Starfish Saponins, LVI[‡]

Three New Asterosaponins from the Starfish *Goniopecten demonstrans*

Simona De Marino,[a] Maria Iorizzi,[b] Franco Zollo,*[a] Charles D. Amsler,[c] Stephen P. Greer,[c] and James B. McClintock[c]

Keywords: Natural products / Saponins / Glycosides / Structure elucidation / Steroids

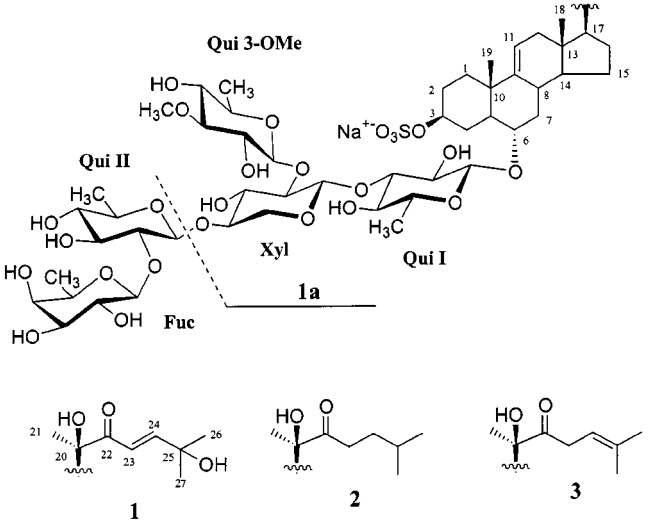
Three new steroidal sulfate pentaglycosides (asterosaponins), goniopectenosides A–C (**1–3**), were isolated from the polar extract of the starfish *Goniopecten demonstrans*. The pentasaccharide moiety linked to C-6 of 3β-sulfated steroidal aglycones, consists of D-xylose, D-fucose, D-quinovose, and the unprecedented 3-*O*-methyl-D-quinovose. The three asterosaponins differ from each other in the side chains. Their structures were elucidated by extensive NMR experiments including ^{1}H-^{1}H (COSY, TOCSY, ROESY) and ^{1}H-^{13}C (HMQC and HMBC) spectroscopy as well as chemical evidence. The isolated asterosaponins have been found to significantly inhibit the settlement of the biofouling marine brown macroalga *Hincksia irregularis*.

Introduction

Goniopecten demonstrans Perrier (Goniopectinidae), a large pinkish sea star with five arms and heavy marginal plates, was collected in the framework of a collaborative program on the search for new biologically active compounds and on the chemical ecology of marine invertebrates. Our investigation on the MeOH extract of this starfish led to the isolation of three new asterosaponins, named goniopectenosides A−C, (**1−3**, respectively). This paper[1] describes the isolation, structure elucidation, and biological activity of these three saponins, possessing the same pentasaccharide moiety linked to C-6 of the $\Delta^{9(11)}$-3β,6α-dihydroxysteroid-3-sulfated aglycones with a 22-oxo functionality in the side chain, which is a structural feature not previously found in glycosides of marine origin.

Results and Discussion

The MeOH extract of the freeze-dried specimens of *Goniopecten demonstrans* collected in June 1995 from deep waters of the Gulf of Mexico, was subjected to gel permeation on Sephadex LH-60 to furnish the crude asterosaponins. Droplet counter current chromatography (DCCC) and reversed phase HPLC gave three pure saponins goniopectenosides A−C (**1−3**, Scheme 1).



Scheme 1. New steroidal oligoglycosides from the starfish *Goniopecten demonstrans*

Goniopectenoside A (1)

1 was obtained as a white amorphous powder and analysed as $C_{57}H_{91}NaO_{28}S$ by its ^{13}C NMR spectroscopic data as well as from the HRFABMS (positive ion mode). Data at *m*/*z* = 1301.5298 $[MNa + Na]^+$ (calcd. 1301.5213) and an intense fragment at *m*/*z* = 1181 $[MNa + Na - NaHSO_4]^+$ confirmed the presence of a sulfate group. The negative-ion FABMS showed a $[M]^-$ anion at *m*/*z* = 1255 and prominent fragment peaks at *m*/*z* = 1141 $[M - 114]^-$ due to the loss of the aglycone side chain, as well as *m*/*z* = 1109 $[M - 146]^-$ and 1095 $[M - 160]^-$ due to the independent losses of a deoxyhexose and a methoxylated deoxyhexose unit, respectively.

The IR spectrum of **1** exhibited characteristic absorptions of a hydroxyl group at 3350 cm^{-1} and an α,β-unsatur-

[‡] Part LV: Ref.[1]

[a] Dipartimento di Chimica delle Sostanze Naturali, Università degli Studi di Napoli "Federico II",
via D. Montesano 49, 80131 Napoli, Italy
Fax: (internat.) + 39-081/678-552
E-mail: fzollo@cds.unina.it
[b] Dipartimento di Scienze e Tecnologie Agro-Alimentari, Ambientali e Microbiologiche, Università degli Studi del Molise
via F. De Sanctis, 86100 Campobasso, Italy
[c] Department of Biology, University of Alabama at Birmingham
Birmingham, Alabama 35294-1170, USA

  1434−193X/00/1224−4093 $ 17.50+.50/0

Table 1. ^1H (500 MHz) and ^{13}C (125 MHz) NMR spectroscopic data for the steroidal aglycone of the asterosaponins **1**, **2**, and **3** (CD_3OD)

Position	**1**, **2** and **3** δ_H[a]	δ_C
1	1.79, 1.48	36.9
2	2.27, 1.68	29.6
3	4.22 m ($W_{1/2}$ = 22 Hz)	79.7
4	2.62, 1.34	31.0
5	1.29	50.0
6	3.60	81.0
7	2.42 dt (4.5, 12.0), 0.99	41.8
8	2.12 m	36.4
9	-	146.6
10	-	39.3
11	5.41 br d (5.5 Hz)	117.8
12	2.32, 2.23	43.3
13	-	43.2
14	1.33	55.0
15		23.4
16	1.74	26.0
17	1.93	56.4
18	0.83 s	13.7
19	1.03 s	19.7

[a] ^1H assignments aided by COSY experiment.

ated carbonyl group at 1678 cm^{-1}. The ^1H and ^{13}C NMR spectra (Table 1) displayed resonances due to two tertiary methyl groups (δ_H 0.83, 1.03; δ_C 13.7, 19.7), one olefinic proton (δ_H 5.41; δ_C 117.8, 146.6), and one multiplet (δ_H 4.22; δ_C 79.7) that was ascribed to a methine proton linked to a carbon bearing a sulfate group, and suggested that the aglycone of **1** has a $\Delta^{9(11)}$-3β,6α-dihydroxysteroidal nucleus.[2−4] The ^1H and ^{13}C NMR spectra exhibited further signals due to three tertiary methyls [δ_H 1.35 (× 2), 1.45; δ_C 29.2, 29.3, 24.4] attached to carbon atoms bearing hydroxyl groups, and two olefinic protons (doublets at δ = 6.84 and 7.07; J = 15.4 Hz of a typical AB pattern) that suggested the existence of a *trans*-α,β-unsaturated carbonyl group (δ_C 204.6). The long-range heteronuclear interactions of H_3-21 with C-17, C-20, and C-22, and of H_3-27 with C-24 and C-25 were observed in the HMBC spectrum (Table 2) and helped to establish the presence of a typical cucurbitacin side chain.[5−7] Based on these findings, the structure of the aglycone was determined to be 5α-cholest-9(11),23-diene-3β,6α,20,25-tetrol-22-one, 3-sulfate.

The ^{13}C NMR spectrum of **1** exhibited signals for five anomeric carbons [δ_C 101.9, 104.6 (× 2) and 106.8 (× 2)], which showed correlations with the corresponding protons at δ 4.56, 4.59, 4.42, 4.43, and 4.57 in the HMQC experiment. Four doublets [(δ_H 1.29, 1.32 (× 2) and 1.39)] in the ^1H NMR spectrum were also present, upfield to the methyl groups of deoxysugars, and at δ = 3.65 a singlet was detected that was ascribed to a methoxyl group. Because all signals of steroidal aglycone match well with literature data,[8] the methoxyl group can be located on oligosaccharide portion.

Acid methanolysis of **1** with MeOH/HCl 1 N at 80 °C and GLC analysis with reference sugars afforded xylose/fucose/quinovose in the ratio 1:1:2. Because the fifth sugar was not detectable in the experimental condition used for GLC analysis, saponin **1**, after methanolysis, was *p*-bromobenzoylated, and the reaction mixture was separated by HPLC. The major UV absorbing HPLC peaks were then subjected to ^1H NMR spectroscopy and methyl 2,4-di-*O*-(*p*-bromobenzoyl)-3-*O*-methyl-α-D-quinovopiranoside was identified by comparison of its ^1H NMR spectrum with that obtained from marthasteroside B.[9]

In order to establish the location of 3-*O*-methylquinovose in the oligosaccharide chain, the natural goniopectenoside A (**1**) was subjected to enzymatic hydrolysis with *Charonia lampas* glycosidase mixture. Along with minor amounts of the starting material, goniopectenoside A (**1**) gave the trisaccharide **1a** showing a molecular anion peak at m/z = 963 in the FAB mass spectrum (negative ion mode). Inspection of ^1H NMR spectrum indicated that all proton signals relative to aglycone moiety were superimposable with the natural asterosaponin. Significant differences were found in the oligosaccharide portion. Only three anomeric proton signals were detectable at δ = 4.55 (× 2) and 4.42, which were correlated, by HMQC, to carbon atom signals at δ = 104.0, 106.4, and 104.2, respectively. ^1H NMR also featured two doublets at δ = 1.39 and 1.29, typical of methyl groups of deoxysugars, as well as a signal at δ = 3.64 due to a methoxyl group. They correlated in the HMQC to the corresponding carbon atoms at δ = 17.7, 18.3, and 60.5, respectively. These data indicated that 3-*O*-methylquinovose unit was located in the trisaccharide portion. The relative position of $-OCH_3$ was also deduced by upfield shift of H-3 of

Table 2. ^1H- (500 MHz) and ^{13}C- (125 MHz) NMR assignments and HMBC correlations of the steroidal side chains in asterosaponins **1** and **2** (CD_3OD)

Position	**1** δ_H[a]	δ_C	HMBC[b]	**2** δ_H[a]	δ_C	HMBC[b]
20	-	80.8		-	80.8	
21	1.45 s	24.4	C17, C20, C22	1.45 s	24.4	C17, C20, C22
22	-	204.6		-	204.6	
23	6.84 d (15.4)	120.3		2.65 t (7.3)	35.3	C24, C25
24	7.07 d (15.4)	156.8		1.45 q (7.3)	33.7	C26/27, C25, C23
25	-	71.5		1.58 m	28.9	C26/27
26	1.35 s	29.3	C24, C25, C27	0.93 d (6.1)	22.9	C27, C25, C24
27	1.35 s	29.2	C24, C25, C26	0.93 d (6.1)	22.9	C26, C25, C24

[a] Coupling constants (in Hz) are given in parentheses. − ^1H assignments aided by COSY experiment.− [b] HMBC optimized for $^{2,3}J_{CH}$ = 10 Hz.

Qui (δ_H 3.09, t, J = 9.0 Hz), which showed a correlation at δ_C = 86.5 in the HMQC experiment. A cross peak, in the HMBC experiment, between H-1 Qui-OMe (δ_H 4.55) and C-2 Xyl (δ_C 84.5) proved that the methoxylated quinovose was linked at C-2 of xylose. The H-5_{eq} of xylose was observed at δ_H = 3.99 (C-5: δ_C = 66.5), upfield shifted when compared with natural saponin **1** (H-5_{eq} xylose δ_H 4.14 dd), suggested that the remaining sugars in goniopectenoside A (**1**) were linked at C-4 of xylose unit.

Assignments of each sugar of oligosaccharide chain of goniopectenoside A (**1**) were made through COSY, TOCSY, HMQC, HMBC, and ROESY experiments[10−12] (Table 3). The COSY experiment allowed the sequential assignment of most of the resonances for each pyranosyl sugar ring, starting from the anomeric signals, because of the overlapping of some signals in the one dimensional spectrum. Complete assignments were achieved by combination of COSY and TOCSY results. Indeed the TOCSY experiment clearly showed correlations for the H-1 to H-5 spin system of quinovose, 3-*O*-methylquinovose, and xylose. The coherence transfer to H-5 of fucose was not obtained because of the small coupling H-4/H-5.

Table 3. NMR spectroscopic data (from 2D-COSY, TOCSY and HMQC experiments) for the oligosaccharide moiety of goniopectenosides **A**−**C** (**1**−**3**) (CD_3OD)

	δ_H	δ_C	HMBC[c]
Qui I			
1	4.42	104.6	C-6 Aglycon
2	3.36	74.6	
3	3.37	90.2[b]	
4	3.06[a]	74.9	
5	3.38	72.6	
6	1.29 d (6.8)	18.2	
Xyl			
1	4.59	104.6	C-3 Qui I
2	3.49	84.8[b]	
3	3.73	75.8	
4	3.74	79.0[b]	
5_{eq}	4.14 dd (11.9, 4.8)	64.7	
5_{ax}	3.45	64.7	
Qui 3-OMe			
1	4.57	106.8	C-2 Xyl
2	3.37	76.2	
3	3.10 t (9.0)	86.5	
4	3.21 t (9.0)	75.8	
5	3.38	76.2	
6	1.39 d (6.8)	17.9	
-OMe	3.65 s	60.5	
Qui II			
1	4.56	101.9	C-4 Xyl
2	3.40	85.0[b]	
3	3.54	77.4	
4	3.08 t (9.0)	76.4	
5	3.40	73.4	
6	1.32 d (6.8)	18.0	
Fuc			
1	4.43	106.8	C-2 Qui II
2	3.57	73.8	
3	3.52	74.9	
4	3.62	73.0	
5	3.69	72.6	
6	1.32 d (6.8)	17.0	

[a] Overlapped with 3.08 (H-4 Qui II). − [b] Glycosidated carbons. − [c] HMBC optimized for $^{2,3}J_{CH}$ = 10 Hz.

A proton-carbon one-bond chemical-shift correlation-experiment via heteronuclear multiple-quantum coherence (HMQC) correlated all proton resonances with those of their corresponding carbons (Table 1−3). The fucose unit was determined as terminal sugar because ^1H and ^{13}C data were not subjected to glycosidation shifts, and matched well with literature data.[8] This has been confirmed during analysis of *p*-bromobenzoylated derivatives since the methyl 2,3,4-tri-*O*-(*p*-bromobenzoyl)-α-D-fucopyranoside was obtained. The excitation-split CD curve[13] of methyl 2,3,4-tri-*O*-(*p*-bromobenzoyl)-α-D-fucopyranoside allowed us to determine the D-configuration of fucose; the common D-configuration for xylose and quinovose were assumed according to those most often encountered among the starfish saponins.[8]

The positions of the glycosidic linkages were established from the connectivities indicated in the HMBC and ROESY spectra. In the HMBC spectrum (Table 4), a cross peak between C-6 (δ_C 81.0) of the aglycone and H-1 of quinovose (Qui-I) (δ 4.42) indicated that quinovose was connected to C-6 of the aglycone. The linkage of the xylose (Xyl) at C-3 of Qui-I was indicated by the cross peaks C-3Qui-I (δ 90.2)/H-1Xyl (δ 4.59). Similarly, the linkages of the terminal fucose (Fuc) at the C-2 of the other quinovose (Qui-II) in turn linked to C-4 of the xylose were indicated by the cross peaks H-1Fuc (δ 4.43)/C-2Qui-II (δ 85.0), C-1Fuc (δ 106.8)/H-2Qui-II (δ 3.40), H-1Qui-II (δ 4.56)/C-4Xyl (δ 79.0). Finally, the location of the terminal 3-*O*-methylquinovose (Qui 3-OMe) at C-2 of the xylose was clearly indicated by the cross peak H-1Qui 3-OMe (δ 4.57)/C-2Xyl (δ 84.8).

The β-configurations for the sugars was deduced from their $^3J_{H1-H2}$ coupling constants (7−8 Hz) and correlations between H-1 and H-5 of Qui-I, Qui-II and Xyl in the ROESY spectrum. On the basis of the foregoing data, and assuming the common *R* configuration of C-20, the structure of **1** was determined to be (20*R*)-6α-*O*-{β-D-fucopyranosyl-(1→2)-β-D-quinovopyranosyl-(1→4)-[3-*O*-methyl-β-D-quinovopyranosyl-(1→2)]-β-D-xylopyranosyl-(1→3)-β-D-quinovopyranosyl}-25-dihydroxy-3β-(sulfoxy)-5α-cholest-9(11),23-dien-22-one.

Table 4. Selected data from HMBC experiment of goniopectenosides **A**−**C** (**1**−**3**); connectivities observed across the glycosidic linkages

C-atom	HMBC[a] (^1H)
81.0 (C-6 aglycone)	4.42 (H-1 Qui I)
90.2 (C-3 Qui I)	4.59 (H-1 Xyl)
84.8 (C-2 Xyl)	4.57 (H-1 Qui 3-OMe)
79.0 (C-4 Xyl)	4.56 (H-1 Qui II)
85.0 (C-2 Qui II)	4.43 (H-1 Fuc)

[a] HMBC optimized for $^{2,3}J_{CH}$ = 10 Hz.

Goniopectenoside B (2)

2 showed, in the negativeion FABMS, an $[M]^-$ anion at m/z = 1241, fourteen mass units shifted relative to **1**, and

gave ^{13}C NMR spectroscopic data consistent with a $C_{57}H_{93}NaO_{27}S$ molecular formula. Comparison of the ^1H and ^{13}C NMR spectra of the sugar portion of **2** with those of **1** indicated that goniopectenosides A and B possess the same aglycone and pentasaccharide chain, but differ in their side chains. ^1H NMR spectrum of **2** showed a singlet at δ = 1.45 relative to CH_3-21 as in the asterosaponin **1**, a triplet at δ = 2.65 (J = 7.3 Hz) and a doublet at δ = 0.93 relative to 6-H was ascribed to the isopropyl group. In the 2D-COSY the protons signal at δ = 2.65 showed a correlation with a second methylene group at δ = 1.45 (q, J = 7.3 Hz), which gave a cross-peak with a proton at δ = 1.58. This last methine proton correlated with the methyl groups at δ = 0.93 (d, J = 6.1 Hz). These data allowed us to construct a 20-hydroxy-22-oxo-saturated side chain (Table 2). Accordingly, goniopectenoside B (**2**) was defined as (20*R*)-6α-*O*-{β-D-fucopyranosyl-(1→2)-β-D-quinovopyranosyl-(1→4)-[3-*O*-methyl-β-D-quinovopyranosyl-(1→2)]-β-D-xylopyranosyl-(1→3)-β-D-quinovopyranosyl}-20-hydroxy-3β-sulfoxy-5α-cholest-9(11)-en-22-one.

The minor compound, goniopectenoside C (**3**), displayed the molecular anion $[M]^-$ at m/z = 1239 in the negative ion FABMS, two mass units fewer than **2**, implying that **3** was the dehydro-derivative of **2**. The ^1H and ^{13}C NMR spectroscopic data for the aglycone and sugar portion of **3** bore a close resemblance to those of **2**, but differences are detected in their steroidal side chains. In the ^1H NMR spectrum of **3**, the appearance of two methyl singlets at δ = 1.65 and 1.76 associated at a methine proton at δ = 5.30, allowed us to locate a 24(25) double bond. The presence of the oxo-function at C-22 (δ_C 204.8) was confirmed by HMBC correlation H_3-21/C-22. (see exp. section)

Thus, the structure of goniopectenoside C (**3**) was determined as (20*R*)-6α-*O*-{β-D-fucopyranosyl-(1→2)-β-D-quinovopyranosyl-(1→4)-[3-*O*-methyl-β-D-quinovopyranosyl-(1→2)]-β-D-xylopyranosyl-(1→3)-β-D-quinovopyranosyl}-20-hydroxy-3β-(sulfoxy)-5α-cholest-9(11),24-dien-22-one.

The antimicrobial activity of each of the isolated compounds was determined as described in the exp. section against the important biofouling marine bacterium model species *Deleya marina.* None of the three compounds had measurable antimicrobial activity. However, all three compounds significantly inhibited the settlement of the biofouling marine brown-macroalga model-species *Hincksia irregularis* (Table 5). This indicates that these compounds may play a role in preventing algal biofouling of *G. demonstrans.* The fact that the compounds were also nontoxic to *D. marina* suggests that they may be useful in biofouling control applications where specificity to marine algae is desirable.

Table 5. Settlement of *Hincksia irregularis* spores in the presence of pure compounds isolated from *Goniopecten demonstrans*

Treatment	Mean settlement[a]	Standard deviation
Control	12.8×10^{-2}	1.2×10^{-2}
Goniopectenoside **A**	6.87×10^{-2}	0.55×10^{-2}
Goniopectenoside **B**	5.81×10^{-2}	1.19×10^{-2}
Goniopectenoside **C**	6.53×10^{-2}	0.72×10^{-2}

[a] Settlement measured as settled spores cm^{-2} (swimming spores $mL^{-1})^{-1}$ over the 30 min settlement intervals. Analysis of variance indicated significant differences between treatments ($F_{3,11}$=30.5, $p < 0.001$) and REGWQ indicated that all three compounds significantly decreased settlement relative to controls ($p < 0.05$) but that there were no significant differences between settlement in the presence of the three compounds.

Experimental Section

All NMR measurements were performed on a Bruker AMX-500 spectrometer equipped with a Bruker X-32 computer, using the UXNMR software package. The ^1H-detected one-bond and multiple-bond ^{13}C multiple-quantum coherence experiments (HMQC and HMBC, respectively) utilized a 5-mm probe with reverse geometry and the sample was not spun. The magnitude of the delay for optimizing one-bond correlations in the HMQC spectrum and suppressing them in the HMBC spectrum was 3.5 ms, and the evolution delay for long-range couplings in the latter was set to 60 ms. − Optical rotation were measured on a Perkin−Elmer 141 polarimeter. − Infrared spectra were recorded on a Bruker IFS-48 spectrometer (KBr pellets). − Fast ion bombardment mass spectra (FAB MS) were recorded in a glycerol matrix on a VG PROSPEC instrument (Cs^+ ions of energy of 4 kV); HRFABMS spectra were recorded in a glycerol matrix on a VG AUTOSPEC instrument; CD spectra were measured with a JASCO 500A polarimeter. − GLC analyses were performed on a Carlo Erba Fractovap 4160 for capillary column (SPB-1, 25 m, 84 °C and 150° C; helium carrier flow 5 mL min^{-1}). − HPLC, C_{18} μ-Bondapak column (300 × 3.9 mm; flow rate 2 mL min^{-1}) and Luna 3 Silica (150 × 4.60 mm; flow rate 1 mL min^{-1}) Waters Model 6000 A pump equipped with U6 K injector and a differential refractometer, model 401, together with a variable-wavelength detector, model 486; DCCC, DCC-A apparatus manufactured by Tokyo Rikakikai Co., equipped with 250 tubes.

Animal Material: The animals, *Goniopecten demonstrans*, 220 g (lyophilized), were collected from deep water of the Gulf of Mexico. Identification was made by Dr. Tom Hopkins of Department of Biological Sciences, University of Alabama, Tuscaloosa, Alabama at the American Museum of Natural History. This large pinkish sea star with five arms and heavy marginal plates is a member of the family Goniopectinidae.

Extraction and Isolation: The freeze-dried organisms (220 g) were cut into small pieces and extracted with MeOH (3 × 3 L) at room temperature. The methanol extracts were taken to dryness to give the glassy material (17.8 g). The remaining solid mass was extracted with Me_2CO (3 L) and the Me_2CO extracts were combined, evaporated under vacuum and partitioned between H_2O and Et_2O. The aqueous residue was then extracted with *n*BuOH. Evaporation of the *n*BuOH extracts afforded 656 mg of a glassy material that was combined with the above MeOH extract and purified by chromatography on a column of Sephadex LH-60 (4 × 80 cm) with MeOH/H_2O (2:1) as eluent.

Fractions (7 mL) were collected and analyzed by TLC on SiO_2 in *n*BuOH/AcOH/H_2O (12:3:5) and $CHCl_3$/MeOH/H_2O (80:18:2).

Fractions 24−69 contained the crude asterosaponins (1 g), which were further purified by DCCC with *n*BuOH/Me_2CO/H_2O (3:1:5) (descending mode; the upper phase was used as the stationary phase; flow rate 14 mL/h; 7 mL fractions were collected) to give

the unresolved saponins (134 mg) in fractions 47−82. These fractions were then separated by reversed-phase HPLC (C_{18} μ-Bondapak 30 cm × 3.9 mm i.d.) with MeOH/H_2O (38:62) as eluent, to give pure saponins: **1** (13 mg), **2** (2 mg) and **3** (4 mg).

Goniopectenoside A (1): Yield 13 mg; $[\alpha]_D$ = +1.9 (*c* = 0.3, MeOH); FABMS in the text. ^1H and ^{13}C NMR of the steroidal aglycone are given in Table 1, and those of the steroidal side chain in Table 2.

Goniopectenoside B (2): Yield 2 mg; $[\alpha]_D$ = −7.5 (*c* = 0.2, MeOH); FABMS in the text. ^1H and ^{13}C NMR of the steroidal aglycone are given in Table 1, and those of the steroidal side chain in Table 2.

Goniopectenoside C (3): Yield 4 mg; $[\alpha]_D$ = +6.6 (*c* = 0.4, MeOH). − ^1H and ^{13}C NMR of the steroidal aglycone are given in Table 1. − ^1H NMR of the steroidal side chain: δ = 1.42 (s, 3 H, CH_3-21), 1.65 (s, 3 H, CH_3-26), 1.76 (s, 3 H, CH_3-27), 3.37 (overlapped with other signals, H_2-23), 5.30 (t, 1 H, *J* = 6.5 Hz, H-24). − ^{13}C NMR of the steroidal side chain: δ = 18.2 (C-26), 25.0 (C-21), 25.8 (C-27), 36.5 (C-23), 80.6 (C-20), 118.7 (C-24), 135.0 (C-25), 204.8 (C-22).

Methanolysis of Saponins 1−3. − Sugar Analysis: A solution (0.5 mg each) of saponins in anhydrous 2 N HCl/MeOH (0.5 mL) was heated at 80 °C in a stoppered reaction vial for 15 h. After cooling, the solution was neutralized with Ag_2CO_3 and centrifuged, then the supernatant was evaporated to dryness under N_2. A minor portion of the residue was reacted with 1-(trimethylsilyl)imidazole in pyridine and analyzed by GLC, the retention times were identical to those of authentic methyl D-quinovoside, methyl D-fucoside, methyl D-xyloside.

Methanolysis of Goniopectenoside A (1), *p*-Bromobenzoylation and Configuration of D-Fucose: A solution (8 mg) of saponin **1** in anhydrous 2 N HCl/MeOH (0.5 mL) was heated at 80 °C in a stoppered reaction vial for 15 h. After cooling, the solution was neutralized with Ag_2CO_3 and centrifuged, then the supernatant was evaporated to dryness under N_2. The residue was treated in dry pyridine (0.5 mL) with *p*-bromobenzoyl chloride (15 mg) and a catalytic amount of 4-dimethylaminopyridine, the mixture was stirred overnight at 60 °C under nitrogen, treated with chilled water and extracted with chloroform. The extract was washed with saturated aqueous $NaHCO_3$, water and then evaporated off under reduced pressure. The *p*-bromobenzoate mixture was separated by HPLC using a Phenomenex Luna 3 Silica column, flow rate 1 mL min^{-1}; elution with 10% diethyl ether in hexane gave 2,3,4-tri-*O*-(*p*-bromobenzoyl)-α-D-fucopyranoside; identified by its ^1H NMR spectrum ($CDCl_3$, 500 MHz): δ_H = 1.29 (3 H, d, *J* = 6.0 Hz, 5-Me), 4.38 (1 H, br q, *J* = 6.0 Hz, 5-H), 5.20 (1 H, d, *J* = 3.7 Hz, 1-H), 5.58 (1 H, dd, *J* = 3.7 and 10.5 Hz, 2-H), 5.71 (1 H, br d, *J* = 3.0 Hz, 4-H) and 5.90 (1 H, dd, *J* = 3.0 and 10.5 Hz, 3-H), 7.41, 7.54 (each 2 H, d, *J* = 9.0 Hz, ArH), 7.61, 7.66, 7.84, and 7.96. − CD (hexane) $\Delta\varepsilon_{254}$ = +99.3, $\Delta\varepsilon_{236}$ = −31.5; calcd. A +140. Elution with 20% diethyl ether in hexane gave methyl 2,4-di-*O*-(*p*-bromobenzoyl)-3-*O*-methyl-α-D-quinovopyranoside. − ^1H NMR: δ_H = 1.32 (3 H, d, *J* = 6.0 Hz, 5-Me), 3.40 (OMe), 3.45 (OMe), 3.99 (1 H, m, 5-H), 3.98 (1 H, t, *J* = 9.0 Hz, 3-H) and 5.01−5.09 (3 H, m, 1-, 2-, and 4-H), 7.58−7.96 (8 H, m, ArH).

Enzymatic Hydrolysis of Goniopectenoside A (1): The saponin **1** (2 mg) in a citrate buffer (1 mL; pH 4.5) was incubated with a glycosidase mixture (5 mg) of *Charonia lampas* (Shikagaku Kogyo) at 37 °C. After two days, the TLC analysis (SiO_2; *n*BuOH/AcOH/H_2O, 60:15:25) displayed two major spots: the starting material (R_f = 0.15) and the trisaccharide **1a** (R_f = 0.46) which was purified by HPLC (C_{18} μ-Bondapack column, 300 × 3.9 mm; MeOH/H_2O 52:48).

Compound 1a: FABMS (negative ion); *m*/*z*: 963 $[M]^-$. − ^1H NMR (aglycone) identical with that of natural **1**, **Qui I:** δ_H (sugar) = 1.29 (d, *J* = 6.8 Hz, CH_3-6), 3.07 (dd, *J* = 9.0, 9.2 Hz, H-4), 3.36 (overlapped with other signals, H-2), 3.37 (overlapped with other signals, H-3), 3.38 (overlapped with other signals, H-5), 4.42 (d, *J* = 7.0 Hz, H-1); **Xyl** δ = 3.23 (t, *J* = 11.9 Hz, H-5), 3.42 (dd, *J* = 9.0, 7.0 Hz, H-2), 3.45 (t, *J* = 9.0 Hz, H-3), 3.57 (m, H-4), 3.92 (dd, *J* = 11.9, 4.8 Hz, H-5), 4.55 (d, *J* = 7.0 Hz, H-1); **Qui 3-OMe** 1.39 (d, *J* = 6.8 Hz, CH_3-6), 3.09 (t, *J* = 9.0 Hz, H-3), 3.20 (dd, *J* = 9.2, 9.0 Hz, H-4), 3.37 (overlapped with other signals, H-2), 3.38 (overlapped with other signals, H-5), 3.64 (s, OCH_3), 4.55 (d, *J* = 7.0 Hz, H-1). − ^{13}C NMR: δ_C (sugar): **Qui I** 18.3 (CH_3-6), 72.5 (C-5), 74.6 (C-2), 74.9 (C-4), 90.2 (C-3), 104.2 (C-1); **Xyl** 66.5 (CH_2-5), 70.3 (C-4), 76.8 (C-3), 83.9 (C-2), 104.1 (C-1); **Qui 3-OMe** 17.7 (CH_3-6), 60.5 (-OCH_3), 75.8 (C-4), 76.2 (C-5), 76.2 (C-2), 86.5 (C-3), 106.4 (C-1).

Antimicrobial Assay: The marine fouling bacterium *Deleya marina* (ATCC 27129) was utilized to assay antimicrobial bioactivity of the purified compounds isolated from *Goniopecten demonstrans*. Paper disks (BBL Microbiology Systems 31039) containing 0.1 mg of each compound were prepared by placing 20 μL of a 5 mg/mL solution in 100% MeOH onto each disk. For bioassays, overnight liquid cultures of the bacteria were spread onto Difco marine Agar 2216 (Difco Laboratories) plates. Pure compound-containing disks were placed onto the culture plates and the cells allowed to grow for 24 h at 26 (±1) °C. Antimicrobial activity was defined as visible inhibition of cell growth in a region surrounding the paper disk. Disks prepared with only the MeOH solvent did not inhibit bacterial growth.

Algal Spore Fouling Assay: *Hincksia irregularis* (strain CDA-Hi2; source: Gulf of Mexico, Florida Panhandle coast) was maintained and utilized in algal biofouling plate experiments described for the related alga *Ectocarpus siliculosus*[14] with the following exceptions. Growth and experimental temperature was 26 °C. Only hydrophobic plates (Corning # 25880; Corning Glass Works, Corning, New York) were utilized. Only 30 min. settlement periods were used. Three replicates each of compounds **1−3** were assayed at a final concentration of 1.0 mg ml^{-1} in artificial seawater and compared to settlement in three replicate artificial seawater only controls. Statistical comparisons of settlement density were done by analysis of variance using the SPSS software (SPSS Inc., Chicago IL) General Linear Model procedure and a Ryan−Einot−Gabriel−Welsch (REGWQ) post hoc test.

Acknowledgments

NMR, IR, UV and MS spectra were performed at "Centro Interdipartimentale di Servizio di Analisi Strumentale", Università di Napoli "Federico II". The assistance of the staff is gratefully appreciated. This work was supported by MURST (PRIN '99) "Chimica dei Composti Organici di interesse biologico" Roma, Italy and Mississippi-Alabama Sea Grant Consortium grant R/MT-40 to CDA and JBM.

[1] S. De Marino, M. Iorizzi, E. Palagiano, F. Zollo, C. Roussakis, *J. Nat. Prod.* **1998**, *61*, 1319−1327.
[2] E. Kitagawa, M. Kobayashi, T. Sugawara, *Chem. Pharm. Bull.* **1978**, *26*, 1852−1863.
[3] Minale, R. Riccio, C. Pizza, F. Zollo, in *Natural Products and Biological Activities. A Naito Foundation Symposium* (Eds.: H.

Imura, T. Goto, T. Murachi, T. Nakajima), University of Tokyo Press: Tokyo, **1986**, pp 59−73.

[4] M. Honda, T. Komori, *Tetrahedron Lett*. **1986**, *27*, 3369−3372.

[5] A. A. Amonkar, T. G. McCloud, C.-J. Chang, J. A. Saenz-Renauld, J. M. Cassady, *Phytochemistry* **1985**, *24*, 1803−1805.

[6] V. V. Velde, D. Lavie, *Tetrahedron* **1983**, *39*, 317−321.

[7] H. Sasamori, K. S. Reddy, M. P. Kirkup, J. Shabanowitz, D. G. Lynn, S. M. Hecht, K. A. Woode, R. F. Bryan, J. Campbell, W. S. Lynn, E. Egert, G. M. Sheldrick, *J. Chem. Soc., Perkin Trans. 1* **1983**, 1333−1347.

[8] L. Minale, R. Riccio, F. Zollo, in *Progress in the Chemistry of Organic Natural Products*, (Eds.: W. Herz, G. W. Kirby, R. E. Moore, W. Steglich, Ch. Tamm), Springer Verlag: Wien, **1993**, *62*, pp.75−308.

[9] I. Bruno, L. Minale, C. Pizza, F. Zollo, R. Riccio, F. M. Mellon, *J. Chem. Soc., Perkin Trans. 1* **1984**, 1875−1883.

[10] A. Bax, D. G. Davis, *J. Magn. Res.* **1985**, *65*, 355−357.

[11] A. Bax, S. Subramanian, *J. Magn. Res.* **1986**, *67*, 365−367.

[12] A. Bax, F. Summers, *J. Am. Chem. Soc.* **1986**, *108*, 2093−2095. A. Bax, A. Aszalos, Z. Dinya, K. Sudo, *J. Am. Chem. Soc.* **1986**, *108*, 8056.

[13] N. Harada, K. Nakanishi, in *Circular Dichroic Spectroscopy-Exiton Coupling in Organic Stereochemistry*, University Science Books, Mill Valley, Calif., **1983**; H. W. Liu, K. Nakanishi, *J. Am. Chem. Soc.* **1982**, *104*, 1178−1185.

[14] C. D. Amsler, K. L. Shelton, C. J. Britton, N. Y. Spencer, S. P. Greer, *Journal of Phycology* **1999**, *35*, 239−244.

Received May 30, 2000
[O00274]