Minutosides A and B, Antifungal Sulfated Steroid Xylosides from the Patagonian Starfish *Anasterias minuta*

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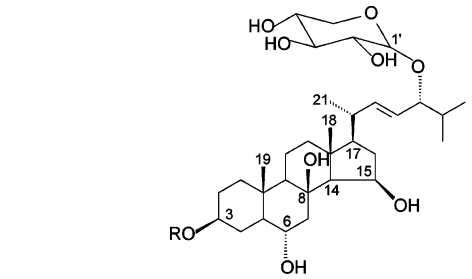
Two new sulfated polyhydroxylated steroidal xylosides, minutosides A (**1**) and B (**2**), together with the known pycnopodioside B (**3**), have been isolated from the brine shrimp active fraction of the ethanolic extract of the starfish *Anasterias minuta.* The structures have been elucidated by extensive 1D and 2D NMR as well as FABMS analysis and chemical methods. Compound **2** is the first example of a polyhydroxylated steroidal xyloside containing an amide function in the aglycon side chain. The three xylosides exhibited antifungal activity against *Cladosporium cucumerinum* and *Aspergillus flavus*.

Starfish are rich in sulfated saponins of the steroidal glycoside type. These compounds comprise asterosaponins (3β-sulfated, 6α-glycosylated steroids), a few examples of steroidal cyclic glycosides, and glycosides of polyhydroxylated sterols.[1,2] Starfish extracts have drawn attention because of their wide spectrum of biological effects: antifungal, cytotoxic, hemolytic, cytostatic, and immunomodulatory activities.[3] In the course of the search for bioactive metabolites from the starfish *Anasterias minuta* (Perrier, 1875) (family Asteriidae, order Forcipulatida), we have isolated two new sulfated steroidal hexaglycosides and established interesting correlations between their structures and antifungal activity.[4] Recently, we have reported a new glucosylceramide containing a C-18 sphingosine-type base from this starfish.[5] In our continuing search for new bioactive metabolites from *A. minuta*, we obtained two new steroidal xylosides, minutosides A (**1**) and B (**2**), together with the known pycnopodioside B (**3**), isolated for the first time from the starfish *Pycnopodia helianthoides*.[6] We describe herein the structural elucidation of **1** and **2** and the antifungal activities of **1**−**3** against the fungi *Cladosporium cucumerinum* and *Aspergillus flavus*.

Bioactivity-guided fractionation of the ethanolic extract of *A. minuta* using the brine shrimp (*Artemia salina* L.) larvae mortality bioassay[7] led us to the isolation of three fractions containing steroidal sulfated glycosides that exhibited significant brine shrimp lethality. Purification of the mixture of saponins by chromatography over Sephadex LH-60 and reversed-phase HPLC yielded pycnopodioside B (**3**) and the new minutosides A (**1**) and B (**2**).

Compound **3** was isolated as a white amorphous powder and identified as pycnopodioside B by comparison of the NMR and FABMS data with those reported previously.[8] Solvolytic desulfation of **3** rendered pycnopodioside A (**3a**), previously isolated from the starfish *Pycnopodia helianthoides* and identified by comparison of the NMR and FABMS data with literature data.[6] The D-configuration of the xylose unit in **3** was determined by GC analysis of the 1-[(*S*)-*N*-acetyl-(2-hydroxypropylamino)]-1-deoxyalditol acetate derivative following the procedure reported previously.[9]

Minutoside A (**1**) was obtained as a white amorphous powder. The molecular formula of **1** was established as $C_{32}H_{53}O_{12}SNa$ on the basis of the pseudomolecular ion peak at *m/z* 707.3046 $[M + Na]^+$ (calcd for $C_{32}H_{53}O_{12}SNa_2$, 707.3053). Comparison of the NMR data of **1** (Table 1) and **3** showed similarities except for the presence of an additional double bond in the side chain of **1**. ^1H and ^{13}C NMR data of compound **1** (Table 1) showed that it shares the same sterol nucleus with **3**. The main difference in the ^1H NMR spectrum consisted in the presence of two doublets of doublets at δ 5.40 and 5.34, which could be assigned to the Δ^{22} protons. The value of the coupling constant $J_{22,23}$ = 15.3 Hz suggested an *E*-configuration of the double bond in the side chain. The HMQC spectrum allowed the identification of the olefinic carbons at δ_C 141.1 (C-22) and



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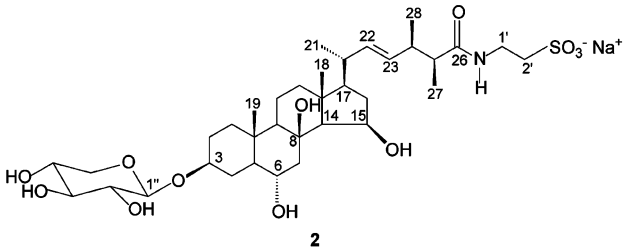
1a R = H



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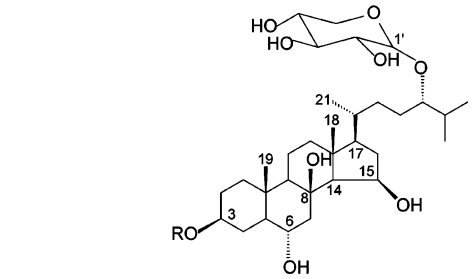

3 R = SO_3^- Na^+
3a R = H

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Table 1. ^{1}H NMR (500 MHz) and ^{13}C NMR (125 MHz) Data of **1** and **1a** in CD_3OD[a]

	1		**1a**
position	δ_H (mult., Hz)	δ_C	δ_C
1	1.71 m	39.4 CH_2	39.4 CH_2
2α	1.82 m	30.1 CH_2	31.6 CH_2
2*β*	1.55 m		
3	4.25 m	80.2 CH	72.1 CH
4	2.43 m	29.1 CH_2	32.3 CH_2
5	1.39 m	53.9 CH	54.0 CH
6	3.69 m	67.6 CH	67.7 CH
7α	1.24 m	49.2 CH_2	49.2 CH_2
7*β*	2.36 dd (12.5, 4.1)		
8		77.5 qC	77.5 qC
9	0.83 dd (12.0, 3.4)	57.3 CH	57.4 CH
10		37.9 qC	37.9 qC
11α	1.28 m	19.7 CH_2	19.7 CH_2
11*β*	2.00 m		
12	1.96 m	43.4 CH_2	43.2 CH_2
13		44.3 qC	44.2 qC
14	1.03 d (5.7)	62.6 CH	62.7 CH
15	4.40 m	71.1 CH	71.2 CH
16α	1.36 m	43.2 CH_2	43.2 CH_2
16*β*	2.24 m		
17	1.28 m	57.6 CH	57.6 CH
18	1.29 s	16.7 CH_3	16.7 CH_3
19	1.00 s	14.0 CH_3	14.0 CH_3
20	2.11 m	40.8 CH	40.8 CH
21	1.01 d (6.6)	20.8 CH_3	20.8 CH_3
22	5.40 dd (15.3, 5.4)	141.1 CH	141.0 CH
23	5.34 dd (15.3, 5.4)	128.1 CH	128.3 CH
24	3.69 m	89.2 CH	89.4 CH
25	1.81 m	33.8 CH	33.9 CH
26	0.86 d (6.8)	18.3 CH_3	18.4 CH_3
27	0.92 d (6.6)	19.3 CH_3	19.3 CH_3
1′	4.22 d (7.5)	104.3 CH	104.4 CH
2′	3.17 dd (9.1, 7.5)	75.4 CH	75.4 CH
3′	3.27 dd (9.1, 9.0)	78.1 CH	77.9 CH
4′	3.45 ddd (10.2, 9.0, 5.2)	71.2 CH	71.1 CH
5′α	3.13 dd (11.4, 10.2)	67.0 CH_2	66.8 CH_2
5′*β*	3.79 dd (11.4, 5.2)		

[a] The assignments were based on ^{1}H−^{1}H COSY, HMQC, DEPT, and NOESY experiments.

128.1 (C-23). The ^{1}H NMR spectrum also showed additional signals for the side chain: three methyl doublets at δ 0.86 and 0.92 (CH_3-26 and CH_3-27) and 1.01 (CH_3-21) and a multiplet at δ 3.69, which correlated in the HMQC spectrum with two signals at δ 67.6 (C-6) and 89.2. Correlations of the multiplet at δ 3.69 with H-25 (δ_H 1.81), H-23 (δ_H 5.34), and H-22 (δ_H 5.40) in the ^{1}H−^{1}H COSY spectrum indicated the presence of a $\Delta^{(22E)}$,24-hydroxycholestane side chain and allowed us to assign the signal at δ 89.2 to C-24. This chain has previously been found in glycosides of *Cosmasterias lurida*[10] and *Certonardoa semiregularis*.[11] Solvolysis of **1** in dioxane/pyridine afforded the desulfated derivative **1a**, whose negative ion FABMS exhibited a pseudomolecular ion peak at *m/z* 581 [M − H]$^-$, accompanied by a fragment ion at *m/z* 449 due to the loss of a xylose unit. An upfield shift of H-3 from δ 4.25 to 3.47 in the desulfated derivative **1a** (see Experimental Section) showed that the sulfate group was located at C-3. This was further confirmed by shifts of −8.1, +3.2, and +1.5 ppm for C-3, C-4, and C-2, respectively, in the ^{13}C NMR spectrum of **1a** (Table 1). Correlation of the signals at δ 4.25 (H-3) and 1.39 (H-5) in the NOESY spectrum of **1** indicated a *β*-configuration for the sulfate group attached to C-3.

In addition to the aglycon signals, the ^{1}H and ^{13}C NMR spectra of **1** showed one anomeric proton at δ 4.22 and an anomeric carbon at δ 104.3. The *β*-configuration of this sugar was deduced from the coupling constant value ($J_{1',2'}$ = 7.5 Hz). The presence of xylose was confirmed by acid hydrolysis of **1** with aqueous 2 N trifluoroacetic acid followed by GC analysis of the corresponding peracetylated alditol. The configuration of xylose was established as D by GC analysis of the 1-[(*S*)-*N*-acetyl-(2-hydroxypropyl-amino)]-1-deoxyalditol acetate derivative. The ^{13}C NMR spectrum established the sugar moiety to be linked, as in previous glycosides, at C-24 due to the downfield shift of C-24 from δ 79.3 in 5α-cholest-22*E*-ene-3*β*,6α,8,15*β*,24-pentol[11] to δ 89.2 in compound **1**. The NOESY experiment showed correlation of H-24 at δ 3.69 to H-1′ of the xylose unit at δ 4.22 and confirmed the attachment of the xylose unit at C-24. The 24*R* configuration is proposed by analogy with the co-occurring pycnopodioside B and the many (24*R*)-polyhydroxylated steroid glycosides isolated from starfishes.[12,13] Minutoside A (**1**) is isomeric with luridoside B,[10] the only difference being the location of the sulfate group at C-3 of **1** instead of C-4′ of the xylosyl moiety as in luridoside B.

Minutoside B (**2**) was obtained as a white amorphous powder. The negative FABMS gave molecular ion peaks at *m/z* 738 [M − H]$^-$ and 716 [M − Na]$^-$, while the positive FABMS gave molecular ion peaks at *m/z* 740 [M + H]$^+$, 762 [M + Na]$^+$, and 778 [M + K]$^+$, where M corresponds to a molecular weight of 739 Da. The molecular formula of **2** was established as $C_{35}H_{58}O_{12}NSNa$ on the basis of the pseudomolecular ion peak at *m/z* 762.3481 [M + Na]$^+$ (calcd for $C_{35}H_{58}O_{12}NSNa_2$, 762.3475) in the HRFABMS. An examination of ^{1}H and ^{13}C NMR spectra (Table 2) indicated that **2** possessed the same 3*β*,6α,8,15*β*-tetrahydroxy steroidal nucleus as minutoside A (**1**) and pycnopodioside B (**3**) bonded to a monosaccharide unit. ^{1}H−^{1}H COSY and HMQC spectra allowed the assignment of all the proton and carbon resonances. The relative stereochemistry of all chiral centers of the aglycon was established with the aid of a NOESY experiment. NOEs on H-9, 18-H_3, and 19-H_3 showed typical steroid configurations for C-9, C-10, and C-13. The hydroxyl groups at C-3 and C-6 were assigned to 3*β* and 6α on the basis of NOEs between H-3 and H-5α and between H_3-19 and H-6 (Figure 1). Correlations between H-14/H-15 and H-15/H-16α revealed the *β*-configuration of the hydroxy group at C-15.

The ^{1}H NMR spectrum contained three methyl doublets at δ 0.95, 0.96, and 1.07 and two multiplets integrating for one proton each at δ 5.20 and 5.22, respectively. In the ^{1}H−^{1}H COSY spectrum, the methyl doublet at δ 0.96 (CH_3-21) was coupled to a methine proton (H-20, δ_H 2.21), which in turn was coupled to one olefinic proton (H-22, δ_H 5.20) and a methine proton (H-17, δ_H 0.96). The ^{1}H−^{1}H COSY spectrum also showed correlations of the olefinic proton at δ 5.22 (H-23) and the multiplet at δ 2.08 (H-24). Further correlation of the latter signal to the methyl protons at δ 0.95 allowed the assignment of this signal to Me-28. Correlation of the methyl signal at δ 1.07 (Me-27) to the carbonyl group at δ 178.5 in the HMBC spectrum of **2** allowed us to assign all the methyl doublets in the side chain. The ^{1}H NMR spectrum also contained two methylene signals at δ 2.96 and 3.53 coupled to each other in the ^{1}H−^{1}H COSY spectrum. These protons correlated in the HMQC spectrum to the signals at δ 51.5 and 36.6, respectively. These data together with the correlation of the triplet at δ 2.96 to the Me-27 (δ_H 1.07) in the NOESY spectrum, the chemical shift of the carbonyl group (δ_C 178.5), and the presence of intense amide bands at 1653 and 1560 cm^{-1} in the FTIR spectrum of **2** suggested the presence of a $\Delta^{(22E)}$,26-amide ergostane side chain.[14] The strong bands at 1212 and 1050 cm^{-1} in the FTIR spectrum, characteristic

Table 2. ^{1}H NMR (500 MHz) and ^{13}C NMR (125 MHz) Data for Minutoside B (**2**) in CD_3OD[a]

position	δ_H (mult., Hz)	δ_C
1	1.51 m	39.4 CH_2
2α	1.78 m	29.8 CH_2
2β	1.58 m	
3	3.58 m	79.7 CH
4α	2.33 m	28.9 CH_2
4β	1.23 m	
5	0.99 m	53.6 CH
6	3.69 dt (10.7, 4.0)	67.7 CH
7α	1.25 m	49.4 CH_2
7β	2.37 dd (12.3, 4.0)	
8		77.5 qC
9	0.82 dd (11.3, 4.0)	57.3 CH
10		38.1 qC
11α	1.50 m	19.7 CH_2
11β	1.79 m	
12α	1.21 m	43.3 CH_2
12β	1.93 m	
13		44.2 qC
14	1.03 d (5.7)	62.7 CH
15	4.39 m	71.0 CH
16α	1.28 m	43.7 CH_2
16β	2.13 m	
17	0.96 m	57.5 CH
18	1.26 s	16.6 CH_3
19	0.97 s	14.0 CH_3
20	2.21 m	41.5 CH
21	0.96 d (6.6)	21.0 CH_3
22	5.20 m	137.9 CH
23	5.22 m	132.5 CH
24	2.08 m	41.1 CH
25	2.15 m	48.2 CH
26		178.5 qC
27	1.07 d (7.0)	18.7 CH_3
28	0.95 d (6.8)	15.7 CH_3
1′	2.96 t (7.1)	51.5 CH_2
2′	3.53 m	36.6 CH_2
1″	4.36 d (7.7)	103.1 CH
2″	3.13 dd (9.1, 7.7)	75.0 CH
3″	3.30 m	77.9 CH
4″	3.47 m	71.3 CH
5″α	3.18 dd (11.3, 10.4)	66.9 CH_2
5″β	3.81 dd (11.3, 5.2)	

[a] The assignments were based on ^{1}H−^{1}H COSY, HMQC, DEPT, and NOESY experiments.

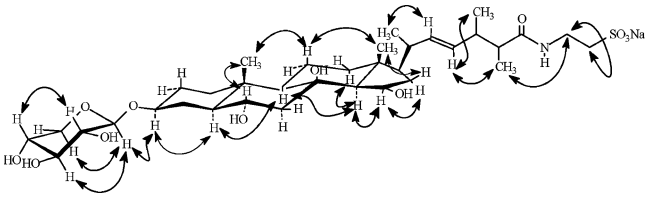

Figure 1. Selected NOESY correlations of **2**.

of a sulfonic acid salt, together with the fragment ion at *m/z* 455 $[M - SO_3Na - Xyl + Na]^+$ in the positive FABMS and the NMR data of **2** suggested the presence of a taurine residue in the side chain. This side chain has been previously identified in a polyhydroxysteroid isolated from the starfish *Myxoderma platyacanthum*.[14] Comparison of the ^{1}H NMR shifts of the olefinic protons of the side chain and those reported for synthetic 26-carboxysteroid models of known configuration[14] allowed us to assign the *threo* configuration at C-24 and C-25. We suggest the 24*R*,25*S* stereochemistry for **2** by comparison of the ^{1}H NMR data for the side chain of **2** and those of the polyhydroxylated steroidal amide isolated from *M. platyacanthum*.[14] The aglycone of **2** is coincident with triseramide, a steroidal conjugate isolated recently from the starfish *Astropecten triseriatus*.[15]

In addition to the aglycon signals, the ^{13}C NMR spectra of **2** showed one methylene carbon signal at δ_C 66.9 and four methine carbon signals at δ_C 103.1, 77.9, 75.0, and 71.3 (Table 2). All proton and carbon chemical shifts of the monosaccharide unit of **2** could be assigned using ^{1}H−^{1}H COSY and HMQC experiments. The chemical shift of the anomeric carbon (δ_C 103.1) and the coupling constant of the anomeric proton (δ_H 4.36, $J_{1',2'} = 7.7$ Hz) suggested that the sugar had a β-configuration. The location of the sugar at C-3 of the aglycon was established on the basis of the correlation between H-1″ and H-3α in the NOESY spectrum of **2**. The presence of xylose was confirmed by acid hydrolysis of **2** with aqueous 2 N trifluoroacetic acid followed by GC analysis of the corresponding peracetylated alditol. The D-configuration was determined by GC analysis of the 1-[(*S*)-*N*-acetyl-(2-hydroxypropylamino)]-1-deoxyalditol acetate derivative as for minutoside A (**1**).

Minutoside B (**2**) is the first example of a steroidal xyloside containing an amide function in the aglycon from a natural source. Only a few examples of polyhydroxylated sterols containing an amide function in their side chains have been reported previously from the starfishes *Myxoderma platyacanthum*[14] and *Styracaster caroli*.[16]

Since some polyhydroxylated steroid glycosides have shown interesting biological activities, such as cytotoxicity[17] and neuritogenic activity,[18] compounds **1**−**3** and the desulfated analogues **1a** and **3a** were examined against the pathogenic fungi *Cladosporium cucumerinum* and *Aspergillus flavus* by a bioautographic technique.[19] Pycnopodioside B (**3**) and minutoside A (**1**) were moderately active (inhibition zones of 7−10 mm) against *C. cucumerinum* at the tested concentrations (10−60 μg/spot), while minutoside B (**2**) was inactive at the lowest concentration (10 μg/spot) and weakly active (inhibition zones of 3−4 mm) at the highest concentrations (20−60 μg/spot). The three glycosides (**1**−**3**) were moderately active against *A. flavus*, showing inhibition zones of 5−10 mm at the highest tested concentrations (20−60 μg/spot). While minutoside B (**2**) was inactive at concentrations of 5−10 μg/spot, minutoside A (**1**) and pycnopodioside B (**3**) were moderately active (inhibition zones of 5−7.5 mm) at these concentrations. The compounds were found to be less active than benomyl, a commercially available fungicide, which showed inhibition zones of 15 and 14 mm at a concentration of 5 μg/spot for *C. cucumerinum* and *A. flavus*, respectively.

The desulfated analogues **1a** and **3a** were inactive against *C. cucumerinum* and *A. flavus* at all the tested concentrations. These results suggest that the presence of a sulfate group in the aglycon moiety might play an important role in the antifungal activity of these monoglycosides.

Experimental Section

General Experimental Procedures. IR spectra were recorded on a Nicolet Magna-550 FT-IR spectrometer. ^{1}H and ^{13}C NMR spectra were recorded in CD_3OD on a Bruker AM 500 spectrometer. FAB mass experiments were recorded in a glycerol matrix on a VG-ZAB mass spectrometer. Optical rotations were measured on a Perkin-Elmer 343 polarimeter. Preparative HPLC was carried out on a SP liquid chromatograph equipped with a Spectra Series P100 solvent delivery system, a Rheodyne manual injector, and a refractive index detector using a C_{18} Bondclone 10 μm column (30 cm × 7.8 mm i.d.). TLC was performed on precoated Si gel F254 (*n*-BuOH−HOAc−H_2O (12:3:5)) and C_{18} reversed-phase plates (65% MeOH−H_2O) and detected by spraying with *p*-anisaldehyde (5% EtOH). GC was performed on a Hewlett-Packard 5890A chromatograph equipped with a flame ionization detec-

tor, an SP-2330 column (25 m × 0.2 mm i.d.) (for analysis of the peracetylated alditol of xylose), and an ULTRA-2 column (50 m × 0.2 mm i.d.) (for analysis of 1-[(*S*)-*N*-acetyl-(2-hydroxypropylamino)]-1-desoxyalditol acetate of xylose).

Animal Material. Specimens of *A. minuta* were collected off the Golfo San Jorge near Comodoro Rivadavia, Chubut Province, Argentina. The organisms were identified by Dr. Alejandro Tablado of the Museo Argentino de Ciencias Naturales "Bernardino Rivadavia", Buenos Aires, Argentina, where a voucher specimen is preserved (MACN no. 34118).

Extraction and Isolation. The starfish (8 kg wet weight) were defrosted, cut into small pieces, homogenized in EtOH (8 L), and centrifuged. The EtOH extract was evaporated, and the aqueous residue was partitioned between H_2O and cyclohexane. The aqueous residue was passed through an Amberlite XAD-2 column (1 kg) and eluted with distilled H_2O (until a negative reaction of chloride was observed) followed by MeOH. The MeOH eluate was evaporated under reduced pressure to give a glassy material (8 g) toxic to the brine shrimp *Artemia salina* (LD_{50}: 540 ppm). The MeOH extract was subjected to vacuum-dry column chromatography[20] on Davisil C_{18} reversed-phase (35−75 μm) using H_2O, H_2O−MeOH mixtures with increasing amounts of MeOH, and finally MeOH as eluents. All the fractions (250 mL) eluted were evaluated for their lethality to *A. salina*. Fractions eluted with 50% (LD_{50}: 280 ppm), 60% MeOH (LD_{50}: 160 ppm), and 70% MeOH (LD_{50}: 80 ppm) contained the sulfated steroidal glycosides. These fractions were combined and chromatographed on a Sephadex LH-60 column with MeOH−H_2O (2:1) as eluent. Fractions containing the crude steroidal monoglycosides were finally submitted to repeated reversed-phase HPLC (ODS, MeOH−H_2O 65%) to give the pure glycosides **1** (6.3 mg), **2** (7.1 mg), and **3** (9.7 mg).

Minutoside A (1): white amorphous powder; $[\alpha]^{20}_D$ −14.7° (*c* 0.32, MeOH); ^{1}H and ^{13}C NMR, see Table 1; FABMS (negative ion mode), *m/z* 683 $[M - H]^-$, 661 $[M - Na]^-$, 511 $[M - Xyl\text{-}O - H - Na]^-$; FABMS (positive ion mode), *m/z* 685 $[M + H]^+$, 535 $[M - Xyl\text{-}O]^+$, 455 $[M - SO_3Na - Xyl + Na]^+$; HRFABMS *m/z* 707.3046 $[M + Na]^+$ (calcd for $C_{32}H_{53}O_{12}SNa_2$, 707.3053).

Minutoside B (2): white amorphous powder; $[\alpha]^{20}_D$ −22.9° (*c* 0.42, MeOH); IR ν_{max} (KBr) cm^{-1} 1653, 1560, 1212, 1050; ^{1}H and ^{13}C NMR, see Table 2; FABMS (negative ion mode), *m/z* 738 $[M - H]^-$, 716 $[M - Na]^-$, 584 $[M - Xyl + H - Na]^-$, 566 $[M - Xyl\text{-}O - H - Na]^-$; FABMS (positive ion mode), *m/z* 778 $[M + K]^+$, 762 $[M + Na]^+$, 740 $[M + H]^+$, 630 $[M - Xyl + H + Na]^+$, 612 $[M - Xyl\text{-}O - H + Na]^+$; HRFABMS *m/z* 762.3481 $[M + Na]^+$ (calcd for $C_{35}H_{58}O_{12}NSNa_2$, 762.3475).

Desulfation of Minutoside A (1) and Pycnopdioside B (3). A solution of each monoglycoside (2.5 mg) in pyridine (0.3 mL) and dioxane (0.3 mL) was heated at 120 °C for 2 h in a stoppered reaction vial. The reaction mixtures were cooled, poured into water (1 mL), and extracted with *n*-BuOH (3 × 0.5 mL). Each butanolic extract was evaporated to dryness at reduced pressure, and the residues were subjected to reversed-phase HPLC to give the pure desulfated glycosides, ds-minutoside A (**1a**) (1.1 mg) and pycnopodioside A (**3a**) (1.3 mg).

Ds-minutoside A (1a): white amorphous powder; ^{13}C NMR, see Table 1; FABMS (negative ion mode), *m/z* 581 $[M - H]^-$, 449 $[M - xylose]^-$; ^{1}H NMR δ_H (multiplicity, J = Hz) 5.39 (dd, 15.3, 5.4, 22-H), 5.34 (dd, 15.3, 5.4, 23-H), 4.39 (m, 15-H), 4.21 (d, 7.5, 1′-H), 3.78 (dd, 11.4, 5.2, 5′-H), 3.69 (m, 6-H), 3.47 (m, 3-H and 4′-H), 3.26 (dd, 9.1, 9.0, 3′-H), 3.17 (dd, 7.5, 9.1, 2′-H), 3.12 (dd, 11.4, 10.2, 5′-H), 1.28 (s, 18-H_3), 1.01 (d, 6.6, 21-H_3), 1.00 (s, 19-H_3), 0.92 (d, 6.6, 27-H_3), 0.85 (d, 6.6, 26-H_3).

Acid Hydrolysis of Compounds 1−3. A solution of each glycoside (1 mg) was heated in a screwcap vial with 2 N trifluoroacetic acid (0.2 mL) at 120 °C for 2 h. The aglycon was extracted with EtOAc, and the aqueous residue was evaporated under reduced pressure. The sugar mixture was treated with 0.5 M NH_3 (0.2 mL) and $NaBH_4$ (2 mg) at room temperature for 18 h. After acidification with 1 M AcOH, the reaction mixture was treated with MeOH (0.3 mL) and evaporated under reduced pressure. The alditol mixture was peracetylated with Ac_2O (0.2 mL) and pyridine (0.2 mL) at 100 °C for 45 min. The reaction mixture was cooled and poured into $CHCl_3$−H_2O (1:1), and the aqueous phase was extracted with $CHCl_3$. The combined chloroform extracts were washed with H_2O (0.3 mL), saturated $NaHCO_3$ solution (0.3 mL), and H_2O (0.3 mL) and evaporated to dryness under nitrogen. The peracetylated alditol was analyzed by GC using standard peracetylated alditols as reference samples.

Determination of the Absolute Configuration of Xylose in Compounds 1−3. A solution of each glycoside (2 mg) was heated in a screwcap vial with 2 N trifluoroacetic acid (0.5 mL) at 120 °C for 2 h. The aglycon was extracted with EtOAc, and the aqueous residue was evaporated under reduced pressure. Then, the following solutions were added: (a) 1:8 (*S*)-1-amino-2 propanol in MeOH (20 μL), (b) 1:4 glacial AcOH−MeOH (17 μL), and (c) 3% $Na[BH_3CN]$ in MeOH (13 μL), and the mixture was allowed to react at 65 °C for 1.5 h. After cooling, 3 M aqueous CF_3CO_2H was added dropwise until the pH dropped to pH 1−2. The mixture was evaporated and further coevaporated with H_2O (2 × 0.5 mL) and MeOH (0.5 mL). The residue was acetylated with Ac_2O (0.5 mL) and pyridine (0.5 mL) at 100 °C for 75 min. After cooling, the derivatives were extracted with $CHCl_3$−H_2O (1:1) (2 × 1 mL). The chloroform extracts were washed with H_2O (0.5 mL), saturated $NaHCO_3$ solution (0.5 mL), and H_2O (0.5 mL) and evaporated to dryness under nitrogen. The 1[(*S*)-*N*-acetyl-(2-hydroxypropylamino)]-1-deoxyalditol acetate derivative of xylose was identified by co-GC analysis with standard xylose derivatives prepared under the same conditions. The derivatives of D- and L-xylose were detected with t_R (min) of 29.75 and 29.95, respectively, while those obtained from compounds **1**−**3** were observed at 29.71 min.

Antifungal Assay. Geometric dilutions were obtained from freshly prepared stock solutions of minutosides A (**1**) and B (**2**), pycnopioside B (**3**), the desulfated analogues ds-minutoside A (**1a**) and pycnopodioside A (**3a**), and reference compound (benomyl) at concentrations of 1−10 mg mL^{-1} in an appropriate solvent. Of these solutions, 10 μL was applied on the TLC plates using graduated capillaries. After that, the plates were sprayed with a suspension of *C. cucumerinum* (DSM 62122) or *A. flavus* (BAFC 589) in a nutritive medium and incubated 2−3 days in a glass box with a moist atmosphere.[19] Clear inhibitions zones appeared against dark gray background. All samples were measured in duplicate. Data given in the text are averages of these measurements.

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