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Patagonicosides B and C, two Antifungal Sulfated Triterpene Glycosides from the Sea Cucumber *Psolus patagonicus*

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Abstract

Two new triterpene glycosides, patagonicosides B (**2**) and C (**3**), together with the known patagonicoside A (**1**), have been isolated from the ethanolic extract of the sea cucumber *Psolus patagonicus.* The structures of the new compounds were established on the basis of extensive NMR spectroscopy (^{1}H and^{13}C NMR,^{1}H–^{1}H COSY, HMBC, HSQC, TOCSY, and NOESY), HRESIMS, and chemical transformations. Compounds **1–3** and their desulfated analogs showed antifungal activity against the phytopathogenic fungus *Cladosporium cladosporoides* in a dose dependent activity.

Keywords

Psolus patagonicus; antifungal activity; sulfated triterpene glycosides

Introduction

Triterpene oligoglycosides are secondary metabolites found in sea cucumbers (Class Holothuroidea). The majority of these saponins contain an aglycon based on a “holostanol” skeleton [3β,20*S*-dihydroxy-5α-lanostano-18,20-lactone] and a sugar chain of two to six monosaccharide units linked to the C-3 of the aglycon [1]. These substances have a wide spectrum of biological effects: antifungal, cytotoxic, hemolytic, cytostatic, and immunomodulatory activities [2]. Recently, we have demonstrated the cytotoxic, hemolytic and antiproliferative activities of patagonicoside A (**1**), the major disulfated triterpene glycoside from the sea cucumber *P. patagonicus* [3–4].

As part of our research on secondary metabolites of biological significance from cold water echinoderms of the South Atlantic [5–8] we have reinvestigated the glycoside fraction of *P. patagonicus* in order to characterize the minor secondary metabolites. We report here the isolation and structure elucidation of two new sulfated triterpene glycosides, patagonicosides B (**2**) and C (**3**), as well as the results of the antifungal activity by bioautography against the phytopathogenic fungus *Cladosporium cladosporoides* of glycosides **1–3** and their desulfated derivatives obtained by solvolysis.

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Results and Discussion

The ethanolic extract of *P. patagonicus* exhibited antifungal activity against *C. cladosporoides*. Antifungal bioautographic assay-guided fractionation of the extract led to the isolation of an active fraction containing sulfated triterpene glycosides. Purification of this fraction by vacuum-dry column chromatography on C_{18} reversed–phase Si gel and finally by reversed-phase HPLC on a Bondeclone C_{18} yielded two new triterpene glycosides, patagonicosides B (**2**) and C (**3**) together with patagonicoside A (**1**), the major component of *P. patagonicus* [3]. The structures of compounds **2** and **3** were elucidated by NMR spectroscopic methods (^{1}H and^{13}C NMR,^{1}H–^{1}H COSY, HMBC, HSQC, TOCSY, NOESY), HRESIMS, and desulfation reactions.

An examination of the^{1}H and^{13}C NMR spectra of patagonicoside B (**2**) suggested the presence of a triterpenoid aglycon with two olefinic bonds, two hydroxy groups, and one lactone carbonyl group bonded to an oligosaccharide chain composed of four sugar units (Tables 1 and 2). The structure of the aglycon of **2** was determined on the basis of spectroscopic data and by their comparison with those of patagonicoside A (**1**). The aglycon of **2** belongs to the holostane type [from the signals of the 18(20)-lactone at δ 177.5 C(18) and 87.3 C(20) ppm in the^{13}C NMR spectrum] and contains a 7(8)-double bond [δ_C 120.7 C(7) and 148.2 C(8); δ_H 5.79 (1H, H-C(7)]. The presence of two hydroxy groups attached to C-12 and C-17 was evidenced by two signals in the downfield region of the^{13}C NMR spectrum, δ_C 72.5 ppm and 90.0 ppm, respectively, together with a signal at δ_H 4.74 ppm H-C(12) in the^{1}H NMR spectrum. NOESY correlations between H-12/H-9 and H-9/H-19 revealed the α configuration of the hydroxy group at C-12 while the correlation between H-12/H-21 supported the α configuration of the hydroxy group at C-17 and consequently the *S* configuration of C-20. In the side chain, a 25(26)-double bond [from signals of C(25) at 145.9 ppm and C(26) at 111.1 ppm] in the^{13}C NMR spectrum and a multiplet at 4.73 H-C(26) ppm in the^{1}H NMR spectrum [6] was indicated (Table 1). The structure of the aglycon of patagonicoside B (**2**), holosta-7,25-diene-3β,12α,17α-triol, is reported for the first time in a natural triterpenoid glycoside.

The carbohydrate chain of **2** consisted of four monosaccharide residues as deduced from the^{13}C NMR spectrum, which showed the signals of four anomeric carbons at 104.7–105.2 ppm, correlated by the HSQC spectrum with the corresponding signals of anomeric protons at 4.66 (d, *J* = 7.2 Hz), 4.79 (d, *J* = 8.1 Hz), 5.01 (d, *J* = 7.8 Hz), and 5.25 (d, *J* = 7.8 Hz) (Table 2). The coupling constants of the anomeric protons were indicative of β-configuration of the glycosidic bonds. In the NMR spectra of the carbohydrate chain of **2**, the signals of two xyloses, one quinovose and a 3-*O*-methylglucose residue were identified. All proton and carbon chemical shifts of the oligosaccharide chain of **2** (Table 2) could be assigned using^{1}H–^{1}H COSY, HSQC, HMBC, TOCSY and NOESY experiments. The^{1}H NMR spectrum of **2** showed the presence of a doublet at δ 1.62 (*J* = 6.1) due to the methyl group of the quinovose unit, and a singlet at δ 3.84 ppm corresponding to the methoxy group of 3-*O*-methylglucose (Table 2). Signals at δ_C 18.2 (c, C-6´´) and 61.2 (c, OCH_3) in the^{13}C NMR spectrum support the presence of a 6-deoxyhexose and a methoxy group. The three carbohydrate units belong to the D-series, as determined by the GC-MS analysis of the mixture of 1-[(*S*)-*N*-acetyl-(2-hydroxypropylamino)]-1-deoxyalditol acetate derivatives following the procedure previously described [8].

The interglycosidic linkages were deduced from NOESY (Figure 1) and HMBC correlations, where cross-peaks between H-C(1')of the xylose residue and H-C(3) of the aglycon, H-1" of the quinovose and H-C(2') of the xylose residue, H-C(1"') of the second xylose (the third monosaccharide unit) and H-C(4") of the quinovose unit and H-C (1"") of 3-*O*-methylglucose and H-C(3"') of the second xylose unit, correspondingly, were observed.

The HRESIMS (negative ion mode) of patagonicoside B (**2**) exhibited a pseudomolecular ion peak at *m/z* 1151.4991 (calc. 1151.49449) $[M - Na]^-$. MS/MS spectrum of this ion indicated the peaks for fragment ions at *m/z* 805.2918 [M - 3-*O*-Me-Glc-*O*-Xyl - 2H – Na]$^-$, 665.1704 [M – OAgl - Na]$^-$, and 423.3297 [M - 3-*O*-Me-Glc-*O*-Xyl-*O*-Qui-*O*-Xyl-$ONaSO_3$ - CO_2]$^-$ confirming the sequence of monosaccharide units in the carbohydrate chain. This and NMR spectroscopic data allowed the determination of the molecular formula as $C_{53}H_{83}O_{25}SNa$. Solvolysis of glycoside **2** gave the desulfated derivative, ds-patagonicoside B (**2a**). Comparison of ^{13}C NMR spectroscopic data of patagonicoside B (**2**) (Table 2) with its desulfated derivative **2a** (Table 3) confirmed the presence of one sulfate group. Thus, the downfield shift (by 4.0 ppm) of C(4') signal and the upfield shift of C(5') (by 3.8 ppm) and C(3') (by 3.5 ppm) signals of the xylose residue in the spectrum of **2** in comparison with those of **2a**, indicated the attachment of one sulfate group to C(4') of xylose. The oligosaccharide part of **2** is identical to the sugar chain of neothyonidioside, isolated from the sea cucumbers *Stichopus mollis* [9] and *Neothyonidium magnum* [10], and of intercedenside A, isolated from *Mensamaria intercedens* Lampert [11].

On the basis of all the above data, the structure of patagonicoside B (**2**) was established as 3-*O*-[3-*O*-methyl-β-D-glucopyranosyl-(1→3)-β -D-xylopyranosyl-(1→4)-β -D-quinovopyranosyl-(1→2)-4-*O*-sodium sulfate-β-D-xylopyranosyl]-holosta-7,25-diene-3β, 12α,17α-triol.

The molecular formula of patagonicoside C (**3**) was established as $C_{54}H_{86}O_{29}S_2Na_2$ by the pseudomolecular ion $[M - Na]^-$ at *m/z* 1285.45487 in the HRESIMS (negative ion mode) and the NMR spectroscopic data. The ^1H and ^{13}C NMR data of patagonicoside C (**3**) (Tables 1 and 2) suggested the presence of the same aglycon as patagonicoside A (**1**) bonded to an oligosaccharide chain composed by four sugar units. All proton and carbon chemical shifts of the oligosaccharide chain of **3** (Table 2) could be assigned using $^1H2013^1H$ COSY, HSQC, HMBC, TOCSY and NOESY experiments. Comparison of the ^{13}C NMR spectrum of the sugar chain of **3** with that of patagonicoside A (**1**) (Table 2) showed that signals of the first and the second monosaccharide units were similar, suggesting a sulfated xylose at C-4 as the first monosaccharide unit and quinovose as the second residue. The differences were in the third (glucose) and fourth (3-*O*-methylglucose) monosaccharide units. In the glucose unit of glycoside **3**, the signal of C-6"' was shifted upfield 6.2 ppm and C(5"') was shifted downfield 3.0 ppm, indicating that the glucose unit in **3** is not sulfated as in glycoside **1**. On the contrary, in the 3-*O*-methylglucose unit, the signal of C(6"") was shifted downfield 5.6 ppm and C(5"") was shifted upfield 3.2 ppm, corresponding to α- and β-shifted effects of sulfate groups [13]. Hence, patagonicosides A (**1**) and C (**3**) are isomers that differ in the position of the second sulfate group in the sugar chain. In order to confirm the position of the sulfate groups in the carbohydrate moiety of patagonicoside C (**3**), we obtained the desulfated derivate (**3a**) by solvolysis of **3** in a dioxane/pyridine mixture. Comparison of ^{13}C NMR spectroscopic data of patagonicoside C (**3**) (Table 2) with those of its desulfated derivate **3a** (Table 3) confirmed the presence of two sulfate groups. Thus, the downfield shift (by 4.6 ppm) of C(4') signal and the upfield shift of C(5') (by 2.1 ppm) and C(3') (by 2.3 ppm) signals of the xylose residue in the spectrum of **3** in comparison with those of **3a**, indicated the attachment of one sulfate group to C(4') of xylose. Similarly, the downfield shift (by 6.0 ppm) of C(6"") signal and the upfield shift (by 2.9 ppm) of C(5"") signal of the 3-*O*-methylglucose residue in the spectrum of **3** in comparison with those of **3a**, indicated the attachment of the second sulfate group to C-6"" of 3-*O*-methylglucose.

The positions of the interglycosidic linkages were corroborated by the NOESY spectrum of **3**, where the cross-peaks between H-C(1')of the xylose residue and H-C(3)of the aglycon, H-C(2') of the xylose and H-C(1") of the quinovose, H-C(4") of the quinovose and H-C(1"')

of the glucose, and H-C(1"") of the terminal 3-*O*-methylglucose and H-C (3"') of the glucose were observed.

On the basis of all the above data, the structure of patagonicoside C (**3**) was established as 3-*O*-[6-*O*-sodiumsulfate-3-*O*-methyl-β-D-glucopyranosyl-(1→3)-β-D-glucopyranosyl-(1→4)-β-D-quinovopyranosyl-(1→2)-4-*O*-sodium sulfate-β-D-xylopyranosyl]-holost-7-en-3β,12α,17α-triol.

Patagonicosides A (**1**), B (**2**) and C (**3**) are the only examples reported so far of holothurins with a Δ^7, 3β,12α,17α-trihydroxy holostane type aglycon.

As patagonicoside A (**1**) and its desulfated analog have shown antifungal activity against the phytopatogenic fungi *Cladosporium cucumerinum* [3], *Cladosporium fulvum*, *Fusarium oxysporum*, and *Monilia sp.* [13], patagonicosides A (**1**), B (**2**) and C (**3**), and their desulfated analogs were examined against the pathogenic fungus *Cladosporium cladosporoides* by a bioautography technique [14]. Benomyl (methyl-1-(butylcarbamoyl)-2-benzimidazolecarbamate) [15], a commercially available fungicide, was used as reference compound. As shown in Figure 2, glycosides **1**–**3** and their desulfated derivatives showed a marked difference in their antifungal properties. Patagonicoside A (**1**) resulted to be considerably active, in a dose dependent activity, showing inhibitions zones of 10–22 mm at the tested concentrations (5–20 μg/spot). Glycosides **2** and **3** were less active than **1** but more active than their desulfated analogs **2a** and **3a**, which were inactive at the lowest concentration (5 μg/spot) and weakly active (inhibition zones of 6 and 7 mm, respectively) at the highest tested concentration (20 μg/spot). As compounds **1** and **3** are isomers and differ only in the position of one of the sulfate groups, these results suggest that not only the presence but also the position of sulfate groups in the oligosaccharide chain plays an important role in the antifungal activity of these triterpene glycosides.

Experimental Part

General

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 Bondclone 10μ column (30 cm×7.8 mm i.d.). The samples were eluted with CH_3CN/H_2O (70:30) with a flow rate of 2 ml/min. TLC was performed on precoated Si gel F254 (n-BuOH/HOAc/H_2O (12:3:5)) and C18 reversed-phase plates (60% MeOH/H_2O) and detected by spraying with p-anisaldehyde (5% EtOH). ^1H and ^{13}C NMR spectra were recorded in C_5D_5N/D_2O (4:1) on a Bruker AM 500 spectrometer. HRESIMS (negative ion mode) were obtained on a Bruker Daltonic microOTOF-QII mass spectrometer (Bruker Daltonic Billerico, MA, USA). Optical rotations were measured on a Perkin-Elmer 343 polarimeter. 1-[(*S*)-N-acetyl-(2-hydroxypropylamino)]-1-deoxyalditol acetate derivates of monosacharides were analyzed using a Shimadzu GCMS-QP5050 chromatograph equipped with a capillary column (Ultra II Hewlett Packard, 50 m×0.20 mm).

Animal Material

Specimens of *P. patagonicus* were collected by scuba diving at a depth of 5–12 m in The Bridges Islands, Ushuaia, (Tierra del Fuego, Argentina) in February 2007. The organisms were identified by Dr. C. Muniain. A voucher specimen is preserved at the Museo Argentino de Ciencias Naturales "Bernardino Rivadavia", Buenos Aires, Argentina (MACN N° 34776).

Extraction and Isolation

The sea cucumbers (70 g), frozen prior to storage, were homogenized in EtOH (2.5 l) and centrifuged. The EtOH was evaporated and the residue was partitioned between MeOH/H_2O (9:1) (400 ml) and cyclohexane (200 ml). The glassy material obtained after evaporation of the methanolic extract was subjected to vacuum dry column chromatography on Davisil C_{18} reversed-phase (35–70 μm) using H_2O, H_2O/MeOH mixtures with increasing amounts of MeOH and finally MeOH as eluents. Fractions (250 ml) were analyzed by TLC, indicating that the triterpene glycosides were eluted with 50% and 40% MeOH. These fractions were combined and submitted to reversed-phase HPLC to give the pure glycosides **1**(45.0 mg; t_R 70 min), **2** (3.9 mg; t_R 24.5 min) and **3**(3.8 mg; t_R 45 min).

Patagonicoside B (2)

white amorphous powder, $[\alpha]^{20}_D$ –40.6 (*c* 0.16, pyridine);^{1}H and^{13}C NMR data, see Tables 1 and 2; (−) HRESIMS, *m/z* 1151.4991 [M - Na]$^-$, $C_{53}H_{83}O_{25}S$, (calcd 1151.49449); (−) HRESIMS/MS of the ion [M - Na]$^-$ at *m/z* 1151.5111, *m/z* 805.2918 [M - 3-*O*-Me-Glc-*O*-Xyl – Na]$^-$, 665.1704 [M – OAgl - Na]$^-$, 423.3297 [M - 3-*O*-Me-Glc-*O*-Xyl-*O*-Qui-*O*-Xyl-$ONaSO_3$ - CO_2]$^-$.

Patagonicoside C (3)

white amorphous powder, $[\alpha]^{20}_D$ –16.9 (*c* 0.36, CH_3OH);^{1}H and^{13}C NMR data, see Tables 1 and 2; (-) HRESIMS *m/z* 1285.45487 [M - Na]$^-$, $C_{54}H_{86}O_{29}S_2Na$, (calcd 1285.45938), 1263.47534 [M - 2Na + H]$^-$, (calcd 1263.47751).

Desulfation of patagonicosides B (2) and C (3)

A solution of **2** (3.0 mg) or **3** (3.5 mg) in pyridine (0.3 ml) and dioxane (0.3 ml) was heated at 120 °C for 2.5 h in a stoppered reaction vial. The reaction mixture was cooled, poured into water and extracted with *n*-BuOH. The butanolic extract was evaporated to dryness at reduced pressure and the residue was subjected to reversed-phase HPLC to give the pure desulfated glycosides, ds-patagonicoside B (**2a**) (2.0 mg) or ds-patagonicoside C (**3a**) (2.3 mg).

Desulfated Patagonicoside B (2a)

white amorphous powder, $[\alpha]^{20}_D$ −26.74 (*c* 0.1, CH_3OH);^{1}H and^{13}C NMR data, see Table 3; (−) HRESIMS *m/z* 1071.5287 [M - H]$^-$, $C_{53}H_{83}O_{22}$ (calcd 1071.53769).

Desulfated Patagonicoside C (3a)

white amorphous powder,^{1}H and^{13}C NMR spectra were identical with literature data for ds-Patagonicoside (**1a**).[3]

Determination of the Absolute Configuration of the Carbohydrate Subunits

A solution of **2a** (1.2 mg) or **3a** (1.4 mg) in 2 N trifluoroacetic acid (1 ml) was heated at 120 °C for 2 h. After extracting with CH_2Cl_2, the H_2O layer was concentrated to furnish the monosaccharide mixture. Then, the following solutions were added: (a) 1:8(*S*)-1-amino-2-propanol in MeOH (20 μl), (b) 1:4 glacial AcOH-MeOH (20 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 whit H_2O (3×0.5 ml) and MeOH (3×0.5 ml). The residue was acetylated with Ac_2O (0,5 ml) and pyridine (0,5 ml) at 100 °C for 0,75 h. After cooling, the derivates were extracted whit $CHCl_3$-H_2O (1:1). The chloroform extracts were washed with H_2O (3×1 ml) and with saturated $NaHCO_3$ solution (3×1 ml) and finally whit

H_2O (1×1 ml).The organic phase was dried with $NaHCO_3$ anhydrous. The mixture of 1-[(*S*)-*N*-acetyl-(2-hydroxypropylamino)]-1-deoxyalditol acetate derivatives of the monosacharides was identified by co-GC-MS analysis with standard sugar derivatives prepared under the same conditions.

Antifungal assay

Solutions of patagonicosides A (**1**), B (**2**), and C (**3**), ds-patagonicoside analogs and reference compound (benomyl) were prepared in an appropriate solvent and applied on the TLC plates (5–20 μg/spot of each compound) using a Hamilton syringe. After that, the plates were sprayed with a suspension of *C. cladosporoides* in a nutritive medium and incubated 2–3 days in a glass box with moist atmosphere [14]. Clear inhibitions zones appeared against dark grey background. All samples were measured in duplicate. Data given in Figure 2 are averages of these measurements.

Acknowledgments

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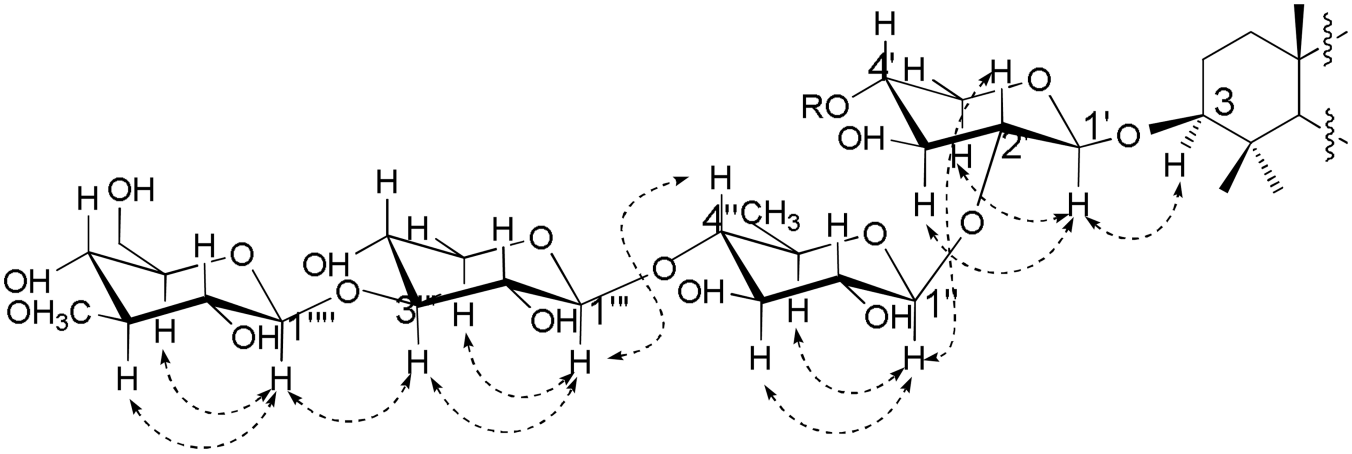


Figure 1.
NOESY correlations of the oligosaccharide chain of patagonicoside B (**2**).

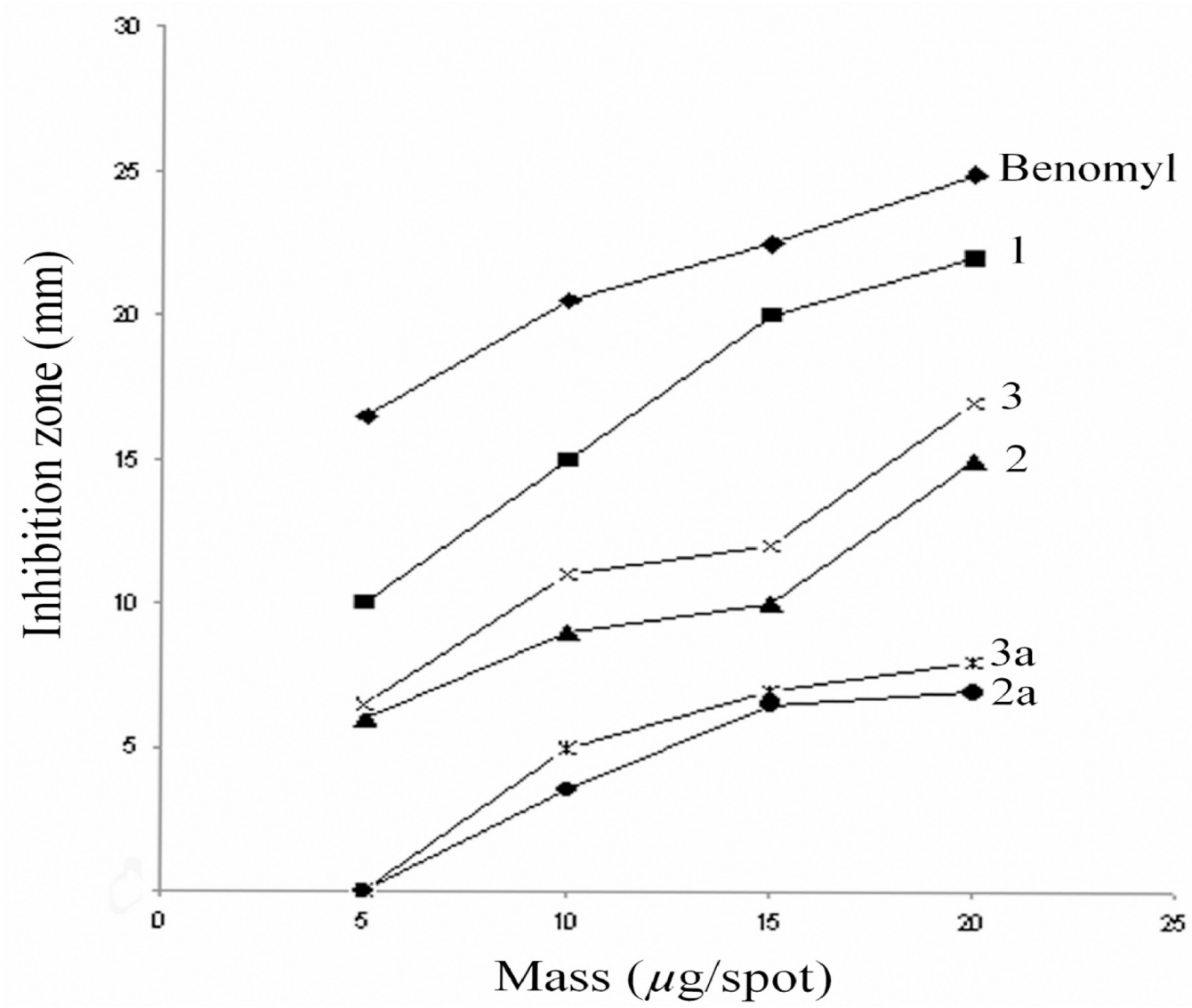
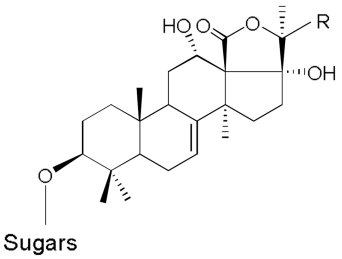
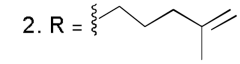


Figure 2.
Dose-response curves for the antifungal activity of patagonicosides A (**1**), B (**2**), C (**3**), desulfated patagonicosides B (**2a**) and C (**3a**), and benomyl against *C. cladosporoides*.

Formulas



2. R =



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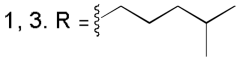
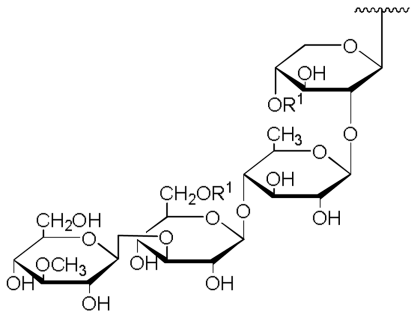
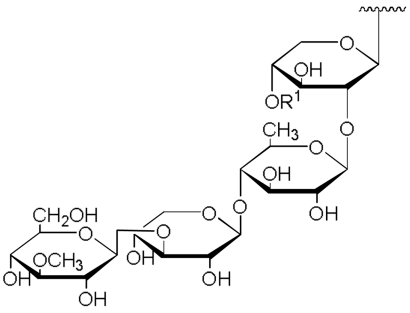




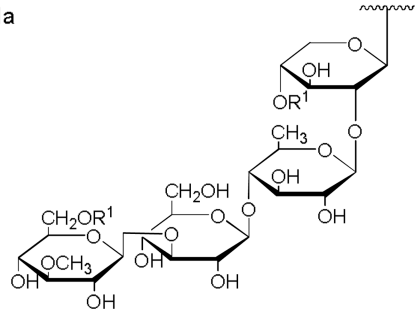
1. $R^1 = SO_3Na$

1a. $R^1 = H$



2. $R^1 = SO_3Na$

2a. $R^1 = H$



3. $R^1 = SO_3Na$

3a. $R^1 = H$

Table 1

NMR Spectroscopic Data of Aglycon Moieties of the Glycosides **2** and **3**[a,b]

	2		**3**	
position	**δ_C, mult.**	**δ_H (*J* in Hz)**	**δ_C, mult.**	**δ_H (*J* in Hz)**
1	36.5, CH_2	1.28–1.36 (m)	36.8, CH_2	1.49–1.53 (m)
2	27.7, CH_2	1.73–1.77 (m), 1.87–1.94 (m)	27.7, CH_2	1.90–1.94 (m), 2.07–2.12 (m)
3	89.5, CH	3.07–3.15 (m)	89.4, CH	3.23–3.30 (m)
4	39.8, qC		40.1, qC	
5	49.3, CH	0.89–0.96 (m)	49.5, CH	0.97–1.14 (m)
6	23.5, CH_2	1.90–1.96 (m)	23.7, CH_2	2.08–2.16 (m)
7	120.7, CH	5.75–5.82 (m)	120.7, CH	5.83–5.89 (m)
8	148.2, qC		148.6, qC	
9	45.6, CH	3.26, brd (15.3)	45.9 CH	3.50–3.57 (m)
10	35.7, qC		36.1, qC	
11	35.7, CH_2	1.90–1.95 (m), 2.43–2.53 (m)	36.4, CH_2	2.07–2.16 (m), 2.51–2.60 (m)
12	72.5, CH	4.72–4.77 (m)	72.6, CH	4.85–4.92 (m)
13	59.4, qC		59.5, qC	
14	51.5, qC		51.8, qC	
15	35.2, CH_2	1.50–1.57 (m), 1.98–2.02 (m)	35.2, CH_2	1.73–1.77 (m), 2.05–2.08 (m)
16	35.9, CH_2	2.24–2.30 (m), 2.43–2.53 (m)	36.5, CH_2	2.34–2.40 (m), 2.62–2.70 (m)
17	90.0, qC		90.2, qC	
18	177.5, qC		177.1, qC	
19	24.4, CH_3	1.09, s	24.7, CH_3	1.27, s
20	87.3, qC		87.1, qC	
21	23.1, CH_3	1.71, s	23.4, CH_3	1.83, s
22	37.8, CH_2	1.70–1.75 (m)	39.0, CH_2	1.82–1.91 (m)
23	22.4, CH_2	1.50–1.56 (m)	22.7, CH_2	1.50–1.56 (m)
24	38.4, CH_2	1.89–1.95 (m)	40.1, CH_2	1.12–1.21 (m)
25	145.9, qC		28.4, CH	1.49–1.54 (m)
26	111.1, CH_2	4.70–4.75 (m)	23.1, CH_3	0.87, s
27	22.5, CH_3	1.64, s	23.0, CH_3	0.86, s
30	17.6, CH_3	0.98, s	17.7, CH_3	1.10, s
31	29.0, CH_3	1.15, s	29.1, CH_3	1.27, s
32	31.3, CH_3	1.75, s	31.6, CH_3	1.91, s

[a] Recorded at 500 MHz (^1H) and 125 MHz (^{13}C) in C_5D_5N/D_2O (4:1).

[b] Assigned by a combination of ^1H-^1H COSY, HMBC, HSQC, TOCSY and NOESY experiments.

Table 2

NMR Spectroscopic Data of the Sugar Moieties of the Glycosides **1**–**3**[a,b]

Carbon		**2**		**3**		**1**
position	**δ_C, mult.**	**δ_H (*J* in Hz)**	**δ_C, mult.**	**δ_H (*J* in Hz)**	**δ_C, mult.**	**δ_H (*J* in Hz)**
1’	105.2, CH	4.66, d (7.2)	105.6, CH	4.66, d (7.3)	105.6, CH	4.79, d (7.3)
2’	82.5, CH	3.99–4.04 (m)	83.6, CH	4.00–4.04 (m)	83.3, CH	4.01–4.08 (m)
3’	75.3, CH	4.27, t (8.9)	75.5, CH	4.27–4.34 (m)	75.7, CH	4.26.4.32 (m)
4’	76.4, CH	4.96–4.99 (m)	76.4, CH	5.10–5.15 (m)	76.6, CH	5.18–5.24 (m)
5’	64.3, CH_2	3.75–3.79 (m), 4.77–4.84 (m)	64.6,CH_2	3.64–3.67 (m), 4.57–4.63 (m)	64.7, CH_2	3.85–3.88 (m), 4.88–4.91 (m)
1”	105.0, CH	5.01, d (7.8)	105.4, CH	4.97, d (7.8)	105.3, CH	5.00, d (7.8)
2”	76.1, CH	3.84–3.90 (m)	76.0, CH	3.87–3.93 (m)	76.2, CH	3.98–4.04 (m)
3”	75.1, CH	3.94–4.01 (m)	75.6, CH	4.00–4.05 (m)	75.6, CH	4.03–4.10 (m)
4”	86.0, CH	3.52, t (9.1)	88.9, CH	3.46–3.52 (m)	88.1, CH	3.48–3.54 (m)
5”	71.9, CH	3.66–3.71 (m)	72.0, CH	3.72–3.76 (m)	72.2, CH	3.71–3.77 (m)
6”	18.2, CH_3	1.62, d (6.1)	18.5,CH_3	1.64, d (6.0)	18.5, CH_3	1.73, d (6.0)
1”’	104.8, CH	4.79, d (8.1)	105.4, CH	4.83, d (7.5)	105.2, CH	4.87, d (7.5)
2”’	73.8, CH	3.88–3.93 (m)	74.3, CH	4.01–4.06 (m)	74.2, CH	4.01–4.07 (m)
3”’	86.5, CH	4.14–4.19 (m)	87.5, CH	4.24–4.29 (m)	86.9, CH	4.34–4.40 (m)
4”’	69.1, CH	3.92–4.00 (m)	70.9, CH	3.85–3.90 (m)	70.3, CH	3.89–3.95 (m)
5”’	66.2, CH_3	4.12–4.17 (m), 3.62–3.67 (m)	78.6, CH	3.97–4.03 (m)	75.6, CH	4.36–4.41 (m)
6”’			62.3,CH_2	4.46–4.51 (m), 4.26–4.32 (m)	*68.5*, CH_2	4.78–4.81 (m), 5.22–5.25 (m)
1””	104.7, CH	5.25, d (7.8)	106.1,CH	5.39, d (8.0)	105.6, CH	5.42, d (7.9)
2””	74.9, CH	3.86–3.92 (m)	75.4, CH	3.97–4.03 (m)	75.5, CH	4.03–4.09 (m)
3””	87.2, CH	3.69–3.74 (m)	88.4, CH	3.68–3.73 (m)	88.1, CH	3.80–3.87 (m)
4””	70.8, CH	3.84–3.90 (m)	70.9, CH	4.09–4.14 (m)	71.2, CH	4.10–4.15 (m)
5””	77.8, CH	3.92–3.96 (m)	75.3, CH	4.01–4.06 (m)	78.5, CH	4.05–4.12 (m)
6””	62.2, CH_2	4.02–4.06 (m), 4.38, br d (10.0)	68.2, CH_2	4.77–4.81 (m), 5.15–5.21 (m)	62.6, CH_2	4.27–4.33 (m), 4.55–4.60 (m)
OCH_3	61.2, CH_3	3.84, s	61.2,CH_3	3.86, s	61.2, CH_3	3.86, s

[a] Recorded at 500 MHz (^{1}H) and 125 MHz (^{13}C) in C_5D_5N/D_2O (4:1).

[b] Assigned by a combination of ^{1}H-^{1}H COSY, HMBC, HSQC, TOCSY and NOESY experiments.

Table 3

NMR Spectroscopic Data of the Sugar Moieties of the Desulfated Glycosides **2a** and **3a**[a,b]

Carbon position	2a		3a	
	δ_C, mult.	δ_H (*J* in HZ)	δ_C mult.	δ_H (*J* in Hz)
1’	106.2, CH	4.88, d (7.2)	105.6, CH	4.78, d (7.3)
2’	84.9, CH	4.13–4.18 (m)	83.6, CH	4.04–4.09 (m)
3’	78.8, CH	4.26–4.30 (m)	77.8, CH	4.24–4.30 (m)
4’	72.4, CH	3.82–3.88 (m)	71.8, CH	3.75–3.79 (m)
5’	68.1, CH_2	3.69–3.76 (m), 4.35–4.40 (m)	66.7, CH_2	3.71–3.76 (m), 4.35–4.39 (m)
1”	106.4, CH	5.27, d (7.6)	105.4, CH	5.15, d (7.5)
2”	77.1, CH	4.15–4.18 (m)	76.2, CH	4.03–4.07 (m)
3”	76.2, CH	4.16–4.18 (m)	75.1, CH	3.96–4.02 (m)
4”	86.6, CH	3.72–3.78 (m)	87.1, CH	3.66–3.71 (m)
5”	69.4, CH	3.81–3.86 (m)	71.8, CH	3.75–3.80 (m)
6”	18.6, CH_3	1.84, d (6.1)	18.5, CH_3	1.74, d (6.1)
1”’	105.9, CH	4.93, (7.9)	104.7, CH	5.00, d (7.8)
2”’	74.1, CH	4.03–4.08 (m)	73.9, CH	4.03–4.08 (m)
3”’	88.1, CH	4.20–4.25 (m)	87.5, CH	4.31–4.34 (m)
4”’	69.7, CH	4.09–4.15 (m)	69.8, CH	3.97–4.02 (m)
5”’	67.2, CH_3	4.26–4.31 (m), 3.70–3.76 (m)	77.8, CH	4.02–4.08 (m)
			62.1, CH_2	4.46–4.53 (m), 4.13–4.19 (m)
1””	106.1, CH	5.39, d (7.8)	105.2, CH	5.34, d (7.8)
2””	75.7, CH	4.06–4.11(m)	75.8, CH	4.08–4.11 (m)
3””	88.7, CH	3.73–3.78 (m)	87.6, CH	3.76–3.81 (m)
4””	71.3, CH	4.22–4.27 (m)	70.9, CH	4.00–4.05 (m)
5””	78.9, CH	4.01–4.06 (m)	78.2, CH	4.02–4.07 (m)
6””	62.8, CH_2	4.52–4.57 (m), 4.33–4.39 (m)	62.2, CH_2	4.46–4.53 (m), 4.13–4.19 (m)
OCH_3	61.4, CH_3	3.94, s	61.2, CH_3	3.90, s

[a] Recorded at 500 MHz (^1H) and 125 MHz (^{13}C) in C_5D_5N/D_2O (4:1).

[b] Assigned by a combination of ^1H-^1H COSY, HSQC and NOESY experiments.