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A SCREEN FOR INHIBITORS OF DNA RECOMBINATION: IDENTIFICATION OF TWO NEW SPIROSTANOL GLYCOSIDES FROM *CHAMAEDOREA LINEARIS*

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ABSTRACT.—Two new glycosides have been isolated from the MeOH extract of the stem wood and stem bark of an Ecuadorian plant *Chamaedorea linearis*, and their structures have been determined by spectroscopic means and X-ray analysis of the aglycone to be 1-*O*-[β-L-fucopyranosyl-(4′-sulfate)]-25*R*,5α-spirostane-1β,3β-diol [**1**] and 1-*O*-[β-L-fucopyranosyl-(4′-sulfate)]-25*R*,5α-spirostane-1α,3β-diol [**2**]. These compounds were identified in a screen for inhibitors of recombinational DNA repair. Cytotoxic activity was also demonstrated.

In our search for biologically active compounds from natural products with potential utility in the treatment of cancer, we established a screen for inhibitors of DNA recombination. Among the most prominent characteristics of transformed cells is genetic plasticity. Tumors appear to evolve toward more aggressive and malignant behavior (1). This progression is associated with increases in genetically altered tumor cell populations; the resultant heterogeneity is recognized as perhaps the major impediment to successful therapy (2). Evidence that genetic recombination plays a central role in the genomic plasticity of the malignant cell includes recombinational deletion of tumor suppressor genes (3), translocation and subsequent activation of protooncogenes (4), and recombinational initiation of gene amplification leading to oncogene overexpression or drug resistance (5).

We hypothesized that specific inhibitors of enzymes involved in recombination could be discovered by mechanism-based screening and that such agents might prove useful in cancer management. An effective inhibitor of recombination might be useful in: (a) controlling the genetic plasticity of tumor cell populations and thus slowing or halting malignant progression, (b) controlling the emergence of drug-resistant populations resulting from gene amplification, and (c) synergizing with established antineoplastic drugs by inhibiting DNA damage repair. Recombination pathways are poorly understood in mammalian cells, and appropriate recombination-deficient mutants are not adequately characterized or available. Thus, we opted to devise a facile screen using the yeast *Saccharomyces cerevisiae*, in which many of the proteins involved in recombination are well defined genetically and biochemically (6).

RESULTS AND DISCUSSION

A total of 3094 extracts of terrestrial plants, 1958 extracts of marine organisms, and 935 extracts of fungal fermentations were screened in the bioassay for inhibitors of DNA recombination. Organisms were extracted sequentially with two or three solvents of increasing polarity. Extracts of 8 plants (0.25%) were identified as having activity. The hit-rate was somewhat higher for marine organisms (0.51%) and fermentation broths (0.53%).

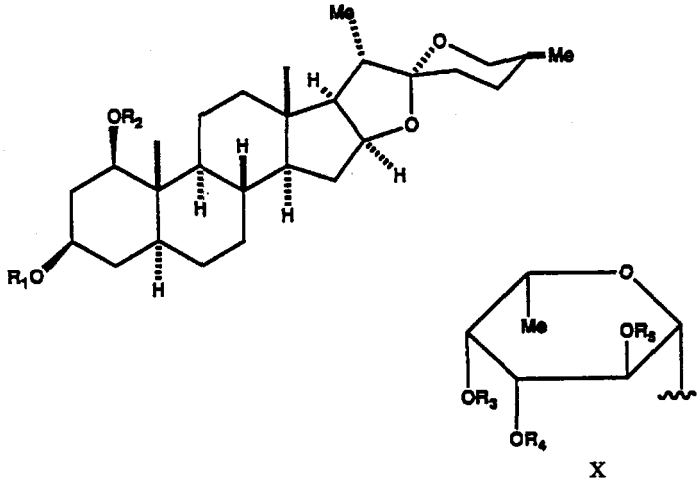
Among the plant extracts with reproducible activity were the MeCOEt and MeOH extracts of *Chamaedora linearis* Mart. (Araceae) collected in Ecuador. The crude MeCOEt extract produced a concentration-dependent zone of inhibition with in IC_{12} of 148 μg per well on anthramycin camptothecin amsacrine (ACA) agar, whereas no zone was

evident at 400 μg per well on control growth medium (i.e., in the absence of DNA-damaging drugs). This activity was used to follow fractionation.

Bioassay-directed fractionation of the crude MeOH extract by open reversed-phase (C_{18}) Si gel cc gave several fractions. Further purification of the combined active fractions by Si gel preparative thick layer chromatography yielded four glycosides, of which two are novel and identified as **1** and **2** on the basis of 2D nmr techniques combined with ms and X-ray analysis of the aglycone obtained from **1**.

The novel glycoside sulfate **1** was isolated as a white amorphous powder, and its ir (KBr) spectrum displayed strong bands at 3561, 3008 (OH), 1385, 1219, 1053 (sulfate), 980, 967, 920, and 900 cm^{-1} (spiroketal moiety). The positive ion fabms yielded $[M+2Na-H]^+$ 703.3109 ($C_{33}H_{53}O_{11}SNa_2$ requires *m/z* 703.3104). Fragment ions at *m/z* 601 and 455 correspond to loss of SO_3 from $[M+Na]^+$ and then further loss of deoxyhexose. Lack of observation of the loss of deoxyhexose directly from the pseudomolecular ion suggests that the sulfate residue is on the sugar moiety. The presence of sulfate was verified by the observation of an ion at *m/z* 97 $[HSO_4]^-$ and 80 $[SO_3]^-$ in the negative ion spectrum. The positive ion ms of the permethylated sample yielded an $[M+H]^+$ at 701, indicating the incorporation of 3 methyl groups. Loss of SO_3 and di-*O*-methyldeoxyhexose sulfate were indicated by ions at *m/z* 621 and 429 with further loss of MeOH to *m/z* 397.

The 400 MHz ^1H nmr (pyridine-d_5) of **1** is well resolved, and most of the protons resonate at slightly lower field compared to the spectrum in MeOH-d_4; such a pyridine-induced shift is well documented (7,8). Methyl singlets observed at δ 0.79 and 0.88 ppm were assigned to H-18 and H-19, respectively, of the aglycone. Methyl doublets appearing at δ 0.66 (J=5.1 Hz) and 1.04 ppm (J=6.91 Hz) were assigned to H-27 and H-21, while the sugar methyl doublet at δ 1.66 ppm (J=6.12 Hz) attests to the presence of deoxysugar. One anomeric proton doublet at 4.75 (J=7.56 Hz) appears in the downfield region. The high coupling constant value together with the ^{13}C-nmr chemical



X

1 R_1=R_4=R_5=H, R_2=X, R_3=SO_3H
2 R_1=R_4=R_5=Ac, R_2=X, R_3=SO_3H
3 R_1=R_2=H
4 R_1=R_3=R_4=R_5=H, R_2=X
5 R_1=R_3=R_4=R_5=Ac, R_2=X

shift (δ 101.5 ppm, Table 1) are consistent specifically with the β configuration (9,10). The ^{1}H-^{1}H 2D COSY together with ^{1}H-^{13}C HETCOR spectra confirmed the connectivities in the β-L-fucopyranosyl unit. After the acetylation of **1** with Ac_2O and pyridine, only the H-2′ and H-3′ of the sugar moiety suffered significant downfield shift (1–2 ppm), which confirmed that the sulfate group is present on C-4′ of the sugar. Furthermore, in the ^{1}H-nmr spectrum of the triacetate **2**, the H-3′ moved downfield by more than 1 ppm to δ 4.91 ppm, confirming that the sugar unit is present on the 1β- OH. The point of attachment of the sugar moiety in **1** is at the 1β-OH rather than the more commonly observed 3β-OH group. This is supported by the ^{13}C-nmr spectrum of the aglycone **3**, in which there is only a shift for C-1 downfield (by ca. 7 ppm) to δ 77.06 ppm.

Hydrolysis of **1** using methanolic HCl yielded aglycone **3** (fabms, *m/z* 433 $[M+H]^+$) identified as brisbagenin (11,15), and a methylated sugar which was subsequently hydrolyzed with 2 N trifluoroacetic acid to obtain a free sugar identified as L-fucose by high performance anion exchange chromatographic detection with pulsed amperometric detector [HPAE-PAD (Dionex Corp.)] as described by Anumula and Taylor (16). The aglycone **3** was crystallized from MeOH as colorless needles. Single

TABLE 1. Nmr Data for Compounds **1**, **3**, and **4** in Pyridine-d_5.

Position	Compound					
	1[a]		**3**		**4**	
	^{13}C (δ, ppm)	^{1}H (δ, ppm)	^{13}C (δ, ppm)	^{1}H (δ, ppm)	^{13}C (δ, ppm)	^{1}H (δ, ppm)
1	70.7 d	3.78 m	77.0 d	3.71 m	70.9 d	3.85 m
2	38.0 t	1.79, 2.75 m	40.0 t		38.3 t	1.76, 2.74 m
3	67.7 d	3.89 m	67.8 d	3.94 m	67.4 d	3.81 m
4	32.6 t	1.58, 1.72 m	32.5 t		32.2 t	1.57, 1.74 m
5	41.9 d	1.82 m	42.1 d		42.8 d	1.80 m
6	28.9 t		29.3 t		29.6 t	
7	32.5 t		31.9 t		32.9 t	
8	36.0 d		36.3 d		36.2 d	
9	54.7 d		56.0 d		54.8 d	
10	36.4 s		39.5 s		39.4 s	
11	23.7 t		24.8 t		23.3 t	
12	39.9 t		41.3 t		39.5 t	
13	41.5 s		41.0 s		41.3 s	
14	55.1 d		55.7 d		56.5 d	
15	38.3 t	1.43, 2.04 m	39.7 t		38.6 t	
16	81.1 d	4.52 (2.6,7.7)	81.1 d	4.58 (2.5,7.0)	81.9 d	4.51 (2.6,7.7)
17	63.2 d	1.81 m	63.2 d		63.9 t	1.83
18	17.3 q	0.79 s	17.3 q	0.80 s	17.1 q	0.84 s
19	16.8 q	0.88 s	16.7 q	1.12 s	17.0 q	1.00 s
20	42.6 d	1.95 m	43.0 d		42.8 d	1.96 m
21	14.9 q	1.04 d (6.9)	15.1 q	1.10 d (6.9)	14.6 q	1.05 d (7.0)
22	109.2 s		109.2 s		109.0 s	
23	32.0 t		32.5 t		31.5 t	
24	30.7 t		29.3 t		30.3 t	
25	30.2 t	1.25 m	29.7 t		30.1 d	
26	66.8 t	3.48, 3.52 m	66.9 t	3.49, 3.54 m	66.5 t	3.48, 3.54 m
27	8.1 q	0.66 d (5.1)	7.7 q	0.65 d (5.0)	8.0 q	0.69 d (5.0)
1′	101.5 d	4.75 d (7.6)			101.4 d	4.80 d (7.7)
2′	72.2 d	4.29 t (7.3)			72.3 d	4.33 t (7.1)
3′	74.1 d	4.20			74.0 d	4.05
4′	78.9 d	5.18 d (2.9)			80.8 d	4.08
5′	82.7 d	3.78 m			81.9 d	3.76 m
Me	17.5 q	1.66 d (6.1)			16.8 q	1.58 d (6.4)

[a]Assignments from 400 MHz. ^{1}H-^{1}H 2D COSY and HETCOR data. Multiplicities were obtained by DEPT spectra.

crystal X-ray diffraction analysis established the structure of **3** (Figure 1), which crystallized as a methanolate. Fractional atomic coordinates are presented in Table 2. The hydroxyl groups at C-1 and C-3 are β-oriented as are the methyl groups at C-10 and C-13. The C-20 methyl group is α-oriented. Thus, glycoside **1** is formulated as 1-*O*-[β-L-fucopyranosyl-(4′-sulfate)]-25*R*,5α-spirostane-1β,3β-diol.

Another novel compound, isolated as a minor metabolite, which displayed repair inhibitor activity, was identified as brisbagenin fucoside **4**, whose positive ion fabms showed an $[M+H]^+$ at *m/z* 579 ($C_{33}H_{55}O_8$) and a fragment ion at *m/z* 433 corresponding to the loss of deoxyhexose. In the ^{1}H-nmr spectrum of **4** (Table 1), because of the absence of a sulfate group, the 4′-H is observed at δ 4.08 ppm, as expected, while the remainder of the proton signals were virtually identical to those found for **1**. The ^{13}C-nmr spectrum of fucoside **4** shows 33 carbon signals (Table 1). The anomeric carbon appears at δ 101.42 ppm, suggesting the β configuration. The DEPT experiment and comparison with ^{13}C-nmr data for **1** result in the facile assignment of 27 of the carbon signals present in the aglycone as shown in Table 1. The remaining 6 carbon signals are in good agreement with those of one β-L-fucopyranosyl unit. Acetylation of **4** yielded a tetraacetate **5**, whose ^{1}H-nmr spectrum shows four singlets (3H each) at δ 2.15–2.17 ppm. Hydrolysis of **4** under conditions identical to those described for **1** gave aglycone **3** as confirmed by direct comparison with the aglycone obtained from the hydrolysis of **1**. The sugar moiety was identified as L-fucose. Thus fucoside **4** is 1-*O*-(β-L-fucopyranosyl)-25*R*,5α-spirostane-1β,3β-diol.

The purified active components demonstrated differential activity on ACA agar but also proved to be inhibitory to the test organism in the absence of DNA-damaging drugs. The IC_{12} values for the sulfated glycoside **1** were 3.5 μg per well on ACA agar and 12 μg per well on control agar (3.4-fold differential). Brisbagenin fucoside [**4**] had an IC_{12} of 2.6 μg per well on ACA agar as compared to 24 μg per well on control medium (9.2-fold differential). The aglycone was inactive up to 800 μg per well. A number of cardiac glycosides (e.g., ouabain, strophanthin, neriifolin, gitoxin, digoxin, and convallatoxin) were evaluated in this assay and all proved to be inactive.

Compounds **1** and **4** were also tested for effects on mammalian cells. The sulfated glycoside **1** had no effect at concentrations up to 32 μM on the proliferation of murine L1210 cells in suspension culture. In contrast, brisbagenin fucoside [**4**] had antiproliferative activity with an IC_{50} of 5.0 μM.

The basis for this screen is our observation that drug-permeable yeast mutants are

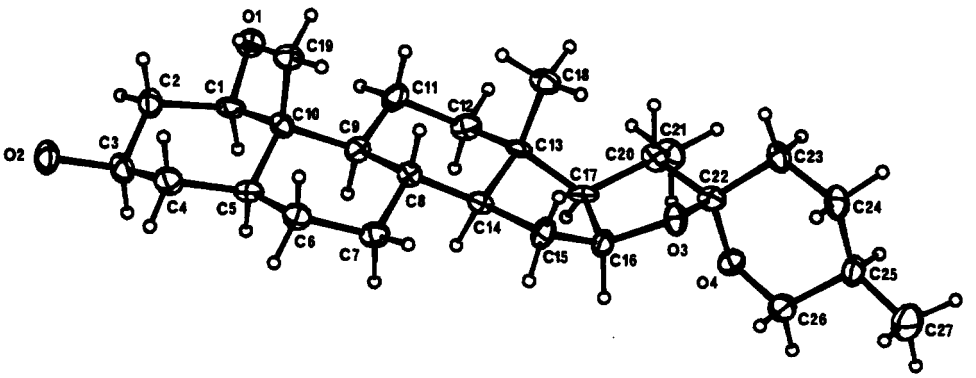


FIGURE 1. An ORTEP drawing of **3** showing the atom labelling scheme. Ellipsoids are drawn at the 50% probability level; hydrogen atoms as spheres of arbitrary size. Hydroxyl hydrogens have been omitted.

TABLE 2. Positional Parameters and ESDs for **3**.[a]

Atom	x	y	z	B(A2)
O-1	−0.1491 (6)	1.0901 (4)	0.8612 (1)	1.96 (8)
O-2	0.1538 (6)	0.8782 (4)	0.9672 (1)	2.35 (9)
O-3	0.1856 (6)	0.8433 (4)	0.5984 (1)	1.75 (8)
O-4	0.3891 (6)	0.9400 (4)	0.6421 (1)	1.78 (8)
O-5	−0.0273 (7)	0.6742 (4)	0.9966 (1)	3.1 (1)
C-1	−0.0264 (8)	0.9846 (5)	0.8700 (2)	1.6 (1)
C-2	0.0012 (9)	0.9786 (6)	0.9134 (2)	1.9 (1)
C-3	0.1274 (9)	0.8709 (6)	0.9264 (2)	2.0 (1)
C-4	0.3148 (8)	0.8854 (6)	0.9062 (2)	1.8 (1)
C-5	0.2870 (9)	0.8811(5)	0.8632 (2)	1.7 (1)
C-6	0.4712 (9)	0.8743 (6)	0.8419 (2)	1.8 (1)
C-7	0.4455 (8)	0.8545 (6)	0.7994 (2)	1.7 (1)
C-8	0.3093 (8)	0.9499 (5)	0.7814 (2)	1.2 (1)
C-9	0.1250 (8)	0.9575 (5)	0.8041 (2)	1.4 (1)
C-10	0.1601 (8)	0.9899 (6)	0.8472 (2)	1.4 (1)
C-11	−0.0200 (9)	1.0416 (6)	0.7836 (2)	1.9 (1)
C-12	−0.0529 (8)	0.9986 (6)	0.7421 (2)	1.8 (1)
C-13	0.1265 (8)	0.9958 (5)	0.7190 (2)	1.4 (1)
C-14	0.2648 (8)	0.9083 (5)	0.7403 (2)	1.3 (1)
C-15	0.4231 (8)	0.8901 (6)	0.7112 (2)	1.8 (1)
C-16	0.3132 (8)	0.8686 (6)	0.6744 (2)	1.6 (1)
C-17	0.1151 (8)	0.9252 (5)	0.6802 (2)	1.5 (1)
C-18	0.2017 (9)	1.1349 (6)	0.7142 (2)	1.8 (1)
C-19	0.2494 (8)	1.1253 (6)	0.8519 (2)	1.8 (1)
C-20	0.0796 (7)	1.0027 (6)	0.6437 (2)	1.5 (1)
C-21	−0.1167 (9)	0.9862 (6)	0.6266 (2)	2.1 (1)
C-22	0.2383 (8)	0.9638 (5)	0.6171 (2)	1.4 (1)
C-23	0.2962 (8)	1.0624 (6)	0.5881 (2)	1.8 (1)
C-24	0.4451 (9)	1.0142 (6)	0.5612 (2)	2.0 (1)
C-25	0.3857 (9)	0.8850 (6)	0.5429 (2)	2.0 (1)
C-26	0.3293 (9)	0.7917 (6)	0.5742 (2)	1.9 (1)
C-27	0.538 (1)	0.8242 (7)	0.5181 (2)	3.4 (2)
C-28	0.095 (1)	0.5670 (7)	0.9994 (2)	3.5 (2)

[a]Anisotropically refined atoms are given in the form of the isotropic equivalent displacement parameter defined as: $(8\pi^2/3)\sum_i\sum_j U_{ij}a_i^*a_j^*\bullet a_ia_j$.

quite refractory to a broad spectrum of DNA-damaging agents that are potent cytotoxins for mammalian cells. However, mutations that render the DNA recombination pathway (RAD52) inoperative result in a dramatic increase in sensitivity to agents that produce DNA damage by a variety of mechanisms. This hypersensitivity extends to ionizing radiation, DNA intercalators, DNA minor groove binders, alkylating agents, inhibitors of topoisomerase I and II, and DNA cleaving agents such as bleomycin. Thus, a potential inhibitor of DNA recombination could be detected by its ability to render a DNA-repair-proficient strain sensitive to such agents.

Although the triterpene glycosides described herein produce a larger zone of inhibition against yeast in the presence of DNA-damaging drugs, they also inhibit the DNA-repair-proficient strain at higher concentrations in the absence of such agents. A selective inhibitor of DNA recombination should not be inherently cytotoxic to yeast since deletion of genes encoding the proteins required for DNA recombination is not lethal. Recombination-deficient mutants proliferate at the same rate as wild-type

strains. It is possible that synergism with DNA-damaging agents could result from some effect of the brisbagenin fucosides other than interference with DNA recombination. Initial studies to assess the effects of the brisbagenin fucosides on the cytotoxicity of anthramycin to L1210 cells were not conclusive.

EXPERIMENTAL

GENERAL EXPERIMENTAL PROCEDURES.—Ir spectra were recorded on a Nicolet Model 20 DXB FTIR spectrometer. ^{1}H- and ^{13}C-nmr spectra, together with homonuclear and heteronuclear correlated 2D spectra, were recorded on a Bruker AM-400 spectrometer. Fabms were obtained on a VG ZAB-HF mass spectrometer. Analytical and preparative tlc were carried out on a precoated Si gel (Kiesel gel G254) and reversed-phase (Whatman $KC_{18}F$) plates. Sugar analysis was performed using high performance anion exchange chromatographic detection with pulsed amperometric detector. Reagent grade chemicals (Fisher and Baker) were used.

BIOASSAY.—DNA-repair-proficient *S. cerevisiae* strain RS188N (MATa ade2-1 his3-11 leu2-3,112 trp1-1 ura3-1 can1-100 nys^1) was used in an agar diffusion bioassay. (RS188N is a derivative of strain W303-1a and is deposited at SmithKline Beecham Pharmaceuticals.) The strain was plated on two media. Both were complete yeast peptone dextrose extract (YPD) growth medium, but, in addition, one (ACA medium) contained three DNA-damaging drugs: a topoisomerase II inhibitor, amsacrine (20 μg/ml); a topoisomerase I inhibitor, camptothecin (2 μg/ml); and the DNA minor groove binder, anthramycin (5 μg/ml). These concentrations of the drugs alone or individually had no effect on the growth of RS188N. However, in an isogenic DNA-recombination-deficient strain in which the RAD52 gene had been deleted, any of these drugs at the given or much lower concentrations completely inhibited growth. Thus, we screened for extracts which gave a zone of inhibition on ACA medium but no zone or a smaller zone of inhibition on control medium, i.e., an extract which made the repair-proficient strain as sensitive to the DNA-damaging agents as the repair-deficient strain.

Natural product extracts dissolved in MeOH-DMSO (1:1) were placed in 5 mm diameter wells punched in the agar. Extracts were tested at 2 mg/ml in a volume of 0.1 ml per well. Plates were incubated in a humidified changer at 30° for 48 h, and zones of inhibition were recorded. In order to be considered positive an extract had to produce at least a 6 mm differential in zone size on ACA medium. Concentration-response studies were performed to determine in IC_{12} (the concentration required to produce a 12 mm zone) by linear regression. For an extract to be considered for fractionation, the IC_{12} on ACA medium had to be at least 3-fold lower than that seen on control medium. In addition, positive extracts were evaluated for synergism with amsacrine, camptothecin, and anthramycin individually since a recombination inhibitor should increase sensitivity to each of these classes of DNA-damaging drugs. Lastly, leads were discriminated for the ability to enhance the sensitivity of the recombination-deficient Δ*rad52* yeast strain to amsacrine, camptothecin, and anthramycin to attempt to eliminate drugs acting by other mechanisms such as increasing permeability to the DNA-damaging agents.

PLANT MATERIAL.—*C. linearis* was collected at the Science Centre, Rio Palenque in Ecuador for phytochemical investigators by USDA under cooperative agreement with the National Cancer Institute in June 1980. A voucher specimen is preserved at the National Herbarium, Washington, DC.

EXTRACTION AND ISOLATION.—The dried stem wood and stem bark (800 g) was extracted three times for 7 day periods with MeOH by cold percolation procedure, yielding 15.8 g of extract. Cc of the MeOH extract [1.5 g, Whatman RP-18, 80 g, MeOH-H_2O (70:30)] gave several fractions (8–9 ml each). Fractions 9–14, which displayed recombinant repair inhibitor activity, were combined (69 mg) and subjected to repeated Si gel preparative thick layer chromatography using MeOH-CH_2Cl_2 (25:75) to get **1** (23 mg). The bioactive fractions 21–34 (48 mg), after repeated Si gel preparative thick layer chromatography employing MeOH-CH_2Cl_2 (15:85) furnished **2** (21 mg) in pure form.

Compound **1**.—Colorless, amorphous powder: ir (KBr) 3561, 3008, 1636, 1385, 1219, 1053, 980, 920, 900, 892 cm^{-1}; ^{1}H nmr and ^{13}C nmr see Table 1; fabms observed *m/z* $[M+2Na-H]^+$ 703.3109 ($C_{33}H_{53}O_{11}SNa_2$ requires *m/z* 703.3104).

Permethylation of **1**.—A portion (200 μg) of **1** was dissolved in DMSO and reacted with NaOH (8 mg). MeI (25 μl) was added and reacted for 20 min. The sample was dried under N_2 and partitioned first between $CHCl_3$ and 30% HOAc and then between $CHCl_3$ and H_2O. The organic layer was evaporated and the permethylated sample analyzed by fabms: *m/z* $[M+H]^+$ 701, $[M+H-$sulfate$]^+$ 621, $[M+H-$deoxyhexose sulfate$]^+$ 429, $[429-$MeOH$]^+$ 397.

Hydrolysis of **1**.—A mixture of fucoside sulfate (9 mg) and methanolic HCl (2.3 ml, prepared from

acetyl chloride and MeOH) was heated in a sealed tube at 80° for 6 h to get brisbagenin [**3**] (4.2 mg) and a methylated sugar, which was subsequently hydrolyzed with 2 N TFA to obtain a free sugar characterized by HPAE-PAD as L-fucose.

Compound **3**.—Colorless, crystalline solid: mp 202–204; ir (KBr) 3400, 1044, 862, 898 cm^{-1}; lr fabms *m/z* $[M+H]^+$ 433; ^1H and ^{13}C nmr see Table 1.

Acetylation of **1**.—A mixture of Ac_2O (0.5 ml), pyridine (0.25 ml), and **1** (4 mg) was stirred at room temperature for 24 h. This mixture was diluted with crushed ice and extracted with $CHCl_3$. Evaporation of the solvent gave a residue which was purified by Si gel preparative thick layer chromatography using MeOH-CH_2Cl_2 (15:85) to yield **2** as white powder (3.8 mg): ^1H nmr (pyridine-d_5) δ 5.89 (M, H-2′), 5.45 (m, H-3′), 5.38 (d, J=2.7 Hz, H-4′), 4.91 (m, H-3), 4.85 (d, J=7.2 Hz, H-1′), 4.44 (m, H-16), 3.89 (m, H-5′), 3.79 (m, H-1), 3.49 and 3.52 (m, 26-CH_2), 2.14, 2.13, 2.12 ppm (OAc); lr fabms *m/z* $[M+2Na-H]^+$ 829.

Compound **4**.—Colorless amorphous powder: ir (KBr) 3500, 2995, 1640, 1225, 1060, 892 cm^{-1}; ^1H and ^{13}C nmr see Table 1; lr fabms *m/z* $[M+H]^+$ 579, $[M-$deoxyhexose$]^+$ 433; hr fabms $[M+H]^+$ 579.3891 ($C_{33}H_{55}O_8$ requires *m/z* 579.3897).

Hydrolysis of **4**.—Fucoside **4** (6 mg) was hydrolyzed with methanolic HCl under conditions identical to those described for **1** to get **3** and a methoxy sugar which was subsequently hydrolyzed with 2 N TFA to get a free sugar which was identified as L-fucose.

Acetylation of **4**.—Fucoside **4** (5 mg) was acetylated with Ac_2O/pyridine under conditions identical to those described for **1** to yield tetraacetate **5** as white powder (4.8 mg): ^1H nmr (pyridine-d_5) δ 2.11, 2.13, 2.15, 2.17 (s, 3H each), 4.91 (m, 3-H).

X-RAY STRUCTURE DETERMINATION[1].—A crystal of approximate dimensions 0.60×0.50×0.15 mm was mounted on a glass fiber with epoxy cement. Lattice parameters were determined at 225 K from the setting angles of 25 high order reflections well distributed in reciprocal space on an Enraf Nonius CAD-4 diffractometer equipped with graphite monochromated molybdenum radiation (λ MoKα=0.71073 Å). A unique octant of data ($2\theta<56°$) was collected on the diffractometer. The 3588 intensities were corrected for background and Lorentz and polarization effects. Intensity standards collected every 3 h of exposure time showed a maximum increase of 8.2%; corrections were made to the data for these changes (min. 0.855, max. 1.131). The structure was solved by direct methods using MULTAN80 (17) and refined by full matrix least squares, which minimized the function $\sum w(|Fo|-|Fc|)^2$ where the weights were defined as $4Fo^2/LpS^2(I)$ with $S^2(I)=[\sigma^2(Ic)+(0.0016Ic)^2]$ using a locally modified version of SDP (18). Non-hydrogen atoms were refined with anisotropic displacements parameters. Hydrogen atom positions, with the exception of those for hydroxyls, were calculated based on geometrical considerations and held fixed along with isotropic temperature factors assigned as 1.3 (Beq) of the attached atoms. One MeOH molecule per molecule of **3** was also located and refined. A total of 1639 observations [$I>3\sigma(I)$] and 298 variables were refined to values of the conventional crystallographic residuals R=0.055, Rw= 0.060, S=1.383, max. Δ/σ=0.005. A final difference Fourier map was featureless ($\Delta\rho$ ±0.287 $eÅ^{-3}$). Neutral atom scattering factors (19) were used throughout.

CRYSTAL DATA.—$C_{27}H_{44}O_4$·MeOH, Orthorhombic, $P2_12_12_1$, Z=4, a=7.210(5), b=10.278(5), c=35.002 (15)Å, V=2593.8(16)$Å^3$, dcalc=1.190 $g\cdot cm^{-3}$, μ=0.742 cm^{-1}, F(000)=1024.

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[1]Atomic coordinates for this structure have been deposited with the Cambridge Crystallographic Data Centre and can be obtained on request from Dr. Olga Kennard, Cambridge Crystallographic Data Centre, 12 Union Road, Cambridge CB2 1EZ, UK.

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