Journal of Natural Products
Vol. 48, No. 5, pp. 826-827, Sep-Oct 1985

A NEW PROSAPOGENIN FROM *GUAIACUM OFFICINALE*

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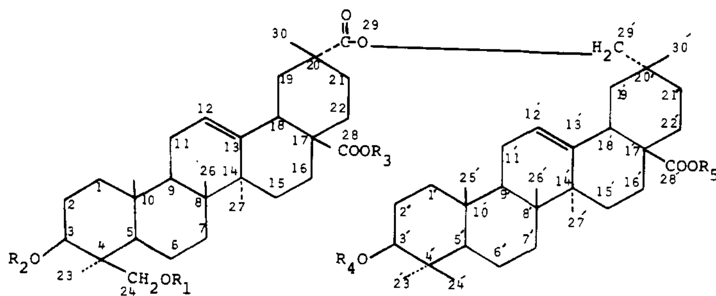
Recently we reported (1) a new sapogenin named officigenin (**1**) from the acid hydrolysate of the saponins from *Guaiacum officinale* L. We have now isolated a new prosapogenin **2** from the same acid hydrolysate. The prosapogenin **2** on further acid hydrolysis furnished officigenin and glucose.

RESULTS AND DISCUSSION

The ir spectrum of **2** exhibited hydroxyl (3420 cm^{-1}) ester (1725 cm^{-1}) and carboxylic (1700 cm^{-1}) absorption bands. Acid hydrolysis of **2** provided an aglycone and a sugar. On the basis of spectroscopic data and mixed tlc, the aglycone was identified as officigenin (**1**) which is an ester of 3β,24-dihydroxy-olean-12-en-28,29-dioic acid and 3β,29-dihydroxyolean-12-en-28-oic acid, previously reported from the same source (1). The sugar was identified as glucose. Treatment of **2** with CH_2N_2 furnished a dimethyl ester **3** which was acetylated with Ac_2O and pyridine to give dimethyl ester hexaacetate **4**. The fabms of **3** showed its highest peak at *m/z* 1169.7 (M^++Na) consistent with the molecular formula $C_{68}H_{106}O_{14}$. The ^{13}C-nmr spectrum of **3** showed a signal that can be attributed to one anomeric carbon. The possible position of the glycosidic linkage is C-3, C-3′, or C-24. The ^1H-nmr spectrum of **4** showed a doublet of doublets due to H-24 at δ 3.36 (J=11 Hz) and at δ 3.60 (the latter doublet overlapped by the signal of the ester group). In the case of officigenin methyl ester acetate (1), this signal appeared as an AB quartet at δ 4.25. This upfield shift of H-24 in **4** clearly indicated that the sugar moiety is attached at C-24. The ^1H-nmr spectrum of **4** showed the anomeric doublet at δ 4.40 with J=8 Hz, which indicated a β-glycosidic linkage for the sugar moiety in compound **2** (2). Comparison of the ^{13}C-nmr spectrum of **3** with officigenin dimethyl ester also confirmed the glycosidic linkage at C-24. The carbon signal due to C-24 appeared at δ 75.73 in **3**, whereas in officigenin dimethyl ester (1), this signal was at 64.40. This downfield shift in **3** is due to the glycosidic linkage.

The spectroscopic evidence described above led us to assign the structure of the prosapogenin as 24-*O*-β-D-glucopyranosyl officigenin.



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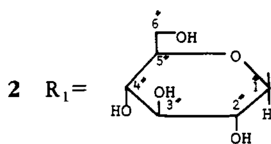
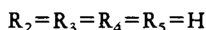
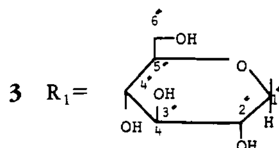
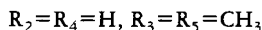
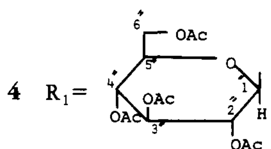
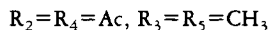
2 R_1= 



3 R_1= 



4 R_1= 



EXPERIMENTAL

GENERAL EXPERIMENTAL PROCEDURES.—^{1}H nmr were recorded on a Bruker AM-300 (300 MHz) and ^{13}C-nmr spectra on a Bruker FT-WP-100 (25.12 MHz). Other equipment used was described in previous communications (3). Tlc was carried out on silica gel plates.

24-*O*-β-D-GLUCOPYRANOSYL OFFICIGENIN.—Elution of the chromatographic column previously described (3) with $CHCl_3$-MeOH (70:30) afforded a mixture of compounds (200 mg) which on repeated flash chromatography using silica gel (60 PF_{254}) with $CHCl_3$-MeOH (90:10) furnished compound **2** (80 mg). It was crystallized from MeOH to yield a white microcrystalline powder, mp 166-169°.

METHYLATION OF **2**.—A solution of **2** (60 mg) in MeOH was treated with CH_2N_2 at room temperature for 30 min. After evaporation of the solvent, the dimethyl ester **3** was crystallized from MeOH as a white microcrystalline powder (58 mg), mp 160-162°; fabms *m/z* 1169.7 (M^++Na), 967, 949, 931, 452, 410, 392; ^{13}C nmr ($CDCl_3$, 25.12 MHz) 79.01 (C-3), 78.92 (C-3′), 75.78 (C-24), 103.71 (C-1″).

ACETYLATION OF **3**.—Compound **3** (45 mg) was treated with Ac_2O and C_5H_5N at room temperature and left overnight. Ice was added to the reaction mixture to yield a white precipitate, which was filtered off, dried, and crystallized from MeOH to give the dimethyl ester hexaacetate as a white powder (**4**) (40 mg), mp 158-159°. ^{1}H nmr ($CDCl_3$, 300 MHz) δ 0.69 (s, 6H, 2× CH_3), 0.82, 0.84, 0.90, 0.91, 0.94, 0.95, 1.09, 1.10, 1.24 (each s, 9×CH_3), 1.97 (s, OAc), 2.00 (s, 6H, 2×OAc), 2.01, 2.03, 2.04 (each s, 3×OAc), 3.36 (d, J=11 Hz, H-24), 3.60 (H-24 overlapped by other signals), 3.61 (s, ester), 3.70 (s, H-29′), 4.40 (d, J=8 Hz, H-1″), 4.5 (m, H-3 and H-3′), 5.28 (t, 2H, H-12 and H-12′); ^{13}C nmr ($CDCl_3$, 25.12 MHz) δ 80.32 (C-3′), 123.10 (C-12), 142.70 (C-13), 75.73 (C-24), 80.82 (C-3′), 122.81 (C-12′), 143.14 (C-13′), 101.05 (C-1″), 71.46 (C-2″), 72.83 (C-3″), 68.74 (C-4″), 71.46 (C-5″), 61.93 (C-6″).

ACID HYDROLYSIS OF **2**.—Compound **2** (20 mg) in methanolic HCl (20 ml MeOH, 2 ml H_2O (2 ml HCl) was refluxed for 3 h. The MeOH was evaporated and H_2O was added, whereby a white precipitate of aglycone (5 mg) was obtained. This aglycone was identical with officigenin by tlc comparison by comparison of its ir, mass, and ^{1}H-nmr spectra. The aqueous layer was evaporated in vacuo, distilled H_2O was added to the residue, and the mixture was evaporated again in vacuo. The residue obtained was compared with standard sugars by tlc on silica gel with EtOAc-H_2O-MeOH-HOAc (65:15:15:20). The sugars were detected with aniline phthalate. Analysis of the sugar by hplc on a carbohydrate column (Waters Associates) using MeCN-H_2O (6:4) showed a single peak with the same retention time as glucose.

LITERATURE CITED

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Received 11 February 1985