Journal of Natural Products
Vol. 49, No. 5, pp. 784-786, Sep-Oct 1986

GUAIANIN, A NEW SAPONIN FROM *GUAIACUM OFFICINALE*

VIQAR UDDIN AHMAD, NASREEN BANO, SHAHEEN BANO, AYJAZ FATIMA,

H.E.J. Research Institute of Chemistry University of Karachi, Karachi-32, Pakistan

and LENART KENNE

Department of Organic Chemistry, Arrhenius Laboratory, University of Stockholm, S-106 91 Stockholm, Sweden

ABSTRACT.—From the stem bark of *Guaiacum officinale* a new nortriterpenoid saponin named guaianin has been isolated. Its structure was elucidated on the basis of chemical and spectral evidence as 3-*O*-[α-L-rhamnopyranosyl (1↦3)-β-D-glucopyranosyl (1↦3)-α-L-arabinopyranosyl]-30-norolean-12,20(29)-dien-28-oic acid (**1**).

Recently, we reported (1) the isolation of a new nortriterpenoid saponin, 3-*O*-[-L-arabinopyranosyl]-30-norolean-12,20(29)-dien-28-oic acid from the stem bark of *Guaiacum officinale* L. (Zygophyllaceae). In this paper we describe the isolation and structure determination of another nortriterpenoid saponin, guaianin, from the same source.

Guaianin (**1**) was eluted with $CHCl_3$-MeOH (75:25) from the silica gel column as described earlier (1) and purified by repeated silica gel column chromatography. On crystallization from MeOH it was obtained as a microcrystalline dextrorotatory powder. The ir spectrum showed carboxylic absorption (1700 cm^{-1}) together with strong hydroxyl (3440 cm^{-1}) absorption bands.

Acid hydrolysis of **1** with methanolic HCl furnished a mixture of five nortriterpenoids. The compounds were identical in tlc to previously obtained (2,3) samples 3β-hydroxy-30-norolean-12,19-dien-28-oic acid, its methyl ester, larreagenin, 3β-hydroxy-20ξ-methoxy-30-norolean-12-en-28-oic acid, and 3β,20ξ-dihydroxy-30-norolean-12-en-28-oic acid. These compounds were all artifact sapogenins.

The ^{13}C-nmr spectrum of **1** showed the presence of four olefinic carbons in its aglycone at 122.99 (CH), 144.26 (quarternary), 149.23 (quarternary), and 107.00 ppm (CH_2). The former two carbons were assigned to C-12 and C-13, respectively, while the latter two olefinic carbons indicated the presence of $C{=}CH_2$ moiety in the compound. It is, therefore, concluded that one of the double bonds present in the aglycone is at C-20(29). From the ^{13}C-nmr spectrum it appeared that the genuine aglycone of the saponin is 3β-hydroxy-30-norolean-12, 20(29)-dien-28-oic acid. The genuine sapogenin has not been isolated so far.

The ^{13}C-nmr spectrum indicated the presence of three sugar residues in **1** as signals from three anomeric carbons were obtained. The sugars released by acidic hydrolysis were analysed by glc-ms as their alditol acetates, and their absolute configuration determined by glc after reaction with (+)-2-butanol and trimethylsilylation (4). Equimolar amounts of L-rhamnose, L-arabinose, and D-glucose were obtained in the analysis.

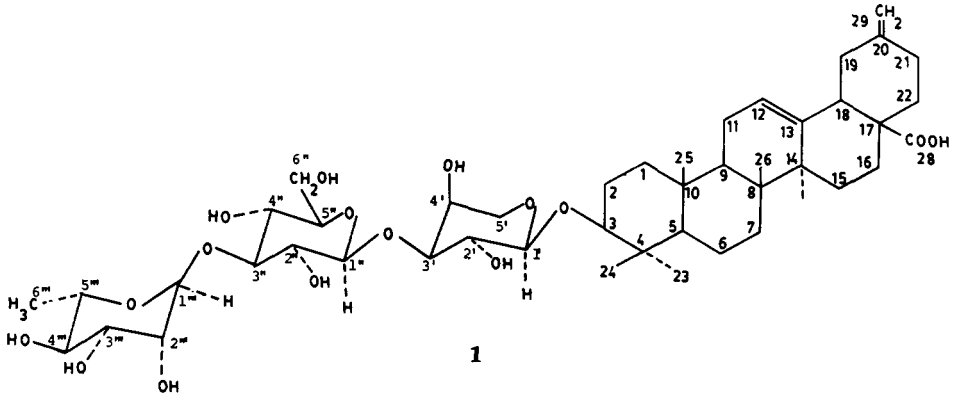
The sites of sugar linkages were determined by methylation analysis. The samples were methylated according to the Hakomori procedure (5), hydrolyzed by acid, and the released partially methylated monosaccharides analyzed as their alditol acetates by glc-ms (6) showing the presence of 2,3,4-tri-*O*-methyl-L-rhamnose, 2,4,-di-*O*- methyl-L-arabinose, and 2,4,6-tri-*O*-methyl-D-glucose in equimolar amounts. This analysis showed that guaianin contained a terminal L-rhamnopyranosyl group, a 3-linked L-arabinopyranosyl, and a 3-linked D-glucopyranosyl residue. As only one terminal sugar was obtained, it was concluded that sugars were present as a linear oligosaccharide linked to C-3 hydroxyl group of the aglycone.

In order to determine the sugar sequence, a Smith degradation (7) of **1** was per-

formed. This includes oxidation of vicinal hydroxyls with periodate, subsequent reduction of the formed aldehyde groups, and mild acid hydrolysis to cleave only the modified terminal L-rhamnopyranosyl linkage. Methylation followed by hydrolysis of the product obtained after the cleavage of terminal rhamnose showed the presence of 2,3,4,6-tetra-*O*-methyl-D-glucose and 2,4-di-*O*-methyl-L-arabinose. These results indicated that L-arabinose is directly linked with the aglycone, and glucose is the terminal sugar in the Smith degradation product.

The anomeric configuration of the sugar be determined from the nmr spectra. The ^{13}C-nmr spectrum showed signals for the anomeric carbon at 107.40, 106.06, and 102.86 ppm. The signals of the anomeric protons in the ^{1}H-nmr spectrum were present at δ 5.23 ($J_{1,2}$=1.4 Hz), 4.61 ($J_{1,2}$=7.7 Hz), and 4.33 ($J_{1,2}$=7.7 Hz). These results showed that two sugars have a 1,2-diequatorial, and the third has a 1,2-diaxial relationship of the hydroxyl groups in the 1 and 2 positions. This means that the H-1′ and H-2′ as well as H-1″ and H-2″ are diaxially oriented (J=7.7 Hz), whereas H-1 and H-2 have a diequitorial relationship (J=1.4 Hz). This is only consistent with the -L-arabinopyranosyl, -D-glucopyranosyl, and -L-rhamnopyranosyl configurations.

From the above results the structure of guaianin (**1**) was concluded to be 3-*O*-[L-rhamnopyranosyl (1↦3)-β-D-glucopyranosyl (1↦3)-α-L-arabinopyranosyl)-30-norolean-12,20(29)-dien-28-oic acid.



1

EXPERIMENTAL

GENERAL EXPERIMENTAL PROCEDURES.—The isolaton of saponins and chromatography of the saponin mixture have been described in an earlier paper (1). The ^{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. Assignments were made using the DEPT technique. For glc a Hewlett-Packard 5830 A instrument fitted with a flame ionization detector was used. Separation of alditol acetates and partially methylated alditol acetates was performed on an SE-54 fused silica capillary column, either isothermally or using a temperature gradient of 150°-220° at 2°/min; glc/ms was performed on a Varian MAT-311A instrument using the same conditions. Identification of mass spectra was unambiguous (6) and is not discussed.

PLANT MATERIAL.—The stem bark of *G. officinale* was collected from trees growing on the Karachi University campus. A voucher specimen (No. 33 KUH) has been deposited in the herbarium of the Botany Department, University of Karachi.

GUAIANIN (**1**).—Compound **1** eluted from the silica gel column with $CHCl_3$-MeOH (75:25). It was further purified by repeated (three times) column chromatography on silica gel 60 (230-400 mesh, Merck); ir λmax (KBr) 3440 (OH), 1700 (COOH) cm^{-1}; ^{13}C-nmr (C_5D_5N): C-1) 38.43[a] 2) 26.69 3) 88.80 4) 39.64[b] 5) 55.96 6) 18.51 7) 33.26 8) 39.84[b] 9) 48.00[c] 10) 37.09 11) 23.82[d] 12) 122.99 13) 144.26 14) 42.20 15) 28.38 16) 23.82[d] 17) 47.11 18) 48.07[c] 19) 42.05 20) 149.23 21) 30.46 22) 38.87[a] 23) 28.22 24) 16.95[e] 25) 15.50[e] 26) 17.41 27)26.18 28) not observed 29) 107.00 1′) 107.40 2′) 72.63 3′) 83.60 4′) 69.22[f] 5′) 66.95 1″) 106.06 2″) 76.00 3″) 84.06 4″) 69.90[f] 5″) 78.55 6″) 62.48 1‴) 102.86 2‴) 72.73[g] 3‴) 71.91[g] 4‴) 74.18 5‴) 69.71[f] 6‴) 18.60.

[1]a,b,c,d,e,f,g values may be interchanged.

ACID HYDROLYSIS OF GUAIANIN.—Compound **1** (15 mg) was refluxed with methanolic HCl (18 ml MeOH, 2 ml H_2O, 3 ml HCl) for 2 h. The reaction mixture was extracted with EtOAc to obtain the corresponding aglycones. The EtOAc layer furnished a mixture of five compounds as shown by tlc on silica gel $CHCl_3$-MeOH (9:1).

SUGAR ANALYSIS.—Compound **1** (1 mg) in 0.04 ml dioxane was treated with aqueous 0.5 M trifluoroacetic acid (0.8 ml) at 100° for 6 h. The reaction mixture was concentrated to dryness by a stream of air, reduced by $NaBH_4$ (5 mg) in H_2O (1 ml) for 2 h, and, then, neutralized with HOAc. The solvent was evaporated, and the H_3BO_3 was removed as the methyl ester by repeated addition of 10% HOAc in MeOH (2×1 ml) and evaporation to dryness. The alditols were acetylated by treatment with Ac_2O and pyridine (1:1,0.6 ml) at 100° for 30 min, concentrated by a stream of air, and partitioned between $CHCl_3$ and H_2O. The organic phase containing the alditol acetates was analysed by glc/ms.

Compound **1** (1 mg) was treated with 1M (+)-2-butanolic HCl (0.5 ml) in a sealed tube at 80° for 8 h. The mixture was cooled and neutralized with Ag_2CO_3, filtered and dried in vacuum. The product was silylated using 0.1 ml hexamethyl disilazane-chlorotrimethylsilane-pyridine (1:1:5) at 25° for 30 min. The sample was analysed by glc and comparison made to reference compounds.

METHYLATION ANALYSIS.—Compound (3 mg) **1**, dissolved in dry DMSO (0.5 ml), was treated with 2 M methylsulfinyl anion in methyl sulfoxide (0.5 ml) in an ultrasonic bath for 20 min, cooled in an ice-bath, and methyl iodide (0.5 ml) was added. After agitation in the ultrasonic bath for 30 min, the methyl iodide was evaporated, and the product was recovered by reversed phase chromatography on a Sep Pak C_{18} cartridge (8). The sample was diluted with an equal volume of H_2O and applied to the cartridge. This was washed with H_2O (10 ml) to remove the methyl sulfoxide, and the methylated saponin was then eluted with MeOH (6 ml). The product was hydrolysed with 2 M trifluoroacetic acid (1 ml) at 125° for 2 h, and the partially methylated sugars were converted to alditol acetates as described but using sodium borodeuteride as reducing agent.

SMITH DEGRADATION OF SAPONIN.—A solution of **1** (10 mg) in a mixture of 0.1 M aqueous sodium metaperiodate (0.5 ml) and MeOH (2.5 ml) was kept in the dark at 4° for 48 h. Excess periodate was destroyed with ethylene glycol (1 drop), and after 2 h the modified saponin was reduced with $NaBH_4$ (20 mg) for 3 h. The reaction mixture was neutralized with HOAc and concentrated to dryness. Boric acid was removed by codistillation with 10% HOAc in MeOH (2×3 ml). The product was isolated by chromatography on a Sep-Pak C_{18} column first washed with H_2O to remove salts and polar compounds, and the saponin was then eluted with MeOH. The product was treated with 0.25 M methanolic HCl (1 ml) at 23° for 6 h, solvents evaporated, and the product isolated by chromatography on a Sep-Pak C_{18} column as described. The Smith degraded saponin was analysed by methylation analysis as described above.

ACKNOWLEDGMENTS

The authors thank the University Grants Commission of Pakistan for financial support of this work.

LITERATURE CITED

1. V.U. Ahmad, N. Bano, and S. Bano, *Phytochemistry*, **25**, 951 (1986).
2. V.U. Ahmad, N. Bano, and S. Bano, *Phytochemistry*, **23**, 2613 (1984).
3. V.U. Ahmad and N. Bano, *J. Chem. Soc.* (Pakistan), **7**, 137 (1985).
4. G.J. Gerwing, J.P. Kamerling, and J.F.G. Vliegenthart, *Carbohydr. Res.*, **77**, 1 (1979).
5. S. Hakomori, *J. Biochem. (Tokyo)*, **55**, 205 (1964).
6. P.E. Jansson, L. Kenne, B. Lindberg, H. Liedgren, and J. Lonngren, *Chem. Commun. Univ. Stockholm*, **15** (8 Oct.) 1976.
7. I.J. Goldstein, G.W. Hay, B.A. Lewis, and F. Smith, *Methods Carbohydr. Chem.*, **5**, 361 (1965).
8. T.J. Waeghe, A.G. Darvill, M. McNeil, and P. Albersheim, *Carbohydr. Res.*, **123**, 281 (1983).

Received 20 December 1985