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INSECT INHIBITORY STEROIDAL SACCHARIDE ESTERS FROM *PHYSALIS PERUVIANA*

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ABSTRACT.—Dry leaves of *Physalis peruviana* gave the glycoside esters **1** and **2** of 24-*E*-22ξ-acetoxy-1α,3β-dihydroxyergosta-5,24-dien-26-oic acid in yields of 170 and 90 mg·kg^{-1}, respectively. Both **1** and **2** reduce the growth of *Helicoverpa zea* larvae to 50% of a control size at a concentration of ca. 35 mg·kg^{-1} in artificial diets.

Physalis peruviana L. (Solanaceae) is native to tropical South America but is cultivated in warmer regions worldwide under such common names as cape gooseberry, ground cherry, and poha berry (1). The plant has found use in traditional medicine in India, and its leaves and roots have been a source of numerous steroidal lactones termed withanolides (2), e.g., Figure 1, some of which have shown physiological activity. A number of withanolides from plants within the Solanaceae including *P. peruviana* have been reported to be significant defensive agents against insects (3,4). We have examined *P. peruviana* as a possible source of insect resistance in intergeneric hybridization and have found that its foliage is highly inhibitory to growth and development of *Helicoverpa zea* (=*Heliothis zea* Boddie), an insect that is an economic pest of numerous crops including the solanaceous plants, tobacco and tomato. Bioassay-directed extraction and fractionation of leaf material led to two steroidal glycoside esters, **1** and **2**, that are strongly active against *H. zea*.

RESULTS AND DISCUSSION

Initial screening of successive $CHCl_3$, Me_2CO, and MeOH leaf extracts indicated that highest inhibitory activity was in the MeOH extract. The last extract was separated from inactive, highly polar substances by selective adsorption from H_2O solution on to nonionic macroreticular XAD-2 resin. Chromatographic fractionation, first on Sephadex, then by preparative hplc, yielded pure **1** and **2** as active compounds accompanied by a number of less pure, unidentified fractions also possessing strong activity.

Compound **1**, obtained in a yield of 170 mg·kg^{-1} (dry wt), gave an empirical formula

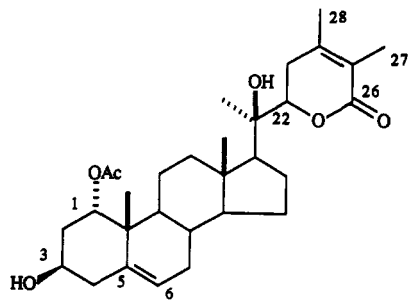
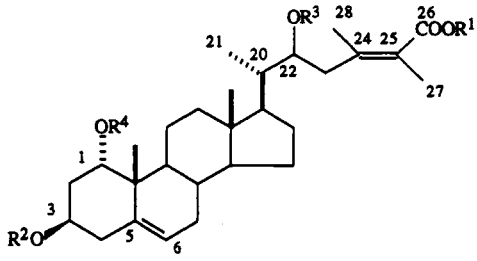


FIGURE 1. Physalolactone B, a typical withanolide.

NOTE ADDED IN PROOF: Contrary to the evidence presented above for the position of the acetylated sugars of **1** and **2**, recent mass spectral information has shown that these acetyl groups are at positions 6′ in **1** and 6′ and 6″ in **2**.



1 R^1=glc-(1↦4)-glc-6″-Ac-(1↦2)-glc, R^2=glc, R^3=Ac, R^4=H
2 R^1=glc-6‴-Ac-(1↦4)-glc-6″-Ac-(1↦2)-glc, R^2=glc, R^3=Ac, R^4=H
3 R^1=Me, R^2=R^3=R^4=H
4 R^1=Me, R^2=R^3=R^4=Ac
5 R^1=Me, R^2=glc, R^3=R^4=H
6 R^1=R^2=R^4=H, R^3=Ac
7 R^1=glc-6″-Ac-(1↦2)-glc, R^2=R^4=H, R^3=Ac
8 R^1=glc-6‴-Ac-(1↦4)-glc-6″-Ac-(1↦2)-glc, R^2=R^4=H, R^3=Ac
9 R^1=glc-(1↦2)-glc, R^2=R^4=H, R^3=Ac

of $C_{56}H_{88}O_{27}$ derived from the major high mass liquid sims peak at *m/z* 1215 $[M+Na]^+$, indicating 13 degrees of unsaturation. The nmr spectra of **1** resemble those of the 3-*O*-glycosides of physalolactone B (5,6), but show significant differences, mainly associated with signals arising from the side chain. Sugar analysis indicated that only glucose was present, and examination of the ^{1}H-nmr spectrum revealed four doublets at δ 4.30, 4.38, 4.70, and 5.64 ppm (J=8 Hz) for anomeric protons, indicative of four β-linked glucose units. The signal at lowest field suggests that one glucose is esterified at its C-1 position as in stevioside and other steviol derivatives, and the correlated carbon (Table 1) at δ_C 93.9 is in good agreement with this assignment (7). The ^{1}H-nmr and ^{13}C-nmr spectra of **1** also showed the presence of two acetoxy groups as well as one tri- and one tetra-substituted double bond, accounting for all degrees of unsaturation and indicating that a lactone of the withanolide type cannot be present. However, the carbonyl carbon of the glycosidic ester gave a signal at δ_C 168.4, and the signals at δ_C 125.5 and 148.0 together with uv absorption at 230 nm were consistent with an open chain α,β-unsaturated ester. Treatment of **1** with MeOH/HCl gave **3**, $C_{29}H_{46}O_5$, whose nmr, uv, and ir spectra (1708 cm^{-1}) indicated formation of an α,β-unsaturated methyl ester with complete deglycosylation and deacetylation. Compound 3 clearly showed nmr signals consistent with 1α,3β-hydroxylation, and ^{1}H COSY indicated connectivity of both H-1 and H-3 to H_2-2. ^{1}H-nmr and ^{13}C-nmr spectra of **3** were essentially identical in the portions of the spectra associated with the steroidal nucleus to those of pubescenolide (8) and deacetylphysalolactone B (9), whose structures are identical to **3** in this region. This was also true for spectra of the triacetate **4** in comparison to those acetates of these two compounds, which clearly showed the seven-line signal of H-3α at ca. δ 4.9. In both the parent triol **3** and the triacetate **4**, partial overlapping of the ^{1}H-nmr signal of H-22 occurred; however, these signals could easily be distinguished in the COSY spectra.

Methanolysis of **1** with NaOMe/MeOH gave the deacetylated α,β-unsaturated methyl ester **5**, $C_{35}H_{56}O_{10}$, retaining one glucose unit at position 3 (H-3, δ 4.04, 7 lines). This conclusively showed that a trisaccharide moiety was originally attached by esterification at position 26. Complete removal of the glucose substituents of **1** with retention of an acetoxy unit at C-22 could be accomplished by Smith degradation using $NaIO_4$ followed by $NaBH_4$ and dilute HCl (10) to give **6**. The position of acetoxylation could be established by COSY more definitively in the case of **6** than in the preceding

TABLE 1. ^{13}C-nmr Data.[a]

Carbon	Compound						
	1	**2**	**3**	**4**	**5**	**7**	**9**
C-1	73.5	73.5	72.9	74.3[b]	73.6	73.5	73.5
C-2	37.6	37.6	38.2	31.9[c]	37.7	39.1	39.2
C-3	ca 75.0	ca 75.0	66.4	69.3	75.0[b]	67.0	67.0
C-4	39.1	39.1	41.3	37.2	39.1	42.4	42.4
C-5	138.9	139.0	137.3	136.0	139.0	139.3	139.3
C-6	125.5	125.5	125.5	125.1	125.5	125.2	125.2
C-7	32.9	32.9	31.7	31.5[c]	32.9	32.9	32.9
C-8	33.1	33.2	31.8	31.6	33.2	33.2	33.2
C-9	42.5	42.5	41.55[b]	42.0	44.2	42.6	42.6
C-10	42.7	42.7	41.7	40.3	42.8	42.5	42.5
C-11	21.0	21.2	20.2	20.4	21.3	21.3	21.3
C-12	40.7	40.8	39.4	39.5	40.9	40.8	40.8
C-13	43.7	43.9	42.7	42.7	43.9	43.9	43.9
C-14	57.5	57.6	56.2	58.3	57.7	57.6	57.6
C-15	25.6	25.6	24.5	24.3	25.6	25.5	25.5
C-16	28.3	28.3	27.4	27.2	28.8	28.3	28.3
C-17	54.1	54.2	53.0	52.9	54.5	54.2	54.2
C-18	12.2	12.3	11.8	11.8	12.3	12.2	12.2
C-19	20.0	20.0	19.4	19.3	20.0	20.0	20.0
C-20	41.0	41.0	41.63[b]	39.5	42.6	41.0	41.0
C-21	13.5	13.5	12.4	13.0	12.8	13.5	13.5
C-22	75.8	75.8	71.2	74.5[b]	72.5	75.8	75.9
C-23	35.0	35.0	36.0	33.4	36.8	35.1	35.1
C-24	148.1	148.1	143.2	142.4	146.1	148.0	148.2
C-25	125.4	125.4	125.5	125.0	125.2	125.5	125.4
C-26	168.3	168.3	170.2	170.29[d]	172.0	168.4	168.6
C-27	15.9	15.9	15.9	15.7	16.1	15.9	15.9
C-28	22.0	22.0	21.2	21.20[e]	21.7	22.0	22.0
OAc (Me)	21.2	21.2	—	21.13[e]	—	21.2	21.2
OAc (Me)	21.0	21.0	—	21.28[e]	—	20.9	—
OAc (Me)	—	20.8	—	21.33	—	—	—
OAc (CO)	172.2	172.2	—	170.05[d]	—	172.3	172.3
OAc (CO)	172.5	172.5	—	170.35[d]	—	172.6	—
OAc (CO)	—	172.8	—	170.38[d]	—	—	—
OMe	—	—	51.4	51.4	51.8	—	—
Sugar Signals							
C-1′	93.8	93.8	—	—	103.0	93.9	94.1
C-2′	81.8	81.9	—	—	74.9[b]	81.7	81.4
C-3′	—	—	—	—	77.9[c]	77.6[b]	77.7[b]
C-4′	70.6	70.6	—	—	71.7	70.7[c]	70.7[c]
C-5′	—	—	—	—	78.1[c]	78.8[b]	78.8[b]
C-6′	62.1	62.2	—	—	62.9	62.2	62.2
C-1″	104.8	104.8[b]	—	—	—	105.0	104.9
C-3″	—	—	—	—	—	78.2[b]	78.1[b]
C-4″	80.9	81.6	—	—	—	71.2[c]	71.2[c]
C-5″	75.3	75.3	—	—	—	75.3	78.0[b]
C-6″	64.5	64.5	—	—	—	64.9	62.6
C-1‴	104.8	104.9[b]	—	—	—	—	—
C-4‴	71.2	71.7[c]	—	—	—	—	—
C-5‴	—	75.4	—	—	—	—	—
C-6‴	62.8[b]	64.9	—	—	—	—	—
C-1⁗	102.6	102.6	—	—	—	—	—
C-4⁗	71.6	71.6[c]	—	—	—	—	—
C-6⁗	62.7[b]	62.9	—	—	—	—	—

[a]In ppm for CD_3OD solutions, except **3** and **4** in $CDCl_3$. Carbons of C-3 glc are indicated by quadruple primed numbers in **1** and **2** and single primed numbers in **5**.

[b-e]Values within columns may be interchanged.

compounds inasmuch as the resonance of H-22, δ 5.17, is well separated from adjoining signals. This showed clearly that both H_3-21 and H-22 are coupled to the same proton at about δ 1.85 (H-20), and that H-22 was also coupled to a methylene (H_2-23) having signals at δ 2.10 and 2.64. The multiplicity of H-22 as a broad double triplet with couplings of about 12 and 3 Hz is consistant with a proton adjacent to one methylene and one methine, and it is very similar to the appearance of H-22 in certain members of the petuniasterone C series (11). It was not possible to assign the configuration of position 22, since the nmr data was inadequate for this purpose and none of the substances derived from **1** gave crystals suitable for X-ray analysis. However, all known withanolides (having monooxygenation at this position) possess the same relative configuration at C-22 (12), and we feel, therefore, that compound **1** has configuration 22*R* as a consequence of identical biogenesis.

We have assigned the *E* configuration to the 24,25 double bond of the above compounds, mainly because lactonization did not occur during degradative treatment of **1**. Both dunawithanines A and B (5) and physalolactone B 3-glucoside (6) did give the lactone rather than the methyl ester after hydrolysis of their glycosides in refluxing methanolic HCl. Additionally, dihydrophysapubenolide could be subjected to hydrolysis of a 15-acetoxy group with methanolic 5% KOH for 12 h, conditions which would be expected to open the lactone ring; nevertheless, the corresponding lactine was obtained upon acidification of the saponification mixture to pH 4 (13). Both of these results suggest that lactone formation is favored when the 24*Z* configuration occurs. Not only did we fail to observe the lactone as a product of either acidic or basic hydrolysis of **1**, but extended treatment of **3** with trifluoroacetic acid in dioxane failed to give the lactone. ^{1}H-nmr evidence supports this configurational assignment in which methyls 27 and 28 are trans oriented. We find that these methyls give much broader and lower peaks in **1** than do methyls 18 and 19, and the evidence for long range coupling is even more obvious in compounds **3** and **4**, in which H_3-27 and -28 appear as apparent doublets (J=ca. 1 Hz). By comparison, the corresponding signals in the nmr spectra of withanolide E and 4β-hydroxywithanolide E, which were obtained under the same conditions, appear as one lower, broad singlet (H_3-27, w 1/2=ca. 5 Hz) and one narrow singlet (H_3-28). In the latter examples these methyls are necessarily cis and long range coupling occurs only between H_2-23 and Me-27, whereas in **3** and **4** homoallylic coupling between the two trans methyls is observed.

Enzymatic hydrolysis of **1** with β-glucosidase gave **7** in which the glucose at position 3 had been removed as well as one glucose from the side-chain ester trisaccharide, but both acetoxy groups were retained. In the ^{1}H-nmr spectrum of **7**, the signals of glucose anomeric protons at δ_H 4.30 and 4.38 were no longer present, permitting full observation of the CH_2OAc methylene protons which appear as non-overlapping multiplets between ca. δ 4.1 and 4.3 and are correlated to a methylene carbon at δ_C 64.9, showing that one acetate is at position 6 of a glucose unit. Compound **7** undergoes loss of this acetate upon extremely mild methanolysis in neutral CD_3OD at ambient temperature ($t_{1/2}$ ca. 10 days) to give **9** with the disappearance of the above H_2-6 signal and of the acetyl resonance at δ 2.06 as well as shift of the methylene carbon to δ_C 62.6. In contrast, **1** is stable under these conditions. This acetoxy group is rendered labile by removal of the terminal glucose unit from the trisaccharide moiety, strongly suggesting that position 6 of the centrally located glucose unit bears this group. It might also be considered that the cessation of β-glucosidase activity at the disaccharide stage of **7** is a consequence of this acetoxy substituent, since it is known that increased steric bulk at position 6 decreases the rate of hydrolysis of β-glucosides by the enzyme (14). However, this result is equivocal inasmuch as the deacetylated analogue **9** is also unaffected upon

enzyme treatment. Nevertheless, the very low solubility of **9** in aqueous solution may be the determining factor in limiting the course of enzymatic hydrolysis in the latter case.

Linkage analysis of the trisaccharide portion of **1** and of the disaccharide portion of **7** was carried out by initial Hakomori methylation in DMSO (15) followed by hydrolysis to the methylated glucoses and conversion to the peracetyl aldonitrile (PAAN) derivatives (16). Under the conditions of methylation, any acetate groups are lost, and their positions are methylated. The Paan derivatives of 2,3,4,6-tetra-*O*-methylglucose (2 mol), 3,4,6-tri-*O*-methylglucose, and 2,3,6-tri-*O*-methylglucose were obtained from **1**, and PAAN's of 2,3,4,6-tri-*O*-methylglucose and 3,4,6-tri-*O*-methylglucose were identified from **7**. This establishes the linkage as $1''\mapsto 2'$ and $1'''\mapsto 4''$ for the respective glucose units of **1** and $1''\mapsto 2'$ for **7** and is consistent with the observation that the ^{13}C nmr of **7** showed a signal at 81.7 for the interglycosidic position (2′) with the corresponding anomeric carbon signal at 105.0 in agreement with values reported for those positions in sophorosides (17). In compound **1**, there are three signals near δ_C 71 that correspond to position 4 in glucosides (18), and a signal occurs at δ_C 80.9 (not present in **7**) for the additional interglycosidic carbon which must correspond to the remaining position 4″.

For compound **2**, obtained in a yield of 90 $mg\cdot kg^{-1}$, an empirical formula of $C_{58}H_{90}O_{28}$ was derived from *m/z* 1257 $[M+Na]^+$. All spectral information from **2** was very similar to that of **1**, but indicated that an additional acetate group was present at position 6 of a glucose unit. Upon incubation of **2** with β-glucosidase, the trisaccharide **8** was obtained that still retained three acetates, and extended enzyme treatment did not cause any further hydrolysis. In this example, it is clear that termination of enzymatic action is a result of acetylation at position 6‴ since the product of monodeglucosylation is completely soluble under the reaction conditions. Further evidence for acetylation at this position is provided by the slow conversion in MeOH at ambient temperature of **2** into **1** which was then stable to further methanolysis. This observation led to the realization that compound **1** might be an artifact of the original extraction, which involved extended exposure to MeOH containing not only the steroidal esters but also various plant acids that could accelerate methanolysis. To examine this point, a crude MeOH extract of plant material was separated from the most nonpolar constituents by partition between H_2O and $CHCl_3$ and examined immediately by hplc. Both **1** and **2** were observed, but compound **2** was present at about four times higher concentration than in the preparative workup. On standing, the proportion of **2** in the crude mixture rapidly declined and **1** increased. It is clear that a substantial amount of the **1** must have arisen by deacetylation of **2** during the original isolation.

It is of interest to compare the biological activity of **1** and **2** with some withanolides that may be present in *P. peruviana*. The $CHCl_3$ and Me_2CO extracts of leaves were observed to contain 4β-hydroxywithanolide E, which had already been shown to possess activity against certain insects (3,4). In our bioassay using *H. zea*, the dietary concentration required to reduce growth to 50% of control values (ED_{50}) was 250 $mg\cdot kg^{-1}$ for 4β-hydroxywithanolide E. A glycosylated withanolide which was present in the MeOH extract, perulactone 3-*O*-β-glucoside, had $ED_{50}=150$ $mg\cdot kg^{-1}$. By comparison, both **1** and **2** had $ED_{50}=35$ $mg\cdot kg^{-1}$, and we have found that semipurified fractions of the most polar material have even higher activity.

EXPERIMENTAL

GENERAL EXPERIMENTAL PROCEDURES.—Ir spectra and rotations were determined on Perkin-Elmer 237 and 241 instruments, and uv spectra were on a Hewlett-Packard 8451 spectrophotometer. ^1H-nmr spectra were obtained on Varian EM-390 and Nicolet NT-200 spectrometers at 90 and 200 MHz, respectively with ^{13}C-nmr spectra at 50 MHz on the latter instrument. Assignments were by H-H and C-

H correlation and by comparison with spectra of withanolides (19). Eims, cims, fabms and liquid sims were determined on a VG Co. Micromass 7070 HS instrument using NH_3 as reagent gas for cims and glycerol as matrix for fabms and liquid sims measurements. Laser desorption mass spectra were run on a Kratos "Kompact MALDI." Gc-ms analyses of methylated glucose PAAN derivatives (16) were obtained on a H-P 5890 gas chromatograph equipped with a Model 5971 mass selective detector using a 0.32 mm×50 m Se-30 column with programming from 105° to 300° at 30° per min. Comparisons were with the NBS mass spectral library and with authentic samples. Low resolution lc was on Sephadex LH-20, and hplc was on Rainin Dynamax C-18 and Altech RSil C-18 columns. Hplc grade solvents were utilized throughout, and detection was by uv at 215 and 228 nm with the activity followed by bioassay.

PLANT MATERIAL.—Seeds of *P. peruviana* were obtained locally, and a voucher specimen (G.D. Barbe, 1990) is at the State of California Division of Plant Industry, Analysis and Identification Branch, Sacramento, CA. Plants were greenhouse grown, and leaves were harvested at intervals and freeze-dried.

BIOASSAYS.—Materials on cellulose powder (5% of final diet wt) were combined with modified Berger-diet premix (20). Neonate larvae of *H. zea* were added, the insects were maintained at 26° for 10 days, and their weights were then compared with those of controls.

EXTRACTION AND FRACTIONATION.—Ground dry leaf material (2 kg) was stirred three times successively for 16 h with 10-liter portions of $CHCl_3$ and filtered. This procedure was then repeated with Me_2CO and MeOH. Bioassay indicated that the MeOH extract possessed strong insect toxicity, and that the $CHCl_3$ and Me_2CO extracts were considerably less active. The MeOH-soluble material (208 g) was dissolved in 500 ml H_2O and applied to a column (50 mm×100 cm) containing XAD-2 resin in H_2O. The column was washed with 2000 ml H_2O to remove nonadsorbed substances and flushed with 2000 ml MeOH to desorb bound material (70 g) which was then chromatographed on Sephadex LH-20/MeOH (50 mm×100 cm) in 10 separate runs to give highly bioactive material in elution volume 500 to 2500 ml (25 g). Hplc separation on Dynamax C-18 (41.2 mm×25 cm) in 1.0 g portions using a gradient of 25 to 40% aqueous MeCN yielded two major active fractions: fraction 6 (720–800 ml) and fraction 7 (800–880 ml), totaling 2.1 and 1.2 g, respectively. These were isocratically rechromatographed using 37.5% aqueous MeCN to give 0.34 g of **1** from fraction 6 (elution volume 296–312 ml) and 0.18 g of **2** from fraction 7 (elution volume 312–352 ml).

Compound **1**.—Amorphous powder: $[\alpha]^{25}D$ −23° (MeOH, c=1.0); ir ν max (KBr) 1725 br, 1630 cm^{-1}; uv λ max (MeOH) 230 nm (log ϵ max 4.06); ^1H nmr δ (CD_3OD) 0.74 (s, H_3-18), 1.02 (s, H_3-19), 1.06 (d, J=7 Hz, H_3-21), 1.92 (br s, H_3-27), 1.97 (s, OAc), 2.02 (br s, H_3-28), 2.07 (s, OAc), 2.68 (br t, J=12 Hz, ½H_2-23), 4.06 (m, H-3), 4.30 (d, J=8 Hz, H-1‴), ca. 4.3 (m, obscured, H_2-6″), 4.38 (d, J=8 Hz, H-1⁗), 4.70 (d, J=8 Hz, H-1″), 5.16 (br d, J=12 Hz, H-22), 5.53 (br d, J=5 Hz, H-6), 5.64 (d, J=8 Hz, H-1′); ^{13}C nmr see Table 1; liquid sims m/z $[M+Na]^+$ 1215 (1192+23), $[M+Na-glc]^+$ 1053, $[M+Na-3\times glc-Ac]^+$ 687, 551 ($C_{30}H_{47}O_9$). $C_{56}H_{88}O_{27}$+Na requires 1215.

Compound **2**.—Amorphous powder: $[\alpha]^{25}D$ −20° (MeOH, c=0.50); ir ν max (KBr) 1730 br, 1630 cm^{-1}; uv λ max (MeOH) 230 nm (log ϵ max 4.06); ^1H nmr δ (CD_3OD) 0.74 (s, H_3-18), 1.02 (s, H_3-19), 1.06 (d, J=7 Hz, H_3-21), 1.92 (br s, H_3-27), 1.97 (s, OAc), 2.02 (br s, H_3-28), 2.06 (s, OAc), 2.09 (s, OAc), 2.68 (br t, J=12 Hz, ½H_2-23), 4.05 (m, H-3), 4.10 (dd, J=12, 6 Hz, ½H-6⁗), 4.25 (dd, J=12, 5 Hz, ½H_2-6″), 4.35 (d, J=8 Hz, H-1‴), 4.38 (d, J=8 Hz, H-1⁗), 4.47 (br d, J=12 Hz, ½H-6⁗), 4.70 (d, J=8 Hz, H-1″), 5.16 (br d, J=12 Hz, H-22), 5.52 (br d, J=5 Hz, H-6), 5.64 (d, J=8 Hz, H-1′); ^{13}C nmr see Table 1; liquid sims m/z $[M+Na]^+$ 1257 (1234+23), $[M+Na-glc-Ac]^+$ 1053, $[M+Na-3\times glc-2Ac]^+$ 687, 593 ($C_{32}H_{49}O_{10}$). $C_{58}H_{90}O_{28}$+Na requires 1257.

Compound **3**.—Compound **1** (30 mg) was dissolved in 10 ml of MeOH to which 0.75 ml of AcCl had been added (ca. 1 M in HCl) and warmed at 65° for 3 h. After evaporation, the mixture was chromatographed on RSil C-18 (10 mm×25 cm, 70% MeCN/H_2O, elution volume 30–34 ml) to give **3** (10 mg): mp 227–229° (EtOAc/heptane); ir ν max ($CHCl_3$) cm^{-1} 3600, 3400 br, 1708, 1620; uv λ max (MeOH) 230 nm; ^1H nmr δ ($CDCl_3$) 0.73 (s, H_3-18), 1.01 (d, J=7 Hz, H_3-21), 1.04 (s, H_3-19), 1.92 (d, J=1 Hz, H_3-27), 2.00 (d, J=1 Hz, H_3-28), 3.74 (s, OMe), 3.86 (br s, H-1), 3.90 (br d partly obscured, H-22), 4.00 (7 lines, J=ca. 5 Hz, H-3), 5.60 (br d, J=5 Hz, H-6); ^{13}C nmr see Table 1; eims m/z $[M]^+$ 474.3324 (0.4), $[M-H_2O]^+$ 456 (4), $[M-C_7H_{11}O_2]^+$ 347 (0.5), $[M-C_7H_{11}O_2-H_2O]^+$ 329 (6), $[M-C_7H_{11}O_2-2H_2O]^+$ 311 (4), $[M-C_7H_{11}O_2-3H_2O]^+$ 293 (2), $[C_7H_{12}O_2]^+$ 128 (100), $[C_7H_{12}O_2-MeOH]^+$ 96 (41). $C_{29}H_{46}O_5$ requires $[M]^+$ 474.3345.

Sugar analysis of **1**.—Compound **1** (3 mg) was hydrolyzed in 1.0 ml of 1 N HCl at 80° for 2 h, and the solution passed through a Waters C-18 Sep Pak with an additional 2 ml of H_2O. After evaporation, the residue was converted to the TMSi ether and analyzed by glc on a 30 m×0.32 mm methyl silicone column. Only the α- and β-anomers of TMSi-glucose were present.

Compound **4**.—Acetylation of **3** (Ac_2O/pyridine) gave **4**, chromatographed on RSil C-18 (10 mm×25 cm, 100% MeCN, elution volume 28–32 ml): mp 183–186° (MeOH); $[\alpha]^{25}D$ −7.6° (MeOH, *c*=0.5); ir ν max ($CHCl_3$) 1720 br cm^{-1}; uv λ max (MeOH) 226 nm (log ε max 4.00); ^1H nmr δ ($CDCl_3$) 0.68 (s, H_3-18), 0.99 (d, *J*=7 Hz, H_3-21), 1.08 (s, H_3-19), 1.89 (d, *J*=ca. 1 Hz, H_3-27), 1.95 (d, *J*=1 Hz, H_3-28), 1.98 (s, OAc), 2.03 (s, OAc), 2.06 (s, OAc), 2.57 (dd, *J*=12, 13 Hz, ½ H_2-23), 3.71 (s, OMe), 4.91 (7 lines, *J*=ca. 5 Hz, H-3), 5.08 (br s, H-1), 5.12 (br d partly obscured, H-22), 5.54 (br d, *J*=5 Hz, H-6); ^{13}C nmr see Table 1; eims *m/z* $[M-HOAc]^+$ 540.3449 (2.0). $C_{33}H_{48}O_6$ requires 540.3451.

Compound **5**.—Compound **1** (60 mg) was dissolved in 5 ml of methanolic 0.1 M NaOMe and warmed at 60° for 3 h. After neutralization with HOAc and evaporation, the mixture was chromatographed on RSil C-18 (10 mm×25 cm, 40% MeCN/H_2O, elution volume 34–38 ml) to give **5** (11 mg): mp 226–229° (dec) (Me_2CO/H_2O); $[\alpha]^{25}D$ −27° (MeOH, *c*=0.5); ir ν max (KBr) 1700 cm^{-1} uv λ max (MeOH) 228 nm; ^1H nmr δ (CD_3OD) 0.76 (s, H_3-18), 1.00 (d, *J*=7 Hz, H_3-21), 1.02 (s, H_3-19), 1.89 (br s, H_3-27), 1.99 (d, *J*=1 Hz, H_3-28), 3.14 (t, *J*=8 Hz, H-2′), 3.70 (s, OMe), 3.82 (br s, H-1), 3.85 (br d partly obscured, H-22), 4.04 (7 lines, *J*=ca. 5 Hz, H-3), 4.37 (d, *J*=8 Hz, H-1′), 5.52 (br d, *J*=5 Hz, H-6); ^{13}C nmr see Table 1; cims (NH_3) *m/z* $[MNH_4]^+$ 654 (100), $[MNH_4-2\ H_2O]^+$ 618 (20), $[MNH_4-C_7H_{12}O_{12}]^+$ 526 (15), $[MNH_4-H_2O-C_6H_{12}O_6]^+$ 456 (62). $C_{35}H_{56}O_{10}$ requires $[MNH_4]^+$ 654.

Compound **6**.—Compound **1** (25 mg) was dissolved in 5.0 ml of MeOH and a solution of 75 mg of KIO_4 in 1.0 ml H_2O added. The mixture was stirred 4.5 h at 25°, and 0.05 ml glycerol was added to consume remaining periodate. After centrifugation to remove precipitate, the supernatant was stirred with 100 mg of $NaBH_4$ and then stored at 5° for 2 days. H_2O (10 ml) was added, the pH was adjusted to 1.0 with concentrated HCl, and the solution was warmed at 60° for 1.25 h. After removal of MeOH in vacuo, the mixture was extracted with $CHCl_3$ (5×10 ml) which was dried over Na_2SO_4 to give 8.0 mg of material upon evaporation. Crystals of **6** deposited from MeCN: mp 232–234° (dec) (MeCN); $[\alpha]^{25}D$ −16° (MeOH, *c*=0.25); ir ν max (KBr) 1685 br cm^{-1}; uv λ max (MeOH) 224 nm (log ε max 3.71); ^1H nmr δ (CD_3OD) 0.73 (s, H_3-18), 1.02 (s, H_3-19), 1.06 (d, *J*=7 Hz, H_3-21), 1.89 (br s, H_3-27), 1.95 (br s, H_3-28), 1.97 (s, OAc), 2.64 (dd, *J*=14, 12 Hz, ½ H_2-23), 3.80 (br s, H-1), 3.90 (7 lines, *J*=ca. 5 Hz, H-3 partly obscured), 5.17 (br dt, *J*=12, 3 Hz, H-22), 5.50 (br d, *J*=5 Hz, H-6); eims *m/z* $[M]^+$ 502.3319 (1.6). $C_{30}H_{46}O_6$ requires 502.3294.

Compound **7**.—Compound **1** (30 mg) was added to a solution of 20 mg β-glucosidase (EC 3.2.1.21, Sigma G 0395) in 10 ml of 0.1 M pH 5 acetate buffer. After 16 h at 35°, the solution was passed through a short column of XAD-2 resin, and the column was washed with H_2O. Bound material was desorbed with MeOH and then chromatographed on Dynamax C-18 (21.4 mm×25 cm, 45% MeCN-H_2O, elution volume 80–90 ml) to give **7** (10 mg) accompanied by **1** and by triglycosidic material. ^1H nmr δ (CD_3OD) 0.74 (s, H_3-18), 1.02 (s, H_3-19), 1.06 (d, *J*=7 Hz, H_3-21), 1.93 (br s, H_3-27), 1.97 (s, OAc), 2.02 (br s, H_3-28), 2.06 (s, OAc), 2.70 (br t, *J*=12 Hz, ½ H_2-23), 3.20 (t, *J*=8 Hz, H-2″), 4.15 (dd, *J*=12, 5, ½ H_2-6″), 4.26 (br d, *J*=12, ½ H_2-6″), 4.67 (d, *J*=8 Hz, H-1″), 5.17 (br d, *J*=12 Hz, H-22), 5.52 (br d, *J*=5 Hz), 5.64 (d, *J*=8 Hz, H-1′); ^{13}C nmr see Table 1; laser desorption ms *m/z* $[M+Na]^+$ 891 (868+23). $C_{44}H_{68}O_{17}$+Na requires 891.

Compound **8**.—Compound **2** (20 mg) was incubated with β-glucosidase as above and isolated by use of XAD-2 resin. Chromatography on RSil C-18 (10 mm×25 cm, 40% MeCN/H_2O, elution volume 24–30 ml) gave **8** (13 mg). Extended enzymatic treatment of **8** did not product further change. ^1H nmr δ (CD_3OD) 0.74 (s, H_3-18), 1.02 (s, H_3-19), 1.06 (d, *J*=7 Hz, H_3-21), 1.92 (br s, H_3-27), 1.97 (s, OAc), 2.02 (br s, H_3-28), 2.06 (s, OAc), 2.08 (s, OAc), 2.68 (br t, *J*=12 Hz, ½ H_2-23), 4.12 (dd, *J*=12, 7 Hz, ½ H-6‴), 4.25 (dd, *J*=12, 5 Hz, ½ H_2-6″), 4.35 (d, *J*=8 Hz, H-1‴), 4.46 (br d, *J*=12 Hz, ½ H-6‴), 4.67 (d, *J*=8 Hz, H-1″), 5.17 (br d, *J*=12 Hz, H-22), 5.50 (br d, *J*=5 Hz), 5.64 (d, *J*=8 Hz, H-1′); fabms *m/z* $[M+Na]^+$ 1095 (1072+23). $C_{52}H_{80}O_{23}$+Na requires 1095.

Compound **9**.—Compound **7** was stored in CD_3OD over a 30 day period, during which time the ^1H-nmr multiplet near δ 4.2 associated with the position 6 glucose acetate disappeared ($t_{1/2}$ ca. 10 days). Chromatography of **9** on RSil C-18 (10 mm×25 cm, 40% MeCN/H_2O, elution volume 19–23 ml). ^1H nmr δ (CD_3OD) 0.74 (s, H_3-18), 1.02 (s, H_3-19), 1.06 (d, *J*=7 Hz, H_3-21), 1.98 (br s, H_3-27), 1.99 (s, OAc), 2.06 (d, *J*=1 Hz, H_3-28), 2.72 (br t, *J*=12 Hz, ½ H_2-23), 3.18 (t, *J*=8 Hz, H-2″), 4.64 (d, *J*=8 Hz, H-1″), 5.17 (br d, *J*=12 Hz, H-22), 5.50 (br d, *J*=5 Hz), 5.64 (d, *J*=8 Hz, H-1′); ^{13}C nmr see Table 1; liquid sims *m/z* $[M+Na]^+$ 849 (826+23), $[M+Na-glc]^+$ 687, $[M+Na-2\times glc]^+$ 525. $C_{42}H_{66}O_{16}$+Na requires 849.

Methanolysis of **2** *to* **1**.—Compound **2** (6 mg) was dissolved in 5 ml of MeOH and stored at ambient temperature. Analysis by hplc indicated $t_{1/2}$ for the conversion to be about 20 days. Isolation of **1** was on RSil C-18 (10 mm×25 cn, 35% MeCN, elution volume 16–20 ml).

Treatment of **3** *with trifluoroacetic acid/dioxane.*—Compound **3** (10 mg) was dissolved in 1.0 ml of dioxane containing 0.5% TFA and allowed to stand 21 h at room temperature. After evaporation, only starting material **3** was recovered.

Sugar linkage analysis.—Permethylation of **1** was carried out in DMSO/dimethylsulfinyl carbanion (15) with MeI under argon. After extractive workup with $CHCl_3$ and evaporation, the sample was treated with 0.25 N H_2SO_4 at 95% for 17 h, neutralized with $BaCO_3$, and centrifuged, and the supernatant was evaporated. This material was dissolved in pyridine, and the PAAN derivatives (16) of the methyl glucoses were prepared by addition of hydroxylamine HCl and warming at 90° for 45 min followed by Ac_2O at 90° for an additional 30 min. Analysis by glc and gc-ms showed that only the PAAN derivatives of 2,3,4,6-tetra-*O*-methylglucose, 3,4,6-tri-*O*-methylglucose and 2,3,6-tri-*O*-methylglucose were present. Similarly, **2** gave the same PAAN derivatives. When applied to **7**, this procedure gave 2,3,4,6-tetra-*O*-methylglucose and 3,4,6-tri-*O*-methylglucose PAAN's.

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