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A SAPONIN FROM THE STEM BARK OF *GUAIACUM OFFICINALE*

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Key Word Index—*Guaiacum officinale*; Zygophyllaceae; nortriterpenoid saponin 3-*O*-[α-L-arabinopyranosyl]-30-norolean-12,20(29)-dien-28-oic acid.

Abstract—A new nortriterpenoid saponin isolated from the stem bark of *Guaiacum officinale* was characterized as the 3-*O*-[α-L-arabinopyranosyl]-30-norolean-12,20(29)-dien-28-oic acid. Sitosterol-D-glucoside was also isolated.

INTRODUCTION

Guaiacum officinale is cultivated as an ornamental plant in many countries including Pakistan. Its wood is used as diuretic, diaphoretic, sudorific and it increases saliva secretion [1]. The bark of *Guaiacum officinale* has been reported to contain saponins. On acid hydrolysis, these saponins release a number of triterpenoid sapogenins. In previous communications, we described the isolation and structure of sapogenins namely 3β-hydroxy-30-norolean-12,19-dien-28-oic acid (**2**), its methyl ester (**3**), 3β-20ζ-dihydroxy-30-norolean-12-en-28-oic acid (**4**) [2], 3β-hydroxy-20ζ-methoxy-30-norolean-12-en-28-oic acid (**5**) [3] and officigenin [4]. Beside these new sapogenins larreagenin, oleanolic acid and sitosterol were also isolated [2].

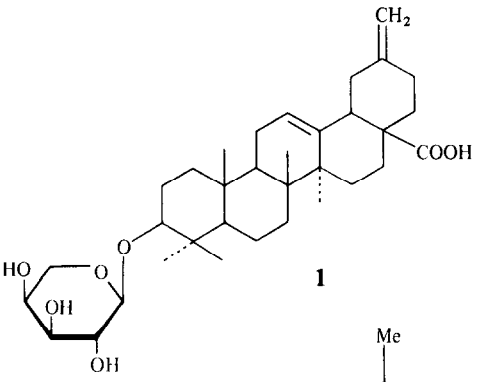
In this paper we report the structure of a new saponin together with sitosterol-D-glucoside isolated from the methanolic extract of *G. officinale*.

RESULTS AND DISCUSSION

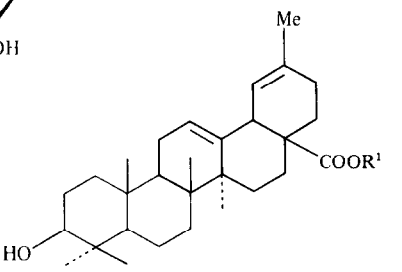
The crude saponin mixture obtained from the methanolic extract of the stem bark of *Guaiacum officinale* was chromatographed on a silica gel column. The fractions obtained from a chloroform–methanol (4:1) eluate contained a mixture of compound **2** and sitosterol-D-glucoside. Repeated flash chromatography using silica gel 60 (PF_{254}) furnished compound **1** and sitosterol-D-glucoside in pure state.

Compound **1** was crystallized from methanol as a white microcrystalline powder, mp 276–279°. Its IR spectrum showed the presence of hydroxyl (3645 cm^{-1}) and carboxyl (1700 cm^{-1}) groups. On acid hydrolysis compound **1** furnished a mixture of two nortriterpenoids, identified by co-TLC and spectroscopic studies as 3β-hydroxy-30-norolean-12,19-dien-28-oic acid (**2**) and its methyl ester (**3**). The sugar residue was identified as arabinose. On treatment with a solution of diazomethane, this mixture yielded pure compound **3**. Its mass spectrum showed a molecular ion peak at *m/z* 454 corresponding to the formula $C_{30}H_{46}O_3$. We have previously reported [2] that **2** and **3** were artifactual genins formed on acid hydrolysis and its genuine sapogenin was considered to be 3β,20ζ-dihydroxy-30-norolean-12-en-28-oic acid (**4**).

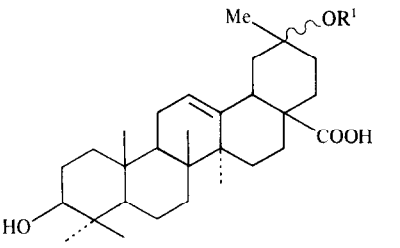
A comparison of the ^{13}C NMR spectrum of compound



1



2 R^1 = H
3 R^1 = Me



4 R^1 = H
5 R^1 = Me

1 with that of 3β,20ζ-dihydroxy-30-norolean-12-en-28-oic acid (4) revealed that signals due to the carbons in ring E of the aglycone moiety appeared at different positions. The ^{13}C NMR spectrum of compound 1 showed the presence of two olefinic bonds. One bond is present between C-12 (δ122.56) and C-13 (144.42). The other two olefinic carbon signals appeared at 149.17 (quarternary carbon) and 107.03 (CH_2) indicating the presence of a $>C=CH_2$ moiety. It is therefore concluded that the other double bond is present between C-20 and C-29. The ^{1}H NMR spectrum of compound 1 in pyridine-d_5 also showed the presence of only five methyl signals at δ0.84, 0.86, 0.94 (6H), 0.97 and a multiplet centred at 4.77 (overlapped with the doublet of the anomeric proton) due to H-29. The presence of only five methyls in the ^{1}H NMR spectrum also confirmed that the other double bond was present between C-20 and C-29. Thus the genuine aglycone of the saponin was 3β-hydroxy-30-norolean-12,20(29)-dien-28-oic acid which has not been isolated previously. The compounds 2, 3, 4 and 5 were artifacts which were formed from compound 1 during acid hydrolysis. Apparently the C-20 (29) double bond is protonated yielding an ion with a positive charge on C-20. This can react with water or methanol or eliminate a proton from C-19 producing the sapogenins 4, 5 and 2 respectively. The ^{13}C NMR spectrum of 1 showed the presence of only one anomeric carbon signal at δ107.39 and other signals due to arabinose were present at δ74.60 (C-3'), 72.93 (C-2'), 69.40 (C-4') and 66.59 (C-5'). These five chemical shifts were identical to the chemical shifts reported for an α-L-arabinopyranosyl moiety [5]. The configuration of the anomeric position of the L-arabinose in compound 1 was α because the C-5 signal of the sugar appeared at relatively low field (66.59) in the ^{13}C NMR spectrum in 1, whereas for the β-configuration this signal appeared at relatively high field (62.3) [5]. The configuration of the sugar molecule was also confirmed by the ^{1}H NMR spectrum of 1 which showed a doublet due to the anomeric proton at δ4.78 (J = 5.79 Hz). The coupling constant was consistent with an α-linkage of the sugar moiety in compound 1. In the ^{13}C NMR spectrum, the C-3 signal of the aglycone appeared at δ88.75 which showed that the arabinose was attached to C-3 of the aglycone. All of the spectroscopic data cited above proved the structure of the saponin to be 3-*O*-[α-L-arabinopyranosyl)-30-norolean-12,20(29)-dien-28-oic acid.

Sitosterol-D-glucoside was purified by repeated flash column chromatography and crystallized from methanol as a white microcrystalline powder, mp 278°. It was identified through direct comparison with an authentic sample as well as on the basis of its ^{1}H NMR and ^{13}C NMR spectra [6].

EXPERIMENTAL

Mps were determined on a Gallenkamp apparatus and are uncorr. ^{1}H NMR (300 MHz) and ^{13}C NMR (75.43 MHz) spectra were recorded on a Bruker AM-300 spectrometer in C_5D_5N. Analytical TLC was carried out on silica gel plates using the following solvent systems, (a) $CHCl_3$–MeOH–H_2O (90:10:1), (b) EtOAc–H_2O–MeOH–AcOH (65:15:15:20), (c) C_6H_6–EtOAc (70:30).

Plant material. The stem bark of *Guaiacum officinale* (20 kg) was collected on the campus of University of Karachi. The powdered stem bark was extracted ×3 with MeOH. The combined MeOH extracts were evaporated at red. pres. to afford a gummy residue (*ca* 600 mg). 100 g of this MeOH extract was chromatographed on a silica gel column using a gradient of MeOH in $CHCl_3$ and finally pure MeOH as eluent. The fractions obtained from $CHCl_3$–MeOH (4:1) was the mixture of compound 1 and sitosterol glucoside. This mixture of glycosides were subjected to repeated flash column chromatography using silica gel 60 (PF_{254}) with a gradient of MeOH (10–15%) in $CHCl_3$ as eluent to yield 3-*O*-(α-L-arabinopyranosyl)-30-norolean-12,20(29)-dien-28-oic acid and sitosterol-D-glucoside.

3-*O*-[α-L-*Arabinopyranosyl*]-30-*norolean*-12,20(29)-*dien*-28-*oic acid* (1). IR ν_{max}^{KBr} cm^{-1}: 3645 (OH), 1700 (COOH). ^{1}H NMR (C_5D_5N, 300 MHz): δ0.84 (*s*, Me), 0.86 (*s*, Me), 0.94 (*s*, 6H, 2 × Me), 0.97 (*s*, Me) 3.22 (*dd*, J = 4.59, 13.98 Hz, H-18), 3.45 (*m*, H-3), 4.10–4.40 (sugar protons), 4.77 (multiplet overlapped with *d* of anomeric signal, 2 × H-29), 4.78 (*d*, J = 5.79 Hz, H-1'), 5.46 (*t*, H-12). ^{13}C NMR (C_5D_5N, 75.43 MHz): δ39.58 (C-1), 26.63 (C-2), 88.75 (C-3), 38.41 (C-4), 55.96 (C-5), 18.54 (C-6), 33.24 (C-7), 39.84 (C-8), 48.06 (C-9), 37.09 (C-10), 23.82 (C-11), 122.56 (C-12), 144.42 (C-13), 42.05 (C-14), 28.36 (C-15), 42.03 (C-16), 47.09 (C-17), 47.97 (C-18), 38.86 (C-19), 149.17 (C-20), 31.00 (C-21), 29.99 (C-22), 28.30 (C-23), 16.92 (C-24), 15.50 (C-25), 17.40 (C-26), 26.17 (C-27), C-28 signal not obs., 107.03 (C-29), 107.39 (C-1'), 72.93 (C-2'), 74.60 (C-3'), 69.40 (C-4'), 66.69 (C-5'), MS *m/z*: 440 [M − arabinose + H]$^+$, 232 (RDA fr. a), 133.

Acid hydrolysis of compound 1. Saponin 1 was refluxed with methanolic HCl (9 ml MeOH, 1 ml H_2O, 1.5 ml HCl) for 3 hr. The MeOH was evaporated from this reaction mixture and H_2O was added which furnished a white ppt. This was filtered and washed with H_2O and gave two spots on TLC in solvent system (c) which were identical to 3β-hydroxy-30-norolean-12,20(29)-dien-28-oic acid (2) and its methyl ester (3). This mixture of aglycones was treated with CH_2N_2 at room temp. for 10 min and worked up in the usual manner. After methylation 2 was converted into 3. On TLC this aglycone gave only one spot (3). MS *m/z*: 454, and the same fragmentation pattern reported for compound 3 [2].

Identification of sugar. The aq. layer was evaporated at red. pres. and the residue obtained was compared with standard sugars on TLC (silica gel with solvent system b). Spots were detected by spraying with aniline phthalate which showed that the sugar was arabinose in saponin 1.

Sitosterol-D-glucoside. EIMS *m/z*: 396 [M − glucose]$^+$. It was identical (TLC, ^{1}H NMR, ^{13}C NMR) with an authenitc sample of sitosterol-D-glucoside.

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