Pergamon 0031-9422(95)00063-9 *Phytochemistry*, Vol. 39, No. 5, pp. 1133–1137, 1995

0031-9422/95 $9.50 + 0.00

ENT-KAURANE GLYCOSIDE FROM *STEVIA SUBPUBESCENS*

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(*Received* 19 *November* 1994)

Key Word Index—*Stevia subpubescens*; Compositae; leaves; *ent*-kaurane glycoside; subpubescensoside.

Abstract—A new *ent*-kaurane glycoside, subpubescensoside, was isolated from the leaves of *Stevia subpubescens*. Its structure was elucidated as 11β,16-oxo-*ent*-kauran-19-oic acid 19-*O*-[β-D-glucopyranosyl(1 → 2)]-[β-D-glucopyranosyl(1 → 3)]β-D-glucopyranosyl ester on the basis of spectroscopic and chemical evidence.

INTRODUCTION

So far, eight sweet *ent*-kaurane glycosides have been isolated from *Stevia rebaudiana* [1], while five non-sweet *ent*-kaurane glycosides, named paniculosides, have been reported to occur in *S. paniculata* [2] and *S. ovata* [3]. The present paper deals with the isolation and structural elucidation of a non-sweet *ent*-kaurane glycoside from the leaves of *S. subpubescens* [4].

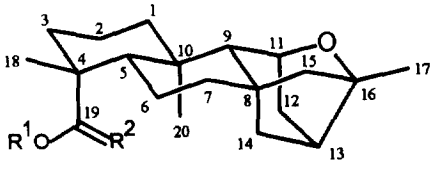
RESULTS AND DISCUSSIONS

Chromatography of the *n*-BuOH-soluble fraction of MeOH extracts of the leaves of *S. subpubescens*, followed by recrystallization, afforded a new glycoside, named subpubescensoside (**1**). Acid hydrolysis of **1** gave D-glucose and aglycone **2**, characterized as 11β,16-oxo-*ent*-kauran-19-oic acid. The same aglycone (**2**) was obtained after alkaline hydrolysis of **1**, suggesting that the sugar residue is linked to the aglycone through an ester bond.

Aglycone **2**, $C_{20}H_{30}O_3$ was obtained as colourless needles, mp 256–258° and $[\alpha]_D$ − 66°. Its IR spectrum exhibited a broad band at 3300–2500 and a band at 1710 cm^{-1} (COOH). The ^1H NMR spectrum (in DMSO-d_6) showed the presence of three tertiary methyl groups at δ1.25, 1.10 and 0.92 and a multiplet at δ4.25. Its ^{13}CNMR (Table 1) and DEPT spectra, which are in agreement with a kauranoic acid structure, show resonances for three methyls, eight methylenes, four methines and five quaternary carbons. The appearance of two carbons bearing oxygen atoms at δ85.8 (*s*) and 76.9 (*d*) and the absence of hydroxyl groups suggest the presence of an ether linkage. The resonance at δ85.8 and the methyl signal at δ1.25 locate one oxygen at C-16. That the ether group is attached to C-11 and not to C-12 is evident by comparison of the ^1H NMR spectrum of **2** with those of related diterpenes isolated from *stevia* species [5]. The β-orientation of the 11 (16) ether linkage follows from the values $J_{9,11} = J_{11,12\beta} = 2.5$ Hz.

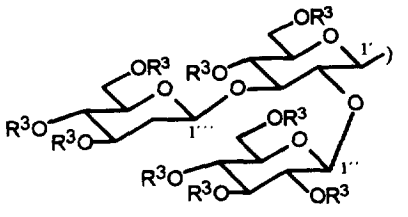
To verify the structure of aglycone **2**, an X-ray analysis of the methyl ester **3** was undertaken, Crystal data are given in the Experimental section and a stereoview of the molecule is shown in Fig. 1.

The absolute stereochemistry of the kaurane derivative **2** can be proposed as an *ent*-type since this substance showed intense laevo-rotations from 589 to 365 mm, as



1: R^1 = A; R^2 = O; R^3 = H
2: R^1 = H; R^2 = O
3: R^1 = CH_3; R^2 = O
4: R^1 = H; R^2 = H_2
5: R^1 = A; R^2 = O; R^3 = Ac
6: R^1 = A; R^2 = O; R^3 = CH_3

A =



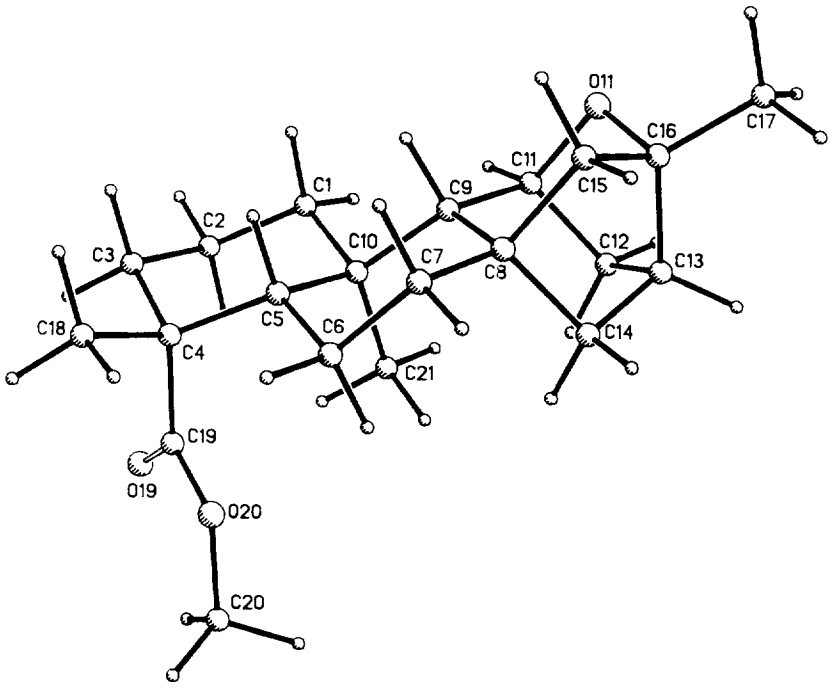


Fig. 1. Perspective view of **3**.

has been consistently observed for other *ent*-kauranoic acid derivatives [6].

Subpubescensoside (**1**) was obtained as prisms from MeOH–H_2O, mp 274–276° and $[\alpha]_D$ −15°. Its IR spectrum showed absorptions at 3500 (OH) and 1720 cm^{-1} (C = O). The ^1H NMR spectrum showed signals for three tertiary methyls and three anomeric hydrogens at δ5.49, 4.86 and 4.48 as doublets (J = 7–8 Hz), indicating three β-linked sugar units. The presence of seven doublets and three triplets, which interchange with D_2O, are due to seven secondary and three primary hydroxyl groups, respectively, indicating the presence of a triscaccharide moiety, This is in agreement with the ^{13}C NMR spectrum of **1** (Table 1) which shows three anomeric carbons at δ102.9, 101.2 and 91.8, the latter signals being characteristic of an esterified anomeric carbon. The signals attributable to the aglycone moiety of **1** are essentially analogous to those of aglycone (**2**), except for the C-19 signal, which confirms the site of glucosidation. Detailed analysis of the ^{13}C NMR spectral data of **1** and the ^1H NMR data of its deca-acetate (**5**), reveals the presence of three glucose units, since only axial–axial coupling constants are observed. The interglycosidic linkages among the three glucose moieties of **1** are suggested from the two resonances at δ88.7 and 79.0 (in pyridine-d_5) due to C-3′ and C-2′ of the first glucopyranose unit. These are deshielded by 10.4 and 4.1 ppm, respectively, compared to the corresponding signals of methyl β-glycopyranoside [6]. Moreover, the chemical shift values of C-3′ and C-2′ are consistent with reported values of triglucosides [7] linked to these positions.

The COSY contour plot of the peracetate **5** showed a correlation between the anomeric proton linked to the carboxyl, H-1′ and the signal at δ4.16, corresponding to the proton at the glycosidation site C-2, thus assigning this signal to H-2′. The COSY plot of the permethylated derivative **6** also showed a correlation between the anomeric proton at δ4.94 and one of the protons of the glycosidation site (δ3.90), which is assigned to H-2′. From these data the sequence Glc (1 → 2) Glc–OOC–R was established.

The third glucose moiety may be attached to the oxygen at C-3′ or at C-3″. The COSY contour plot of **5** shows a correlation of H-2′ with the proton at the other glycosidation site (δ4.09), therefore assigned to H-3′.

On the other hand, methanolysis of the decamethyl ether (**6**) yields methyl 2,3,4,6-tetra-*O*-methylglucopyranoside and methyl 4,6-di-*O*-methylglucopyranoside, identified by NMR spectroscopy. From the above data, it follows that the third glucose unit is linked to C-3′ and consequently glycoside **1** is 11β,16-oxo-*ent*-kauran-19-oic acid 19-*O*-[β-D-glucopyranosyl (1 → 2)-β-D-glucopyranosyl (1 → 3]-β-D-glucopyranosyl ester (1).

The presence of the two terminal glucose units was further confirmed by periodate oxidation of **1** followed by partial hydrolysis, which furnished a monoglucoside, thus confirming that one glucose unit is the branching point of the sugar chain, as found in rebaudioside isolated from *S. rebaudiana* [1].

EXPERIMENTAL

Mps: uncorr. ^1H (300 MHz) and ^{13}C (75 MHz) NMR spectra were recorded in pyridine-d_5, DMSO-d_6 or $CDCl_3$ using TMS as int. standard. CC: silica gel 60 (70–230 mesh). TLC: cellulose F (0.1 mm). Spots were

Table 1. ^{13}C NMR data of compounds **1–6**

	1		2		3	4	5	6
C	C_5D_5N	DMSO-d_6	C_5D_5N	DMSO-d_6	$CDCl_3$	$CDCl_3$	$CDCl_3$	$CDCl_3$
1	41.3	40.6	41.8	40.9	41.4	41.0	41.1	41.3
2	19.8	18.9	19.8	18.7	19.0	18.1	19.1	19.5
3	37.3	37.7	38.7	37.5	38.0	35.5	37.2	37.2
4	43.7	43.1	43.8	42.8	43.6	38.5	43.7	43.9
5	57.9	56.5	57.5	56.4	57.2	57.2	57.3	57.9
6	21.8	20.8	22.4	21.3	21.6	20.1	21.8	21.3
7	38.6	38.5	38.6	37.8	38.3	38.3	38.5	38.4
8	45.2	44.4	45.3	44.5	45.0	44.8	45.1	45.1
9	58.6	57.8	58.8	57.9	58.3	59.3	58.5	58.3
10	37.7	36.5	37.4	36.6	36.9	36.7	37.7	37.1
11	76.7	75.6	76.8	75.6	76.8	76.8	76.7	77.1
12	40.7	—*	40.8	40.0	40.4	40.3	40.7	40.5
13	45.8	44.9	45.9	45.0	45.5	45.5	45.9	45.5
14	44.1	42.9	43.8	42.6	43.4	43.7	43.9	43.6
15	57.2	56.8	57.3	56.5	56.9	57.2	57.8	57.0
16	85.5	84.7	85.5	84.8	85.8	85.5	85.4	85.8
17	23.6	23.0	23.7	23.1	23.2	23.2	23.4	23.3
18	29.1	28.2	29.5	28.7	28.9	27.4	28.5	28.9
19	176.2	175.0	180.2	178.6	178.0	65.5	175.6	176.2
20	18.2	17.6	17.9	17.2	17.4	19.2	18.2	18.2
Glc-OOC								
1′	93.4	91.8	—	—	—	—	92.3	91.8
2′	79.0	77.3	—	—	—	—	77.8	77.1
3′	88.7	86.7	—	—	—	—	80.8	82.5
4′	69.4	67.9	—	—	—	—	67.7	80.1
5′	76.7	74.7	—	—	—	—	73.0	74.8
6′	63.5	61.7	—	—	—	—	62.0	71.8
Glc (1 → 2)†								
1	103,5	101.2	—	—	—	—	99.6	101.1
2	75.9	73.9	—	—	—	—	72.5	86.5
3	78.6	76.9	—	—	—	—	72.5	86.5
4	72.8	69.9	—	—	—	—	68.5	79.8
5	78.5	76.5	—	—	—	—	73.4	75.4
6	62.3	60.8	—	—	—	—	61.9	71.4
Glc (1 → 3)†								
1	104.6	102.9	—	—	—	—	99.8	101.8
2	75.4	73.6	—	—	—	—	72.4	84.5
3	78.6	76.9	—	—	—	—	72.5	86.4
4	71.5	69.9	—	—	—	—	68.3	79.8
5	78.3	76.5	—	—	—	—	73.4	75.3
6	61.2	60.2	—	—	—	—	61.7	71.0

*Overlapped with solvent signal.
†Assignments may be interchanged.

visualized by spraying with naphthoresorcinol reagent followed by heating.

Plant material. Leaves of *S. subpubescens* Lag. were collected in July 1992 near Lagunillas, Mich. A voucher specimen is deposited in the Herbarium of the Instituto de Ecologia, A. C., Pátzcuaro, Mich., México, where Prof. Jerzy Rzedowski identified the plant material.

Isolation of subpubescensoside (**1**). Fr. leaves (100 g) were extracted with hot MeOH. The extract was evapd under red. pres. and the residue suspended in H_2O, washed with Et_2O and then extracted with *n*-BuOH satd. with H_2O. The *n*-BuOH fr., after evapn under red. pres. to dryness, yielded 21 g of an amber syrup. Part of this extract (9 g) was subjected to CC (150 g, silica gel 160) and eluted with $CHCl_3$–MeOH–H_2O (200:30:1, 800 ml; 50:50:1, 400 ml; 25:75:2, 200 ml) and MeOH (200 ml); eluates were collected in 100 ml frs. Frs 8–16 yielded 3.5 g of crude glycoside **1** as a brown solid which was repeatedly recrystallized from MeOH–H_2O, affording 2.1 g of subpubescensoside (**1**).

Subpubescensoside (**1**). White prisms, mp 274–276°. $[\alpha]_{589}$ − 15°, $[\alpha]_{578}$ − 17°, $[\alpha]_{546}$ − 19°, $[\alpha]_{436}$ − 34°. $[\alpha]_{365}$ − 55° (EtOH; c = 0.1), IR ν_{max} cm^{-1}: 3500 (OH), 1700 (C = O). ^1H NMR (300 MHz, pyridine-d_5): δ6.20

(1H, *d*, J = 8.0 Hz, H-1'), 5.84 (1H, *d*, J = 8.0 Hz, H-1''), 5.30 (1H, *d*, J = 7.8 Hz, H-1'''), 1.39 (3H, *s*, Me-18), 1.37 (3H, *s*, Me-17), 0.98 (3H, *s*, Me-20).^{1}H NMR (DMSO-d_6 + D_2O): δ5.49 (1H, *d*, J = 7 Hz, H-1'), 4.86 (1H, *d*, J = 8 Hz, H-1''), 4.48 (1H, *d*, J = 7 Hz, H-1'''), 4.24 (1H, *m*, H-11), 3.84 (1H, *t*, J = 9 Hz, H-2'), 3.77 (1H, *t*, J = 9 Hz, H-3'), 3.36 (1H, *t*, J = 8 Hz, H-4'), 3.23 (1H, *t*, J = 9 Hz, H-3''), 3.20 (2H, 2*t*, J = 9 Hz, H-4'' and H-3'''), 3.11 (1H, *t*, J = 9 Hz, H-2'''), 2.97 (1H, *t*, J = 9 Hz, H-4'''), 2.89 (1H, *t*, J = 9 Hz, H-2''), 1.25 (3H, *s*, Me-17), 1.16 (3H, *s*, Me-18), 0.86 (3H, *s*, Me-20). ^{13}C NMR: see Table 1.

Acid hydrolysis of **1**. A soln of **1** (100 mg) in 4N H_2SO_4–MeOH (1:1, 12 ml) was heated under reflux for 1 hr and left at room temp. overnight. The ppt. was filtered and recrystallized to give aglycone **2** (30 mg). The filtrate was deionized with Amberlite MB-3A, concd under red. pres. and tested for sugars by TLC (cellulose) against authentic samples using i-PrOH–pyridine–HOAc–H_2O (8:8:1:4) and detecting by spraying with naphthorescorcinol reagent followed by heating. The sugar was identified as glucose.

Aglycone **2**. White needles from CH_2Cl_2–hexane, mp 255–256°. $[\alpha]_{589}$ − 66°, $[\alpha]_{578}$ − 70°, $[\alpha]_{546}$ − 75°, $[\alpha]_{436}$ − 137°, $[\alpha]_{365}$ − 217° ($CHCl_3$; c = 0.1). IR ν_{max} cm^{-1} 3300–2500 (OH carboxyl), 1690 (CO_2H), ^{1}H NMR (pyridine-d_5): δ4.44 (1H, *br t*, J = 2.5 Hz, H-11), 2.46 (1H, *br d*, J = 12.7 Hz, H-3α), 2.11 (1H, *t*, J = 6.3 Hz, H-13), 1.40 (3H, *s*, Me-17), 1.34 (3H, *s*, Me-18), 1.33 (1H, *d*, J = 10.7 Hz, H-15β), 1.14 (3H, *s*, Me-20). ^{1}H NMR ($CDCl_3$): δ4.38 (1H, *m*, H-11), 2.22 (1H, *t*, J = 6.5 Hz, H-13), 2.19 (1H, *br d*, J = 13.7 Hz, H-3α), 2.02 (1H, *d*, J = 11.0 Hz. H-12α), 1.96 (1H, *dd*, J = 11.0 and 3.5 Hz, H-14α), 1.91 (1H, *m*, H-12β), 1.82 (1H, *br d*, J = 13.0 Hz, H-1α), 1.82 (1H, complex *m*, H-6β), 1.72 (1H, complex *m*, H-7α), 1.55 (1H, *dd*, J = 11.2 and 3.5 Hz, H-15β), 1.52 (1H,*m*, H-9), 1.48–1.38 (4H, complex *m*, H-1β, H-2β, H-6α H-7β), 1.35 (1H, *d*, J = 11.2 Hz, H-15α), 1.35 (3H, *s*, Me-17), 1.24 (1H, *br dd*, J = 11.0, 6.5 Hz, H-14β), 1.24 (3H, *s*, Me-18), 1.16–1.02 (2H, complex *m*, H-2α and H-3β), 1.09 (1H, *br d*, J = 11.5 Hz, H-5), 0.98 (3H, *s*, Me-20). ^{1}H NMR (DMSO-d_6):δ4.24 (1H, *m*, H-11), 2.15 (1H, *t*, J = 6.5 Hz, H-13), 2.05 (1H, *br d*, J = 13.7 Hz, H-3α), 1.95 (1H, *d*, J = 11.0 Hz, H-12α), 1.89 (1H, *dd*, J = 11.0 and 3.5 Hz, H-14α), 1.80–1.55 (4H, complex *m*, H-1α, H-6β, H-7α and H-12β), 1.40 (1H, *dd*, J = 11.2 and 3.5 Hz, H-15β), 1.40–1.35 (5H, complex *m*, H-1β, H-2β, H-6α, H-7β and H-9), 1.31 (1H, *d*, J = 11.2 Hz, H-15α), 1.25 (3H, *s*, Me-17), 1.21 (1H, *br dd*, J = 11.0, 6.5 Hz, H-14β), 1.10 (3H, *s*, Me-18), 1.10–0.95 (3H, complex *m*, H-2α, H-3β and H-5), 0.92 (3H, *s*, Me-20). ^{13}C NMR: see Table 1. (Found C, 75.43; H, 9.491 0, 15.07. $C_{20}H_{30}O_3$ requires: C, 75.70; H, 9.46; O, 14.74%.)

Methyl ester **3**. The Me ester **3** prepd with CH_2N_2 had mp 138–139° $[\alpha]_{589}$ − 127° ($CHCl_3$; c = 0.1). IR ν_{max} cm^{-1}: 1710 (CO_2Me). ^{1}H NMR ($CDCl_3$): δ4.35 (1H, *t*, J = 3.1 Hz, H-11), 3.64 (3H, *s*, OMe), 2.20 (1H, *t*, J = 6.5 Hz, H-13), 2.20 (1H, *br d*,J = 12.9, H-3α), 2.0 (1H, *d*, J = 11.1 Hz), H-12α), 1.95 (1H, *dd*, J = 11.7 and 3.4 Hz, H-14α), 1.89 (1H, *m*, H-12β), 1.54 (*dd*, J = 11.4 and 3.5 Hz, H-15α), 1.44 (1H, *br s*, H-9), 1.41 (1H, *m*, H-7), 1.34 (1H, *d*, J = 11.4 Hz, H-15β), 1.34 (3H, *s*, Me-17), 1.24 (1H, *m*, H-14β), 1.17 (3H, *s*, Me-18), 0.87 (3H, *s*, Me-20). ^{13}C NMR: see Table 1.

X-ray analysis. Single crystals of **3** were grown by slow recrystallization from CH_2Cl_2–hexane. They were orthorhombic, space group $P2_12_12_1$ with a = 6.881 (3), b = 9.898 (5), c = 27.625 (12) Å, and d_{calc} = 1.17 g cm^{-3} for Z = 4 (M_r 332.48). The intensity data were measured on a Nicolet R3m four-circle diffractometer with Cu-Kα monochromated radiation in the θ:2θ scan-mode. The size of the crystal used for data collection was *ca* 0.3 × 0.2 × 0.3 mm^3. No absorption correction was necessary (μ = 5.7 cm^{-1}). A total of 1496 reflections was measured for 3° ≤ θ ≤ 110°, of which 1176 reflections were considered to be observed [$T \geq 2$ (I)]. The structure was solved by the direct method using the software provided by the manufacturer and refined by full-matrix least-squares assuming anisotropic temperature factors for non-hydrogen atoms and isotropic ones for hydrogen atoms. The final discrepancy indices were R = 4.6%, using a unit weight for 1063 reflections. The final difference Fourier map was essentially featureless, the highest residual peaks having intensities of 0.4 e$^-$/Å^3. Lists containing atomic coordinates and thermal parameters, bond distances, bond angles, anisotropic temperature factors, hydrogen atom coordinates, torsion angles and comparison of the observed and calculated structure factors have been deposited at the Cambridge Crystallographic Data Centre.

Reduction of methyl ester **3** *with* $LiAlH_4$. Compound **3** (500 mg) in dry THF (50 ml) was heated under reflux with $LiAlH_4$ (500 mg) for 2 hr. Excess reagent was decomposed with H_2O, acidified with 5% H_2SO_4 and extracted with EtOAc. The organic layer was dried over Na_2SO_4 and evapd. Recrystallization of the residue from acetone–hexane gave 210 mg of **4** as white flakes, mp 130–132°. $[\alpha]_{589}$ − 35°, $[\alpha]_{578}$ − 37°, $[\alpha]_{546}$ − 42°, $[\alpha]_{436}$ − 70, $[\alpha]_{365}$ − 106° ($CHCl_3$; c = 0.17). IR ν_{max} cm^{-1}: 3620 (*s*, free OH), 3360 (*br*, bonded OH). ^{1}H NMR ($CDCl_3$: δ4.34 (1H, *t*, J = 3.1 Hz, H-11), 3.78 (1H, *d*, J = 11.0 Hz, H-19), 3.44 (1H, *d*, J = 11.0 Hz, H-19'), 2.20 (1H, *t*, J = 6.6 Hz, H-13), 2.05 (1H, J = 11.3 Hz, H-12α), 1.94 (1H, *dd*, J = 11.7 and 3.4 Hz, H-14α), 1.88 (1H, *m*, H-12β), 1.58 (1H, *br d*, J = 12.0 Hz, H-3α), 1.54 (1H, *dd*, J = 9.6 and 3.6 Hz, H-15α), 1.52 (1H, *br s*, H-9), 1.36 (1H, *d*, J = 9.6 Hz, H-15β), 1.34 (3H, *s*, Me-17), 1.25 (1H, *m*, H-14β), 1.06 (3H, *s*, Me-18), 0.97 (3H, *s*, Me-20). ^{13}C NMR: see Table 1.

Alkaline hydrolysis of glycoside **1**. A soln of **1** (50 mg) in MeOH (20 ml) was heated under reflux with 10% KOH for 4 hr. The soln was neutralized with 1N HCl and extracted with EtOAc. The organic layer was dried over Na_2SO_4 and evapd to give aglycone **2** (10 mg), which was identical to the compound obtained by acid hydrolysis of **1**. From the mother liquors, 30 mg of **1** were recovered.

Reduction of glycoside **1** *with* $LiAlH_4$. Glucoside **1** (100 mg) in dry THF (30 ml) was reduced with $LiAlH_4$ as described above. Recrystallization of the residue gave alcohol **4** (30 mg) identical to that obtained by $LiAlH_4$ reduction of Me ester **3**.

Peracetylation of glycoside **1**. Compound **1** (100 mg) was dissolved in pyridine–Ac_2O (3 ml each) and left overnight at room temp. Work-up in the usual manner gave peracetate **5** as a white powder, mp 101–103°. ^{1}H NMR (C_6D_6): δ5.89 (1H, *d*, J = 6.9 Hz, H-1′), 5.48 (1H, *t*, J = 8.9 Hz, H-3‴), 5.39 (1H, *t*, J = 9.5 Hz, H-4‴), 5.54 (1H, *t*, J = 9.2, H-3″), 5.33 (1H, *t*, J = 8.8 Hz, H-4′), 5.32 (1H, *t*, J = 9.2 Hz, H-4″), 5.30 (1H, *t*, J = 8.9 Hz, H-2″), 5.27 (1H, *t*, J = 8.7 Hz, H-2‴), 5.13 (1H, *d*, J = 7.6 Hz, H-1″), 5.05 (1H, *d*, J = 7.0 Hz, H-1‴), 4.54 (1H, *dd*, J = 12.5 and 4.3 Hz, H-6a″), 4.50 (1H, *dd*, J = 12.7 Hz, 4.0, H-6a‴), 4.41 (1H, *m*, H-11), 4.32 (1H, *dd*, J = 12.4, 5.3 Hz, H-6a′), 4.16 (1H, *t*, J = 8.0 Hz, H-2′), 4.13 (1H, *dd*, J = 12.2, 2.2 Hz, H-6b″), 4.09 (1H, *t*, J = 8.6 Hz, H-3′), 4.08 (1H, *dd*, J = 12.2, 2.2 Hz, H-6b′), 4.03 (1H, *dd*, J = 12.1, 1.8 Hz, H-6b‴), 3.80 (1H, *ddd*, J = 7.0, 3.9 and 2.0 Hz, H-5″), 3.71 (1H, *ddd*, J = 10.0, 4.1 and 1.4 Hz, H-5‴), 3.54 (1H, *ddd*, J = 9.6, 4.9 and 2.4 Hz, H-5′), 1.41 (3H, *s*, Me-17), 1.23 (3H, *s*, Me-18), 1.08 (3H, *s*, Me-20). ^{13}C NMR: see Table 1 (Found: C, 56.57; H, 6.77 $C_{58}H_{80}O_{28}$ requires: C, 56.86; H, 6.58%).

Permethylation of **1**. A soln of glycoside **1** (50 mg) in dry DMSO (4.7 ml) was treated with *t*-BuONa (500 mg), finely powdered NaOH (144 mg) and MeI (4 ml). The reaction mixt. was stirred at room temp. for 1 hr, poured into ice–H_2O and extracted with Et_2O. Flash CC of the residue ($CHCl_3$–EtOAc, 1:1) gave permethyl derivative **6** (40 mg) as an oil; R_f 0.33. ^{1}H NMR ($CDCl_3$): δ5.52 (1H, *d*, J = 7.7 Hz, H-1″), 5.50 (1H, *d*, J = 7.7, H-1‴), 4.94 (1H, *d*, J = 7.8, H-1′), 4.34 (1H, *m*, H-11), 3.94 (1H, *t*, J = 8.2 Hz, H-3′), 3.90 (1H, *t*, J = 8.2 Hz, H-2′), 2.90 (1H, *t*, J = 8.4 Hz, H-2″), 2.76 (1H, *t*, J = 7.8 Hz, H-2‴), 1.35 (3H, *s*, Me-17), 1.19 (3H, *s*, Me-18), 0.93 (3H, *s*, Me-20). ^{13}C NMR: see Table 1.

Acid hydrolysis of permethylate **6**. A soln of permethylate **6** (70 mg) in MeOH (4 ml) and 6% H_2SO_4 (6 ml) was refluxed for 4 hr. After evapn, H_2O was added and the soln extracted with $CHCl_3$. Flash CC of the residue (EtOAc) gave aglycone **2** (20 mg), Me 2,3,4,6-tetra-*O*-methylglucopyranoside (α- and β-form) and Me 4,6-di-*O*-methylglucopyranoside (α- and β-form) which were identified by ^{1}H NMR.

Periodate oxidation of **1**. Glycoside **1** (100 mg) and $NaIO_4$ (200 mg) were suspended in H_2O (10 ml) and kept in the dark for 64 hr. Excess $NaIO_4$ was decomposed with ethylene glycol and hydrolysed by refluxing with 1M H_2SO_4 for 1 hr. The aq. extract was neutralized and extracted with *n*-BuOH. Evapn of the organic layer gave the monoglucoside of aglycone **2** identified by NMR.

Acknowledgements—We wish to thank Q.F.B.G. Morán and Q.F.B.I. Tapia (Universidad Michoacana) for technical assitance and CoNaCyT and SEP México for partial financial support.

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