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GUAIANIN N, A NEW SAPONIN FROM FLOWERS OF *GUAIACUM OFFICINALE*

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A new triterpenoidal saponin, guaianin N, (1) has been isolated from the butanolic extract of the flowers of *Guaiacum officinale*. It showed antibacterial activity against *Pseudomonas pseudomaliae* as well as brine shrimp toxicity [1]. Spectroscopic methods have been used to characterize compound 1 as 3-O-[β-D- glucopyranosyl (→3)α-L-arabinopyranosyl]-oleanolic acid.

Key words: Guaianin N, Saponin, *Guaiacum officinale*, Zygophyllaceae.

Introduction

The flower extract of *Guaiacum officinale* L. has been subjected to intensive investigation. The butanolic extract has shown significant activity against both gram positive and gram negative bacteria as well as toxicity against shrimps [1].

Guaiacum officinale L. (Zygophyllaceae) is both a medicinal as well as an ornamental plant and has been introduced into Pakistan from Africa and West Indies [2]. Both its resin and wood possess medicinal and pharmacological applications [3].

A number of saponins have already been reported from bark and leaves of *Guaiacum officinale*. The new oleanolic acid saponin guaianin N, 1, isolated from the flowers of *Guaiacum officinale* L. resembles Akebia saponin P_E [4] and Scabioside B isolated from *Patrinia scabiofolia* [5] with the difference in the linkages between the two sugars. The former has (1→ 2) and the latter has (1→ 4) linkage between the two sugars glucose and arabinose while 1 has (1→ 3) linkage between them.

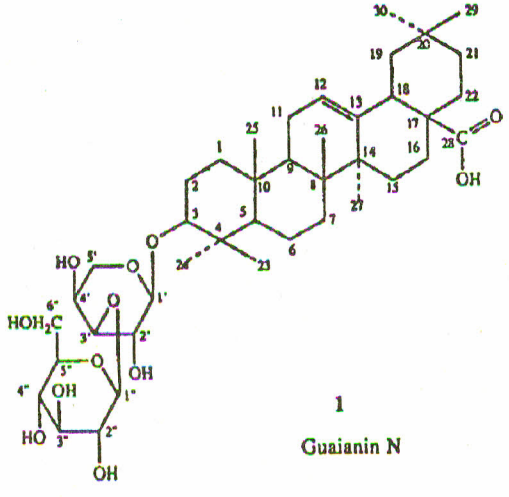
Experimental

Melting point was recorded on Gallenkamp microscope. The ^{1}H- NMR and ^{13}C-NMR spectra were recorded in CD_3OD on Bruker Aspect AM-3000 spectrometer at 300 MHz and 75.43 MHz respectively. Mass spectrum was recorded on JEOL, JMS, HX-110 connected with DA 5000 (Data system LS1 11/73 (CPU) DEC. E. Merck. Kiesel gel 60 GF_{254} Art. 5719 glass plates were used for TLC analysis. Shimadzu liquid chromatography 6A HPLC was used for final purification.

Collection. The flowers of *Guaiacum officinale* L. were collected from the premises of Karachi University.

Extraction and isolation. 5 kilogram (wet wt.) of the flowers were crushed in methanol by Ultraturex homogenizer (Janke & Kunkel Co., Stanfen, Germany) and were extracted. The methanolic extract was evaporated under reduced pressure. The extractive was dissolved in water and then shaken with chloroform to remove terpenes and chlorophyll etc. The remaining water soluble extract was shaken with butanol and the butanolic extract was also evaporated under reduced pressure and subjected to column chromatography on silica gel. The column was eluted with chloroform and with chloroform : methanol with increasing polarity. The fractions isolated with 7% methanol were subjected to repeated silica gel column chromatography and flash chromatography and finally guaianin N was isolated via HPLC using semi-preparative RP-18 column and differential refractometer detector. The solvent system used was 20% water in methanol; m.p.: 255-265° (decomposed); [α]D : + 140 (c=0.27, MeOH); ^{1}H-NMR : δ0.84 (s, CH_3), 0.84 (s, CH_3), 0.90 (s, CH_3), 0.94 (s, CH_3), 0.94 (s, CH_3), 1.05(s, CH_3) and 1.09 (s, CH_3) indicatidng seven tertiary methyl groups. The anomeric signals at δ4.28 (d,$J_{1,2}$=7.1 Hz, H-1') and 4.50 (d,$J_{1,2}$=7.5 Hz, H-1") show 1,2-diaxial relationship which is consistent for α-L-arabinopyranosyl and β-D-glucopyranosyl respectively and a distorted triplet at δ5.23 (t, H-12) indicates endocyclic double bond. ^{13}C-NMR data of a glycone moiety is given in Table 1 while that of the two sugar moieties β-D-glucopyranosyl-(1→3)-α-L-arabinopyranosyl is given in Table 2 FAB-MS (negative mode high field) *m/z* 749 $(M-H)^-$, $C_{41}H_{78}O_{12}$, 587 $(M\text{-glucose-H})^-$, 455 $(M\text{-glucose-arabinose-H})^-$.

Acid hydrolysis. 5 mg of compound 1 was refluxed with methanolic HCl (9ml MeOH, 1ml H_2O and 1.5 ml HCl) for 3 hrs. The reaction mixture was concentrated under reduced



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Guaianin N

pressure to remove methanol. It was then diluted with water, extracted with ethyl acetate. The aqueous layer was concentrated at reduced pressurre and the residue obtained was compared with standard sugar on silica gel TLC. The solvent system for TLC was *n*-butanol : ethyl acetate : 2-propyl alcohol : acetic acid in 8.75 : 25 : 15 : 8.75 : 7.5 ratio. TLC card 20 cm long was run twice. Sugars spots were detected by spraying sugar reagent (orcinol + H_2SO_4 + $FeCl_3$). It also showed that sugars in compound **1** were arabinose and glucose.

Results and Discussion

The ^{1}H-NMR (C_5D_5N) exhibited signals at δ0.83 (s, 3 x H-25), 0.95 (s, 3 x H-29), 0.96 (s, 3 x H-24), 0.98 (s, 3 x H-26), 1.00 (s, 3 x H-30), 1.29 (s, 3 x H-23) and 1.30 (s, 3 x H-27) indicated 7 methyl group while the ^{1}H-NMR (CD_3OD) exhibited signals at δ0.84 (s, CH_3), 0.84 (s, CH_3), 0.90 (s, CH_3), 0.94 (s, (H_3, 0.94, (s, CH_3), 1.05 (s, CH_3) and 1.09 (s, CH_3) due to 7 tertiary methyl groups. The anomeric signals at δ4.28 (d, $J_{1,2}$=7.1 Hz, H-1') and 4.50 (d, $J_{1,2}$=7.5 Hz, H-1") show diaxial relationship which is consistent for α-L-arabinopyranosyl and β- D-glucopyranosyl respectively and a distorted triplet appeared at δ5.23 (t, H-12) due to endocyclic double bond.

TABLE 1. ^{13}C-NMR CHEMICAL SHIFTS OF AGLYCONE MOIETY OF COMPOUND 1 IN CD_3OD.

Carbon	Shift	Carbon	Shift	Carbon	Shift
1	39.6	11	24.5	21	35.0
2	27.0	12	123.5	22	34.0
3	90.5	13	145.37	23	28.5
4	40.0	14	42.6	24	17.0
5	57.1	15	28.9	25	15.9
6	19.3	16	24.93	26	17.8
7	33.9	17	47.6	27	26.4
8	37.5	18	39.6	28	180.8
9	48.7	19	47.39	29	33.5
10	38.1	20	-	30	24.0

TABLE 2. ^{13}C-NMR CHEMICAL SHIFT OF α-L-ARABINOPYRANOSYL AND β-D- GLUCOPYRANOSYL.

	(1"—2') in C_5D_5N		(1"—3') in MeOH	(1"—4') in C_5D_5N
Carbon	Chemical shift	Chemical shift	Chemical shift	Chemical shift
α-L-Arabinopyranosyl				
1'	104.7	107.0	106.7	106.8
2'	80.6	72.1	73.6	72.8
3'	72.5	83.6	74.6	74.5
4'	68.1	69.5	79.9	69.0
5'	64.7	66.6	66.2	66.1
β-D-Glucopyranosyl				
1"	105.7	106.4	106.3	
2"	75.1	75.7	75.7	
3"	78.3	78.3	78.6	
4"	71.5	71.6	71.4	
5"	78.3	78.8	78.3	
6"	62.5	62.8	62.6	

The ^{13}C-NMR spectrum of aglycone moiety is given in Table 1 and Table 2 contains a list of ^{13}C-NMR signals of β-D- glucopyranosyl and α-L-arabinopyranosyl having (1"→2'), (1"→3') and (1"→4') linkages and has also ^{13}C-NMR signals of only α-L- arabinopyranosyl. It is obvious from the chart of Table 2 that when C-1 of glucose is linked with any of the carbon of arabinose then ^{13}C-NMR signal of that carbon of arabinose shifts downfield about 8-10 ppm.

When there is (1→2) linkage as in Akebia saponin P_E then C- 2' signal is shifted from 72.8 - 80.6 ppm, a 7.8 ppm downfield shift, while Scabioside B isolated from *Patrinia scabiofolia* [5] and its ^{13}C-NMR signals [6] have [1→4] linkages and the signal of C-4' is shifted from 69.0 - 79.7 ppm, a 10.7 ppm downfield shift. Like this in guaianin N, ^{13}C-NMR signal of C-3' is shifted from 74.4 - 83.6 ppm, a 9.2 ppm downfield shift which indicates that C-1" β-D-glucopyranosyl is attached with C-3' of α-L- arabinopyranosyl.

There are 7 methyl peaks in aglycone region of ^{13}C-NMR ranging from δ15.9 - 33.5 ppm and a peak of carboxylic acid carbon (C-28) is found at 180.8 ppm. Unsaturated C-12 and C-13 carbons gave signals at 123.5 and 145.37 ppm respectively. A comparison of ^{13}C-NMR spectrum of **1** with that of Akebia saponin P_E [4] showed that the chemical shifts due to aglycon moieties in both the saponins are almost the same so the ^{13}C-NMR spectrum of the aglycon of compound **1** was established to be oleanolic acid.

The sequence of sugars has been established through FAB mass spectrum (negative mode) which exhibits molecular ion peak at *m/z* 749 $(M-H)^-$ corresponds to the formula $C_{41}H_{66}O_{12}$ of compound **1**. The prominent fragment ion peak at *m/z* 587 results from the expected cleavage of glucose moiety i.e.$[M\text{-glucose-H}]^-$ corresponds to the formula $C_{35}H_{55}O_7$, and the next prominent ion peak at *m/z* 455 showing cleavage of arabinose i.e. (M-glucose- arabinose-H)$^-$ and also corresponds to the formula of aglycon moiety $C_{30}H_{47}O_3$.

From all these ^{13}C-NMR, ^{1}H-NMR and FAB negative mass spectroscopic evidences mentioned above, the structure of guaianin N was determined as 3-O-(β-D-glucopyranosyl-(1→3)-α-L- arabinopyranosyl)-oleanolic acid. Compound **1** was found to be active against gram negative *Pseudomonas pseudomaliae*.

Brine shrimp toxicity test [1] was also performed. Its LC_{50} was found to be 621.1715 mg/ml.

References

1. J.T. Arnason, B.J.R. Philogene, P. Morand, *Insecticides of Plant Origin* (1989), Chap. 3, pp. 25.
2. A. Ghafoor, E. Nasir and S.I. Ali, *Flora of West Pakistan*

(Department of Botany, University of Karachi, Karachi-75270, Pakistan, No. 76, Dec. 1974), pp. 2.
3. H.A. Hoppe, *Drogenkunde, Angiosperm* (Walter De Gruyter, Berlin, 1975), Vol. I, 8th ed., pp. 551.
4. R. Ryuichi, K. Toshio, Chem. Pharm. Bull., **24**, 1021 (1976).
5. V.G. Bukherov, V.V. Karlin and T.N. Sidorowich, Khim. Prir. Soedin, **6**(1), 69 (1970).
6. X.C. Li, D.Z. Wangi, S.G. Wu and C.R. Yang, Phytochemistry, **29**(2), 595 (1990).