J. Nat. Prod. **2008,** *71,* 793–798

Steroidal Triglycosides, Kurilensosides A, B, and C, and Other Polar Steroids from the Far Eastern Starfish *Hippasteria kurilensis*

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Received November 11, 2007

Three novel steroidal triglycosides, designated as kurilensosides A, B, and C (**1**–**3**), were isolated along with a new steroidal diglycoside, kurilensoside D (**4**), and two new (**6**, **7**) and one known (**5**) polyhydroxysteroid from the alcoholic extract of the Far Eastern starfish *Hippasteria kurilensis*. Compounds **1**–**3** are the first triglycosides containing two carbohydrate chains found from starfish. The structures of **1**–**7** were elucidated by spectroscopic methods (mainly 2D NMR) and chemical derivatization. Glycosides **1**–**4** and steroids **6** and **7** inhibited sea urchin egg fertilization by sperm preincubated with these compounds.

Starfish (phylum Echinodermata, class Asteroidea) have proven to be an especially rich source of structurally unusual and biologically active polar steroids. Often these steroids occur as complicated mixtures of highly oxygenated sterols, very difficult to separate into individual compounds. In a continuation of our search for new polar steroidal metabolites from Far Eastern starfish,[1] we have examined the alcoholic extract of the spiny red starfish *Hippasteria kurilensis* Fisher, 1911 (order Valvatida, family Goniasteridae). Employing column chromatography on silica gel and Florisil followed by a final separation using reversed-phase HPLC, we have isolated four new steroidal glycosides, designated as kurilensosides A, B, C, and D (**1**–**4**), along with two new (**6**, **7**) and one known (**5**) polyhydroxysteroids. Kurilensosides A, B, and C (**1**–**3**) are unprecedented examples of steroidal triglycosides, containing two carbohydrate chains, in which a monosaccharide unit is attached to C-3 and a disaccharide unit is located at C-24 of a cholestane aglycon. Earlier only three triglycosides, monilosides G, H, and I, with a carbohydrate chain only at C-29 were known from the starfish *Fromia monilis,*[2] in contrast with more than two hundred different mono- and diglycosides of polyhydroxysteroids isolated from starfish so far.

Results and Discussion

The molecular formula of kurilensoside A (**1**) was determined as $C_{43}H_{73}O_{21}SNa$ from the $[M + Na]^+$ sodiated molecular ion at *m/z* 1003.4223 (calcd for $C_{43}H_{73}O_{21}SNa_2$, 1003.4160) in the HR (+)-MALDI-TOFMS and the $[M - Na]^-$ ion at *m/z* 957 in the (−)-MALDI-TOFMS. The fragment ion peak at *m/z* 901 $[(M + Na) - SO_3Na + H]^+$ in the (+)-MALDI-TOFMS exhibited the presence of a sulfate group in the glycoside. The ^1H, ^{13}C, and DEPT NMR spectra of **1** showed the presence of 43 carbon atoms, including five methyl groups, 11 methylenes, 23 methines, two quaternary carbons, one oxygenated quaternary carbon, and one methoxyl group (Tables 1 and 3). The ^1H NMR spectrum of **1** exhibited three resonances in the downfield region due to anomeric protons (δ 5.12, 5.18, and 4.46) that correlated in the HSQC experiment with carbon signals at δ 108.0, 109.6, and 102.6, respectively. Along with mass spectra, these data revealed the existence of three monosaccharide residues and a hexahydroxy-substituted steroidal moiety in glycoside **1**. $^1H-^1H$ COSY cross-peaks indicated sequences of protons at C-1 to C-7, C-9 to C-12 through C-11, C-14 to C-17, C-20 to C-21, and C-22 to the end of the side chain. The planar structure of **1** was determined on the basis of the correlations in the HMBC spectrum (Table 1). The analysis of the NMR spectral data of **1** indicated that kurilensoside A was related to linckoside L_1,[3] containing the same 3β,4β,6β,8,15α,24-hexahydroxy cholestane aglycon bearing a 2-*O*-methyl-β-D-xylopyranosyl residue at C-3. However, **1** also has a disaccharide chain at C-24. Glycosidation at C-24 was supported by the shifts of the C-23, C-24, and C-25 signals (δ 28.4, 83.8, and 31.3) in the ^{13}C NMR spectrum of **1** in comparison with the corresponding values (δ 31.6, 78.1, and 34.6) for steroids having unsubstituted 24-hydroxycholestane side chains.[3] Acid hydrolysis of **1** with aqueous 2 M CF_3COOH gave arabinose and 2-*O*-methylxylose in a 2:1 ratio that was confirmed by TLC and GC analysis of the corresponding aldononitrile peracetates. The (+)-MALDI-TOFMS showed a series of fragmentations with the following sugar losses: *m/z* 871 $[(M + Na) - 132]^+$ loss of arabinose; 857 $[(M + Na) - 146]^+$ loss of 2-*O*-methylxylose, 769 $[(M + Na) - 102 - 132]^+$ loss of a sulfate group and arabinose. The (−)-MALDI-TOFMS also indicated a series of fragmentations with the following sugar losses: *m/z* 825 $[(M - Na) - 132]^-$ loss of arabinose; 811 $[(M - Na) - 146]^-$ loss of 2-*O*-methylxylose; 679 $[(M - Na) - 132 - 146]^-$ loss of arabinose and 2-*O*-methylxylose. The common L-configuration for two arabinose units and D-configuration for the 2-*O*-methylxylose unit were preferred according to those most often encountered among the starfish steroidal glycosides.[4] The carbon and proton signals as well as the corresponding coupling constants of the terminal monosaccharide in the disaccharide moiety at C-24 in the NMR spectra of **1** strictly coincided with those of the α-L-arabinofuranosyl residue.[5] The signals of an internal monosaccharide coincided with those of a 2′-substituted 3′-*O*-sulfate-α-L-arabinofuranosyl unit reported for the first time for miniatoside B[6] and differed from those of a nonsulfated 2′-substituted α-L-arabinofuranosyl unit.[4] So, the signal of C-3′ in the ^{13}C NMR spectrum of **1** was downfield shifted (δ 77.6 → 83.3) and the signal of C-2′ was upfield shifted (δ 92.4 → 87.9) in addition to those of the nonsulfated 2′-substituted α-L-arabinofuranosyl unit, which confirmed the location of sulfate group at C-3′. By careful examination of monosaccharide signals with the aid of $^1H-^1H$ COSY and HMBC data, the α-L-Ara_f-(1 → 2)-3′-*O*-sulfate-α-L-Ara_f disaccharide unit attached to C-24 was determined. The linkage of terminal α-L-Ara_f at C-2′ was indicated by the cross-peak H-1″/C-2′, the linkage of internal 3′-*O*-sulfate-α-L-Ara_f at C-24 was confirmed by the cross-peak H-1′/C-24, and the attachment of a 2-*O*-Me-Xyl_p to C-3 was fixed by the long-range correlations of H-1‴/C-3 in the HMBC spectrum. Kurilensoside A was subjected to partial hydrolysis with aqueous acetic acid to give monoside

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10.1021/np070637x CCC: $40.75 © 2008 American Chemical Society and American Society of Pharmacognosy
Published on Web 03/22/2008

Table 1. ^{1}H NMR and HMBC Data for Compounds **1–3** (500 MHz, CD_3OD)[a]

	1		**2**		**3**	
position	δ_H (J in Hz)	HMBC[b]	δ_H (J in Hz)	HMBC[b]	δ_H (J in Hz)	HMBC[b]
1	1.73 m 1.01 m		1.70 m 0.99 m		1.70 m 0.99 m	
2	1.98 m 1.70 m		1.91 m 1.62 m		1.91 m 1.63 m	
3	3.65 m		3.58 m		3.58 m	
4	4.25 m		4.40 br s		4.40 br s	
5	1.22 m	10, 19	1.46 dd (2.3, 11.7)	6, 10, 19	1.46 dd (2.3, 11.7)	6, 10, 19
6	4.25 m		4.26 dd (2.9, 11.6)		4.26 dd (2.9, 11.6)	
7	2.40 dd (2.9, 15.1) 1.59 dd (3.3, 15.2)		3.92 d (2.9)	5, 6, 8, 9	3.91 d (2.8)	6, 8
8						
9	0.97 m		1.09 dd (3.3, 12.6)		1.09 dd (3.4, 12.7)	
10						
11	1.82 m 1.48 m		1.82 m 1.43 m		1.80 m 1.42 m	
12	1.96 m 1.23 m		1.95 m 1.13 m		1.95 m 1.13 m	
13						
14	1.16 d (9.5)	13, 15, 18	1.40 d (5.4)	8, 13, 15, 17, 18	1.40 d (5.4)	8, 13, 15, 17, 18
15	4.28 dt (3.0, 9.4)		4.53 ddd (2.1, 5.1, 7.6)	13	4.53 ddd (1.8, 5.5, 7.5)	
16	1.91 m 1.76 m		2.40 m 1.38 m		2.40 m 1.38 m	
17	1.32 m		0.99 m		1.00 m	
18	0.95 s	12, 13, 14, 17	1.26 s	12, 13 14, 17	1.26 s	12, 13, 14, 17
19	1.43 s	1, 5, 9, 10	1.16 s	1, 5, 9, 10	1.16 s	1, 5, 9, 10
20	1.33 m	17	1.52 m		1.52 m	
21	0.90 d (6.8)	20, 22	0.93 d (6.5)	17, 20	0.93 d (6.5)	17, 20, 22
22	1.61 m 0.96 m		1.63 m 0.98 m		1.63 m 1.00 m	
23	1.59 m 1.28 m		1.59 m 1.32 m		1.58 m 1.34 m	
24	3.34 m		3.30 m[b]		3.30 m[b]	
25	1.87 m		1.83 m		1.83 m	
26	0.90 d (6.8)	24, 25, 27	0.90 d (6.8)	24, 25, 27	0.90 d (6.8)	24, 25, 27
27	0.88 d (6.8)	24, 25, 26	0.89 d (6.8)	24, 25, 26	0.89 d (6.8)	24, 25, 26
1′	5.12 br s	24, 3′	4.93 d (1.5)	24, 4′	4.90 d (2.1)	24, 4′
2′	4.32 d (1.9)	3′, 1″	3.97 dd (1.7, 3.5)		3.96 dd (2.1, 4.2)	3′
3′	4.59 dd (1.9, 5.9)	2′	3.94 dd (3.5, 6.2)		3.85 m	2′
4′	4.16 dt (3.0, 5.5)	3′	4.06 dt (4.0, 6.2)		4.05 ddd (3.4, 4.7, 6.9)	
5′	3.88 dd (3.1, 12.1) 3.72 dd (5.4, 12.1)	4′	3.96 m 3.73 dd (3.8, 11.3)	3′, 4′, 1″	3.91 dd (5.5, 11.3) 3.72 dd (3.4, 11.3)	1″ 3′, 4′, 1″
1″	5.18 d (1.5)	3″, 4″, 2′	4.28 d (7.5)	5′	4.33 d (7.6)	5′
2″	3.98 dd (1.5, 3.4)	3″	3.18 t (9.0)	1″	2.87 dd (7.5, 9.0)	1″, 3″, 2″-OMe
3″	3.86 dd (3.4, 6.3)	2″	3.30 m[c]		3.32 t (8.7)	
4″	3.90 ddd (3.4, 4.7, 6.3)	3″	3.48 m		3.48 m	
5″	3.70 dd (3.4, 12.1) 3.62 dd (4.7, 12.1)	3″, 4″	3.86 dd (5.3, 11.3) 3.20 dd (10.8, 11.3)	1″, 4″	3.84 dd (5.5, 12.0) 3.16 dd (10.0, 11.7)	1″, 3″ 1″
2″-OMe					3.58 s	2″
1‴	4.46 d (7.4)	3	4.43 d (7.4)	3	4.45 d (7.6)	3
2‴	2.90 dd (7.4, 8.9)	1‴, 3‴, 2‴-OMe	2.91 dd (7.6, 9.0)	1‴, 3‴, 2‴-OMe	2.89 dd (7.5, 9.0)	1‴, 3‴, 2‴-OMe
3‴	3.35 t (8.9)	2‴, 4‴	3.41 t (8.9)	2‴, 4‴	3.34 t (8.9)	2‴, 4‴
4‴	3.47 m		3.17 m		3.47 m	
5‴	3.82 dd (5.1, 11.3) 3.17 dd (10.1, 11.4)	1‴, 3‴, 4‴ 1‴, 3‴, 4‴	4.00 dd (4.7, 11.0) 3.11 m	1‴, 3‴, 4‴ 1‴, 4‴	3.82 dd (5.3, 11.9) 3.15 dd (10.2, 11.7)	1‴, 3‴ 1‴
2‴-OMe	3.61 s	2‴	3.61 s	2‴, 4‴	3.61 s	2‴
4‴-OMe			3.45 s	4‴		

[a] Assignments from ^{1}H−^{1}H COSY and HSQC data. [b] HMBC correlations, optimized for 8 Hz. [c] Overlapped with the solvent signal.

1a, which was identified by ^{1}H NMR data as linckoside L_1.[3] The stereochemistry of C-24 was determined as *S* by analysis of the ^{1}H NMR spectrum of (*R*)-MTPA ester obtained by reaction of **1a** with *S*-(+)-α-methoxy-α-(trifluoromethyl)phenylacetyl (MTPA) chloride in dry pyridine. The H_3-26 and H_3-27 signals were observed at δ 0.80 and 0.82, which coincided with those reported for (*R*)-MTPA derivative (24*S*)-24-hydroxysteroids.[3,4] Hence, the structure of kurilensoside A (**1**) was elucidated as sodium (24*S*)-3-*O*-(2-*O*-methyl-β-D-xylopyranosyl)-24-*O*-[α-L-arabinofuranosyl-(1 → 2)-3-*O*-sulfate-α-L-arabinofuranosyl]-5α-cholestane-3β,4β,6β,8,15α,24-hexol.

The molecular formula of kurilensoside B (**2**) was determined as $C_{44}H_{76}O_{19}$ from the $[M + Na]^+$ sodiated molecular ion at *m/z* 931.4821 (calcd for $C_{44}H_{76}O_{19}Na$, 931.4879) in the HR (+)-MALDI-TOFMS and the $[M - H]^-$ ion at *m/z* 907 in the (−)-LSI MS. The ^{1}H NMR spectrum of **2** showed signals for three anomeric protons at δ 4.93, 4.28, and 4.43, which were correlated in the HSQC experiment with the corresponding carbons at δ 109.5, 105.4, and 102.3, respectively. The ^{1}H, ^{13}C, DEPT NMR, and MS spectra exhibited the presence three monosaccharide residues and a heptahydroxy-substituted steroidal moiety in glycoside **2**. The ^{1}H NMR data of the steroidal moiety of **2** were related to those of compound **5a** (derived from co-occurring steroid **5** by solvolysis; see Experimental Section), and thus **2** was expected to have the same 3β,4β,6α,7α,8,15β,24-heptahydroxy substitution pattern. The proton and carbon signals attributable to the aglycon moiety were assigned by the application of 2D NMR experiments including ^{1}H−^{1}H COSY, HSQC, and HMBC. Glycosidation at C-3 and C-24 was supported by the downfield shifts of the H-3 (δ 3.58) and H-24 (δ 3.30) in the ^{1}H NMR spectrum of **2** with respect to the corresponding values of δ 3.44 and 3.20 in **5a**. The (+)-LSIMS showed a fragmentation with the following sugar losses: *m/z* 753 $[(M + Na) - 178]^+$ loss of 2,4-di-*O*-methylxylose; 621 $[(M + Na) - 178 - 132]^+$ loss of 2,4-di-*O*-methylxylose and xylose. The (−)-

Table 2. ^{1}H NMR Data for Compounds **4**, **6**, and **7** (500 MHz, CD_3OD, *J* in Hz)[a]

position	4[b]	6	7
1	1.70 m 0.96 m	1.71 m 1.01 m	1.72 m 1.00 m
2	1.82 m 1.54 m	1.83 m 1.57 m	1.88 m 1.62 m
3	3.42 m	3.47 ddd (3.8, 4.9, 11.9)	3.49 ddd (3.9, 5.2, 11.9)
4	4.25 br s	4.21 br s	4.05 br s
5	0.93 m	1.72 dd (2.3, 12.2)	1.23 m
6	4.15 td (4.3, 10.8)	5.04 dd (2.8, 12.0)	4.24 m
7	2.44 dd (4.3, 12.3) 1.30 m	4.23 d (2.9)	2.40 dd (3.0, 15.0) 1.59 dd (2.9, 14.9)
8			
9	0.81 dd (3.2, 12.6)	1.09 m	0.97 m
10			
11	1.78 m 1.43 m	1.82 m 1.43 m	1.81 m 1.47 m
12	1.97 dd (3.0, 12.4) 1.15 m	1.95 m 1.15 m	1.96 m 1.23 m
13			
14	0.98 d (5.7)	1.42 d (5.8)	1.17 d (9.7)
15	4.41 ddd (2.1, 5.6, 7.7)	4.48 ddd (2.1, 5.7, 7.7)	4.27 dt (3.2, 9.5)
16	2.38 m 1.39 m	2.23 m 1.37 m	1.91 m 1.69 m
17	0.97 m	1.01 m	1.34 m
18	1.25 s	1.28 s	0.95 s
19	1.15 s	1.22 s	1.42 s
20	1.51 m	2.20 m	1.36 m
21	0.92 d (6.6)	1.02 d (6.6)	0.91 d (6.0)
22	1.62 m 0.98 m	5.42 dd (8.0, 15.2)	1.60 m 0.99 m
23	1.57 m 1.33 m	5.37 dd (6.8, 15.5)	1.55 m 1.21 m
24	3.30 m[c]	3.67 t (6.5)	3.19 m
25	1.82 m	1.65 m	1.61 m
26	0.90 d (6.8)	0.90 d (6.7)	0.90 d (6.7)
27	0.89 d (6.8)	0.86 d (6.7)	0.88 d (6.7)

[a] Assignments from ^{1}H−^{1}H COSY and HSQC data. [b] ^{1}H NMR data for the disaccharide moiety are identical with those of kurilensoside C (**3**); see Table 1. [c] Overlapped with the solvent signal.

LSIMS also indicated a peak at *m/z* 729 [(M − H) −178]$^{-}$ coinciding with the loss of 2,4-di-*O*-methylxylose. By analysis of the ^{1}H and ^{13}C NMR data, a 2,4-di-*O*-methyl-*β*-D-xylopyranosyl residue[7,8] attached to C-3 and *β*-D-xylopyranosyl-(1 → 5)-α-L-arabinofuranosyl disaccharide unit attached to C-24 were identified. The signals of the *β*-D-Xyl$_p$-(1 → 5)-α-L-Ara$_f$ disaccharide unit in the ^{13}C NMR spectrum coincided well with those reported for mediasteroside M_2.[7] The attachment of the carbohydrate fragments and the presence of the (1 → 5) interglycosidic linkage was confirmed by the HMBC experiment, which showed the following cross-peaks: H-1′(Ara)/C-24, H-1″(Xyl)/C-5′, and H-1‴(2,4-di-*O*-Me-Xyl)/C-3. The stereochemistry at C-24 was expected to be *S* by analogy with co-occurring compound **1**. Thus, the structure of kurilensoside B was defined as **2**.

The HR (+)-MALDI-TOFMS of kurilensoside C (**3**) showed a sodiated molecular ion peak at *m/z* 931.4934 [M + Na]$^{+}$ (calcd for $C_{44}H_{76}O_{19}Na$, 931.4879). The detailed comparison of its NMR data with those of **2** clearly indicated that glycoside **3** has the same steroidal aglycon with the 5′-substituted α-L-arabinofuranosyl unit attached to C-24 and differs from **2** only in the signals of the terminal monosaccharide units. By analysis of the ^{1}H−^{1}H COSY, HSQC and HMBC experiments, two 2-*O*-methyl-*β*-D-xylopyranosyl residues attached to C-3 of the steroid nucleus and to C-5′ of the α-L-arabinofuranosyl unit were identified. The fragment ion peak in the (−)-LSIMS at *m/z* 743 [(M − H) − 164]$^{-}$ corresponding to the loss of 2-*O*-methylxylose was confirmed at its terminal position. The location of the methoxy groups at C-2″ and C-2‴ of the corresponding xylopyranose units (2 and 3 in the formula) was supported by the reciprocal HMBC correlations between the methoxy groups and C-2″ and C-2‴, respectively. The signals of the 2-*O*-Me-*β*-D-Xyl$_p$-(1 → 5)-α-L-Ara$_f$ disaccharide unit in the ^{13}C NMR spectrum corresponded well to those reported for mediasteroside M_1.[7] Accordingly, the structure of kurilensoside C was established as **3**.

The HR (+)-MALDI-TOFMS of kurilensoside D (**4**) showed a sodiated molecular ion peak at *m/z* 769.4394 [M + Na]$^{+}$ (calcd for $C_{38}H_{66}O_{14}Na$, 769.4351). The fragment ion peak in the (−)-LSI MS at *m/z* 599 [(M − H) − 164]$^{-}$ corresponded to the loss of a methoxylated pentose. Comparison of the NMR data of compound **4** with those of **3** revealed the presence of the same 2-*O*-Me-*β*-D-Xyl$_p$-(1 → 5)-α-L-Ara$_f$ disaccharide unit attached to C-24 of the steroid side chain (Tables 2 and 3). The generality of the signals in the NMR spectra of **4** attributable to the steroid nucleus was similar to those of **3**. However, the signal of H-6 in **4** appeared as triplet doublets at *δ* 4.15 instead of doublet doublets at *δ* 4.26 of **3** due to the lack of a hydroxyl group at C-7. Furthermore, the signals of C-2 (*δ* 26.2), C-3 (*δ* 73.7), and C-4 (*δ* 69.1) in **4** were shifted in comparison with the analogous signals in **3** (*δ* 24.8, 81.2, 66.5, respectively) due to the absence of a monosaccharide residue at C-3. 2D NMR (^{1}H−^{1}H COSY, HSQC, and HMBC) analysis indicated that the aglycon moiety contains a 3*β*,4*β*,6α,8,15*β*,24-hexahydroxyoxidation pattern, and the structure of kurilensoside D was proposed as **4**.

Kurilensosides A, B, and D (**1**, **2**, and **4**) contain rare carbohydrate moieties with (1 → 5) bonds between the monosaccharide units. Earlier this type of asterosaponin carbohydrate chain was known only for mediasterosides M_1−M_3 from *Mediaster murrayi*[7] and crossasteroside P_4 from *Crossaster papposus*.[8]

The known compound **5** was identified by comparison of the MS and NMR data with those reported.[9] It was previously isolated from the Far Eastern starfish *Hippasteria phrygiana*.[9]

The HR (+)-MALDI-TOFMS of compound **6** showed a sodiated molecular ion peak at *m/z* 607.2587 [M + Na]$^{+}$ (calcd for $C_{27}H_{45}O_{10}SNa_2$, 607.2529). The fragment ion peaks at *m/z* 505 [(M + Na) − 102]$^{+}$ and 487 [(M + Na) − 120]$^{+}$ in the (+)-LSIMS and at 97 [HSO_4]$^{-}$ and 80 [SO_3]$^{-}$ in the (−)-LSIMS exhibited the presence of a sulfate group in the steroid. Detailed comparison of NMR data of **6** with those of **5** clearly indicated that compound **6** contained the identical 3*β*,4*β*,6α,7α,8,15*β*-hexahydroxysteroidal nucleus with a sulfoxy group at C-6 and differed from **5** only in the presence of the signals attributable to a 22(23)-double bond (δ_H 5.42 and 5.37, δ_C 139.3 and 130.1) in the 24-hydroxyholestane side chain. The sequence of H-17 to H-27 correlating with the corresponding carbon atoms was revealed using ^{1}H−^{1}H COSY and HSQC experiments. The HMBC correlations supported the total structure of the side chain: Me-21/C-17, C-20, C-22; H-22/C-23; H-23/C-17, C-24; Me-26/C-24, C-25, C-27; Me-27/C-24, C-25, C-26. In addition, the $J = 15.2$ Hz suggested a *trans*-configuration for a 22(23)-double bond. Thus, steroid **6** was determined to be a Δ^{22}-derivative of **5**.

The HR (+)-MALDI-TOFMS of compound **7** exhibited a sodiated molecular ion peak at *m/z* 491.3369 [M + Na]$^{+}$ (calcd for $C_{27}H_{48}O_6Na$, 491.3349). The NMR data of **7** were very similar to those of the aglycon moiety of kurilensoside A (**1**). The signals of H-3, H-24, C-3, and C-24 of steroid **7** were shifted from δ_H 3.49 to 3.65 and 3.19 to 3.34, and from δ_C 73.1 to 80.6 and 78.1 to 83.8, respectively, in comparison with glycoside **1**, as a result of lack of carbohydrate units at the C-3 and C-24 positions. The total structure of **7** was confirmed by the HMBC correlations H-5/C-10, C-19; H-6/C-8, C-10; H-7eq/C-5; H-14/C-13, C-18; H-16/C-17; H-18/C-12, C-13, C-14, C-17; H-19/C-1, C-5, C-9, C-10; H-21/C-17, C-20, C-22; H-26/C-24, C-25, C-27; H-27/C-24, C-25, C-26. Thereby, the steroid **7** was proved to be a free aglycon of **1**. Although some glycosides of polyhydroxysteroids with the same aglycon have been previously reported from starfish,[3–5] the native steroid **7** was encountered for the first time.

Glycosides **1**−**4** and steroids **6** and **7** were assayed for inhibition of the egg fertilization by sperm of the sea urchin *Strongylocentrotus nudus* preincubated with these compounds (sperm test). Kurilensosides B, C, and D (**2–4**) and polyhydroxysteroid **7** exhibited 100% inhibition at 5.5×10^{-5}, 5.5×10^{-5}, 6.7×10^{-5}, and 1.3×10^{-5}

Table 3. ^{13}C NMR Data for Compounds **1–4**, **6**, and **7** (CD_3OD, 125.8 MHz)

position	**1**	**2**	**3**	**4**	**6**	**7**
1	41.0	39.7	39.7	39.7	39.6	41.0
2	25.9	24.9	24.8	26.2	26.6	26.5
3	80.6	81.3	81.2	73.7	73.0	73.1
4	74.7	66.5	66.5	69.1	69.4	77.5
5	50.5	47.4	47.4	57.2	46.7	50.6
6	76.2	66.6	66.7	64.8	76.6	76.2
7	45.3	76.7	76.7	49.8	75.0	45.2
8	76.8	79.3	79.3	77.4	79.4	76.8
9	57.7	51.2	51.2	58.4	51.2	57.6
10	36.9	37.9	37.9	38.2	38.5	36.7
11	19.3	18.9	18.9	19.2	18.8	19.3
12	42.7	43.0	43.0	43.4	42.8	42.7
13	45.4	44.2	44.2	44.4	44.1	45.5
14	66.4	56.6	56.6	62.8	56.5	66.5
15	70.1	71.2	71.2	71.1	71.0	70.1
16	41.9	42.8	42.8	42.6	43.4	41.7
17	56.0	58.0	57.9	58.0	57.7	55.8
18	15.3	16.5	16.5	16.5	16.5	15.3
19	18.6	16.9	16.9	17.0	16.8	18.7
20	36.5	36.5	36.5	36.5	40.6	36.3
21	19.0	19.1	19.1	19.0	20.8	19.0
22	33.0	32.9	32.9	32.8	139.3	33.3
23	28.4	28.8	28.7	28.8	130.1	31.5
24	83.8	85.0	84.8	84.9	78.9	78.1
25	31.3	31.9	31.7	31.8	35.3	34.6
26	18.5	18.4	18.4	18.4	18.9	19.5
27	18.1	18.3	18.3	18.3	18.7	17.5
1′	108.0	109.5	109.3	109.3		
2′	87.9	83.8[a]	83.9	83.9		
3′	83.3	79.2	79.0	79.0		
4′	84.0	83.9[a]	83.0	83.0		
5′	62.8	69.8	70.1	70.1		
1″	109.6	105.4	102.3	105.3		
2″	83.7	74.9	84.7	84.6		
3″	78.6	77.7	77.7	77.2		
4″	85.7	71.2	71.1	71.2		
5″	62.7	67.0	66.8	66.9		
2″-OMe			61.0	61.1		
1‴	102.6	102.3	105.4			
2‴	84.7	84.7	84.6			
3‴	77.6	76.8	77.2			
4‴	71.3	81.0	71.3			
5‴	66.8	64.2	66.9			
2‴-OMe	61.0	61.1	61.1			
4‴-OMe		59.0				

[a] Assignment may be reversed.

M, respectively. The sulfated substances **1** and **6** were nonactive in the sperm test below 5×10^{-5} M and showed weaker activities, with EC_{100} values of 8.9×10^{-5} and 11×10^{-5} M, respectively.

Experimental Section

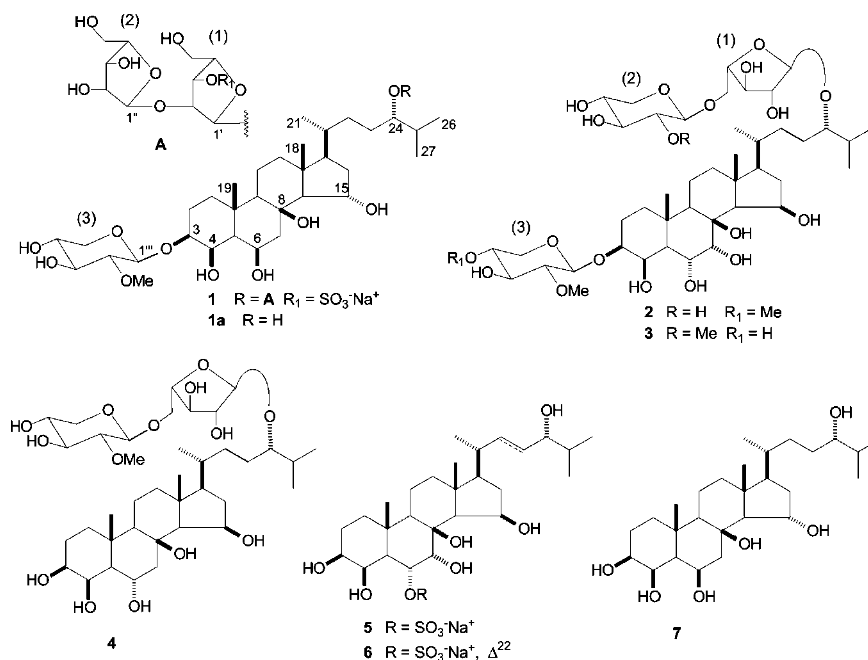
General Experimental Procedures. Optical rotations were determined on a Perkin-Elmer polarimeter model 343. The ^{1}H and ^{13}C NMR spectra were recorded on a Bruker DPX 300 spectrometer at 300 and 75.5 MHz, respectively, and on a Bruker DRX 500 spectrometer at 500 and 125.8 MHz, respectively, using signals of CD_3OD (3.30 ppm in the ^{1}H NMR spectrum and 49.0 ppm in the ^{13}C NMR spectrum) as the internal standard. MALDI-TOF mass spectra were recorded on a Bruker Biflex III laser-desorption mass spectrometer coupled with delayed extraction using a N_2 laser (337 nm). Samples of the tested compounds were dissolved in MeOH (1 mg/mL), and 1 μL aliquots were analyzed using a α-cyano-4-hydroxycinnamic acid (CCA) matrix. LSI mass spectra were recorded on an AMD-604S mass spectrometer (AMD, Germany) with an accelerating voltage of 8 keV and an energy of Cs^+ ions of 10–12 keV. For recording the mass spectra, a sample was dissolved in MeOH (10 mg/mL) and an aliquot (1 μL) was analyzed using glycerol (Sigma) as the matrix.

HPLC separations were carried out on a Agilent 1100 Series chromatograph equipped with a differential refractometer. Diasfer-110-C18 (10 μm, 250 × 15 mm) and Kromasil 100A-C18 (5 μm, 250 × 4.6 mm) columns were used. Low-pressure column liquid chromatography was performed using Amberlite XAD-2 (20–80 mesh, Sigma, Chemical Co.), Si gel KSK (50−160 μm, Sorbpolimer, Krasnodar, Russia), and Florisil (200–300 mesh, Aldrich Chemical Co.). Sorbfil Si gel plates (4.5 × 6.0 cm, 5–17 μm, Sorbpolimer, Krasnodar, Russia) in the eluent system BuOH/EtOH/H_2O (4:1:2) were used for thin-layer chromatography.

Animal Material. Specimens of *Hippasteria kurilensis* Fisher, 1911 (order Valvatida, family Goniasteridae) were collected by dredging at a depth of 100 m near Matua Island (Kuril Islands) in the Sea of Okhotsk (research vessel *Akademik Oparin*, 29th scientific cruise) in July 2003. Species identification was carried out by Dr. A. V. Smirnov (Zoological Institute of the Russian Academy of Science, St. Petersburg, Russia). A voucher specimen [no. 029-26] is on deposit at the marine specimen collection of the Pacific Institute of Bioorganic Chemistry, Vladivostok, Russia.

Extraction and Isolation. The fresh animals (770 g) were chopped and extracted twice with EtOH at 20 °C. The H_2O/EtOH layer was evaporated, and the residue was dissolved in H_2O (1 L). The H_2O-soluble fraction was passed through an Amberlite XAD-2 column (7 × 20 cm) and eluted with distilled H_2O until a negative chloride ion reaction was obtained, followed by elution with EtOH. The combined EtOH eluate was evaporated to give a brownish material (3.4 g). The resulting total fraction of steroidal compounds was chromatographed on a Si gel column (4 × 18 cm) using $CHCl_3$/EtOH (stepwise gradient, 4:1 → 1:6), and then obtained fractions were purified on a Florisil

Chart 1



column (2.5 × 15 cm) using $CHCl_3$/EtOH (stepwise gradient, 4:1 → 1:2). HPLC separation of collected subfractions, containing nonsulfated steroids, on a Diasfer-110-C18 column (10 μm, 250 × 15 mm, 2.5 mL/min) with 65% EtOH as the eluent system, followed by purification on a Kromasil 100A-C18 column (5 μm, 250 × 4.6 mm, 0.5 mL/min) with 80% MeOH as the eluent system, yielded pure **2** (1.4 mg, R_f 0.65), **3** (2.2 mg, R_f 0.65), **4** (1.7 mg, R_f 0.75), and **7** (1.0 mg, R_f 0.85). HPLC separation of collected subfractions, containing sulfated steroids, on a Diasfer-110-C18 column (10 μm, 250 × 15 mm, 2.5 mL/min) with 55% EtOH as the eluent system gave pure **1** (3.7 mg, R_f 0.55), **5** (1.0 mg, R_f 0.78), and **6** (1.1 mg, R_f 0.78).

Kurilensoside A (1): amorphous powder; $[\alpha]^{25}_D$ −48.0 (*c* 0.1; H_2O/MeOH, 3:1); ^{1}H NMR data, see Table 1; ^{13}C NMR data, see Table 3; MALDI-TOFMS (+) *m/z* 1003 [M + Na]$^+$, 901 [(M + Na) − 102]$^+$, 871 [(M + Na) − 132]$^+$, 857 [(M + Na) − 146]$^+$, 769 [(M + Na) − 102 − 132]$^+$, 637 [(M + Na) − 102 − 2 × 132]$^+$, 619 [637 − H_2O]$^+$, 601 [637 − 2 × H_2O]$^+$; MALDI-TOFMS (−) *m/z* 957 [M − Na]$^-$, 825 [(M − Na) − 132]$^-$, 811 [(M − Na) − 146]$^-$, 679 [(M − Na) − 132 − 146]$^-$; HR MALDI-TOFMS (+) *m/z* 1003.4223 [M + Na]$^+$ (calcd for $C_{43}H_{73}O_{21}SNa_2$, 1003.4160).

Kurilensoside B (2): amorphous powder; $[\alpha]^{25}_D$ −16.8 (*c* 0.1; H_2O/MeOH, 3:1); ^{1}H NMR data, see Table 1; ^{13}C NMR data, see Table 3; LSIMS(+) *m/z* 931 [M + Na]$^+$, 753 [(M + Na) − 178]$^+$, 621 [(M + Na) − 178 − 132]$^+$; LSIMS(−) *m/z* 907 [M − H]$^-$, 729 [(M − H) −178]$^-$; HR MALDI-TOFMS (+) *m/z* 931.4821 [M + Na]$^+$ (calcd for $C_{44}H_{76}O_{19}Na$, 931.4879).

Kurilensoside C (3): amorphous powder; $[\alpha]^{25}_D$ −23.2 (*c* 0.1; H_2O/MeOH, 3:1); ^{1}H NMR data, see Table 1; ^{13}C NMR data, see Table 3; LSIMS(+) *m/z* 931 [M + Na]$^+$; LSIMS(−) *m/z* 907 [M − H]$^-$, 743 [(M − H) − 164]$^-$; HR MALDI-TOFMS (+) *m/z* 931.4934 [M + Na]$^+$ (calcd for $C_{44}H_{76}O_{19}Na$, 931.4879).

Kurilensoside D (4): amorphous powder; $[\alpha]^{25}_D$ −4.6 (*c* 0.1; H_2O/MeOH, 1:6); ^{1}H NMR data, see Table 2; ^{13}C NMR data, see Table 3; LSIMS(+) *m/z* 769 [M + Na]$^+$; LSIMS(−) *m/z* 745 [M − H]$^-$, 599 [(M − H) −146]$^-$; HR MALDI-TOFMS (+) *m/z* 769.4394 [M + Na]$^+$ (calcd for $C_{38}H_{66}O_{14}Na$, 769.4351).

Compound 5: amorphous powder; $[\alpha]^{25}_D$ +8.1 (*c* 0.1, MeOH); ^{1}H NMR (CD_3OD, 500 MHz) δ 0.89 (3H, d, *J* = 6.7 Hz, H_3-27), 0.91 (3H, d, *J* = 6.7 Hz, H_3-26), 0.94 (3H, d, *J* = 6.6 Hz, H_3-21), 1.23 (3H, s, H_3-19), 1.26 (3H, s, H_3-18), 1.41 (1H, d, *J* = 5.8 Hz, H-14), 1.72 (1H, dd, *J* = 2.3, 12.2 Hz, H-5), 2.35 (1H, m, H-16), 3.20 (1H, m, H-24), 3.48 (1H, m, H-3), 4.21 (1H, br s, H-4), 4.24 (1H, d, *J* = 2.7 Hz, H-7), 4.50 (1H, ddd, *J* = 2.1, 5.5, 7.6 Hz, H-15), 5.03 (1H, dd, *J* = 2.7, 12.1 Hz, H-6); ^{13}C NMR data were identical with those reported by Levina et al.;[9] MALDI-TOFMS (+) *m/z* 609 [M + Na]$^+$, 507 [(M + Na) − 102]$^+$, 489 [(M + Na) − 120]$^+$.

Compound 6: amorphous powder; $[\alpha]^{25}_D$ ±0 (*c* 0.1, MeOH); ^{1}H NMR data, see Table 2; ^{13}C NMR data, see Table 3; LSIMS(+) *m/z* 607 [M + Na]$^+$, 505 [(M + Na) − 102]$^+$; 487 [(M + Na) − 120]$^+$, 454 [(M + Na) − 153]$^+$; LSIMS(−) *m/z* 561 [M − Na]$^-$, 408 [(M − Na) −153]$^-$, 97 [HSO_4]$^-$, 80 [SO_3]$^-$; HR MALDI-TOFMS (+) *m/z* 607.2587 [M + Na]$^+$ (calcd for $C_{27}H_{45}O_{10}SNa_2$, 607.2529).

Compound 7: amorphous powder; $[\alpha]^{25}_D$ +14.7 (*c* 0.1, MeOH); ^{1}H NMR data, see Table 2; ^{13}C NMR data, see Table 3; EIMS *m/z* (%) 468 [M]$^+$ (3), 450 [M − H_2O]$^+$ (75), 432 [M − $2H_2O$]$^+$ (100), 414 [M − $3H_2O$]$^+$ (75), 396 [M − $4H_2O$]$^+$ (30), 378 [M − $5H_2O$]$^+$ (8), 321 (78); HR MALDI-TOFMS (+) *m/z* 491.3369 [M + Na]$^+$ (calcd for $C_{27}H_{48}O_6Na$, 491.3349).

Acid Hydrolysis of 1. A solution of glycoside **1** (1 mg) in aqueous 2 M CF_3COOH (1 mL) was heated at 100 °C for 2 h in a sealed ampule. The reaction mixture was evaporated in vacuo, and the residue was dissolved in H_2O and extracted with $CHCl_3$ twice. The monosaccharides 2-*O*-methylxylose and arabinose were identified in the aqueous layer by TLC on Si gel in BuOH/EtOH/H_2O (4:1:2). The aqueous layer was evaporated to dryness. Then, pyridine (0.5 mL) and $NH_2OH \cdot HCl$ (2 mg) were added to the dried residue, and the mixture was heated at 90 °C for 40 min. After that time, 0.5 mL of Ac_2O was added and the heating at 90 °C was continued for a further 1 h. The solution was concentrated, and the resulting aldononitrile peracetates were analyzed by GLC using standard aldononitrile peracetates as reference samples.

Partial Acid Hydrolysis of 1. A solution of **1** (2 mg) in 50% aqueous CH_3COOH (1 mL) was stirred at 54 °C for 48 h. The reaction mixture was evaporated in vacuo, and the residue was subjected to HPLC separation on a Diasfer-110-C18 column (5 μm, 250 × 4 mm, 0.5 mL/min) with 65% EtOH as the eluent system to give **1a** (0.5 mg). ^{1}H NMR data of **1a** were identical with those of linckoside L_1.[3]

Preparation of (*R*)-MTPA Ester of Compound 1a. Compound **1a** (0.5 mg) was treated with *S*-(+)-α-methoxy-α-(trifluoromethyl)phenylacetyl (MTPA) chloride (3 μL) in dry pyridine (100 μL) for 4 h at room temperature. After removal the solvent, the product was purified on a Si gel column (0.8 × 9 cm) using hexane/$CHCl_3$ (1:4) to obtain the corresponding (*R*)-MTPA ester of **1a** (0.3 mg): selected ^{1}H NMR (CD_3OD, 500 MHz) δ 0.80 (3H, d, *J* = 6.7 Hz, H_3-27), 0.82 (3H, d, *J* = 6.7 Hz, H_3-26), 0.92 (3H, d, *J* = 6.2 Hz, H_3-21), 0.98 (3H, s, H_3-18), 1.37 (3H, s, H_3-19).

Solvolysis of 5. A solution of **5** (1.0 mg) in a mixture of pyridine (0.5 mL) and dioxane (0.5 mL) was heated at 120 °C for 5 h. The reaction mixture was evaporated to dryness and purified on a Florisil column (0.8 × 2 cm) using $CHCl_3$/EtOH (3:1) to obtain the desulfated derivative **5a** (0.5 mg): ^{1}H NMR (CD_3OD, 500 MHz) δ 0.88 (3H, d,

$J = 6.7$ Hz, H_3-27), 0.90 (3H, d, $J = 6.7$ Hz, H_3-27), 0.94 (3H, d, $J = 6.6$ Hz, H_3-21), 1.15 (3H, s, H_3-19), 1.26 (3H, s, H_3-19), 1.40 (1H, d, $J = 5.5$ Hz, H-14), 1.47 (1H, dd, $J = 2.2$, 11.6 Hz, H-5), 2.36 (1H, m, H-16), 3.20 (1H, m, H-24), 3.44 (1H, m, H-3), 3.91 (1H, d, $J = 2.9$ Hz, H-7), 4.18 (1H, br s, H-4), 4.26 (1H, dd, $J = 2.8$, 11.6 Hz, H-6), 4.52 (1H, ddd, $J = 2.0$, 5.5, 7.5 Hz, H-15).

Sperm Test. The sea urchin *Strongylocentrotus nudus* sperm and eggs were used as test material for bioassays according to the method of Kobayashi[10] with minor modification. Two drops of the sperm solution was added to 25 mL of sea water and mixed just prior to each test. Then 0.9 mL of sperm suspension was added to each well of a 24-well microplate, including 0.1 mL of toxicant solution in distilled H_2O. After 15 min of exposure, approximately 1000 prewashed eggs were added to the sperm−toxicant mixture and fertilization was allowed to proceed for 15 min. The eggs were then preserved in 2% formaldehyde, and fertilization was tabulated by scoring the presence or absence of a complete fertilization membrane in a subsample of about 100 eggs. Fertilization in seawater controls was $\geq 98\%$. Data for every compound were analyzed from three independent measurements. The results were expressed as a percentage relative to the controls. Means and standard errors for each treatment were calculated, using SigmaPlot 3.02 software (Jandel Corporation).

Acknowledgment. The research described in this publication was made possible in part by Program of Presidium of RAS "Molecular and Cell Biology", Grant No. 08-04-00599 from the RFBR, Grants No. 06-II-CM-05-001 and 06-III-B-05-128 from FEB RAS, and Grant No. 2813.2008.4 supporting leading Russian scientific schools.

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NP070637X