleanolic acid, was crystallised and compared with an

authentic sample, m.p. 302–304°C. It was treated with diazomethane to obtain a methyl ester, m.p. 196–198°C. The aqueous layer was evaporated under reduced pressure. The sugars were identified as arabinose, rhamnose and glucose by comparison with authentic samples on cellulose TLC. The TLC solvent system consisted of ethyl acetate : water : methanol : acetic acid (65 : 15 : 15 : 20). Spots were detected by spraying it with aniline phthalate.

3.5. *Alkaline hydrolysis of 1*

About 10 mg of **1** was dissolved in 5 mL methanol and 5 mL solution of 20% sodium hydroxide was added. The whole mixture was refluxed for 2 h on water bath and then neutralised with Dowex 50. It was extracted with *n*-butanol. The aqueous layer was concentrated under reduced pressure and the residue was identified as glucose by comparing it with an authentic sample on cellulose TLC plate, developed in ethyl acetate : water : methanol : acetic acid (65 : 15 : 15 : 20). Spots were detected by spraying the TLC plate with aniline phthalate. The *n*-butanol extract was also concentrated under reduced pressure. The FAB negative ion mass spectrum of the prosapogenin of **1** indicated ion peaks at *m*/*z* 895, 749, 733, 587 and 455, indicating the loss of only one glucose moiety.

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