Four new steroid glycosides from the Vietnamese starfish *Linckia laevigata*

A. A. Kicha,[a]★ N. V. Ivanchina,[a] A. I. Kalinovsky,[a] P. S. Dmitrenok,[a] E. V. Sokolova,[a] I. G. Agafonova,[a] J. Morre,[b] and V. A. Stonik[a]

[a]*Pacific Institute of Bioorganic Chemistry of the Far Eastern Branch of the Russian Academy of Sciences, 159 prosp. 100 let Vladivostoku, 690022 Vladivostok, Russian Federation. Fax: +7 (423 2) 31 4050. E-mail: kicha@piboc.dvo.ru*

[b]*Department of Chemistry and Environmental Health Sciences Center, Oregon State University, Corvallis, Oregon 97331, USA*

Eighteen steroid compounds, including four new steroid glycosides, *viz.*, linckosides L3—L6, along with the previously known nine glycosides and five free polyhydroxysteroids, were isolated from the starfish *Linckia laevigata* collected on the Vietnamese coast. New compounds contain the 2-*O*-methyl-β-D-xylopyranosyl unit at the C(3) atom of polyhydroxylated steroidal aglycone. Two of these compounds are monosides, and the other two belong to biosides and have an additional β-D-xylopyranosyl residue at C(26) in the side chain of the aglycone. The structures of the new compounds were determined by NMR spectroscopy, mainly by 2D NMR, and mass spectrometry.

Key words: starfish, *Linckia laevigata*, polyhydroxylated steroid, steroid glycoside, NMR spectra.

Free polyhydroxylated steroids and related glycosides are the most widespread secondary metabolites of starfishes. These compounds attract interest not only because of their unusual chemical structure but also due to their various biological properties, including embryotoxic, antifungal, antiviral, antifouling, and other activities.[1,2] In continuation of search for new steroid compounds from starfishes,[3] we studied the steroid composition of the starfish *L. laevigata* (the order Valvatida, the family Ophiodiasteridae) collected in the Van Phong Bay of the South China Sea on the Vietnamese coast.

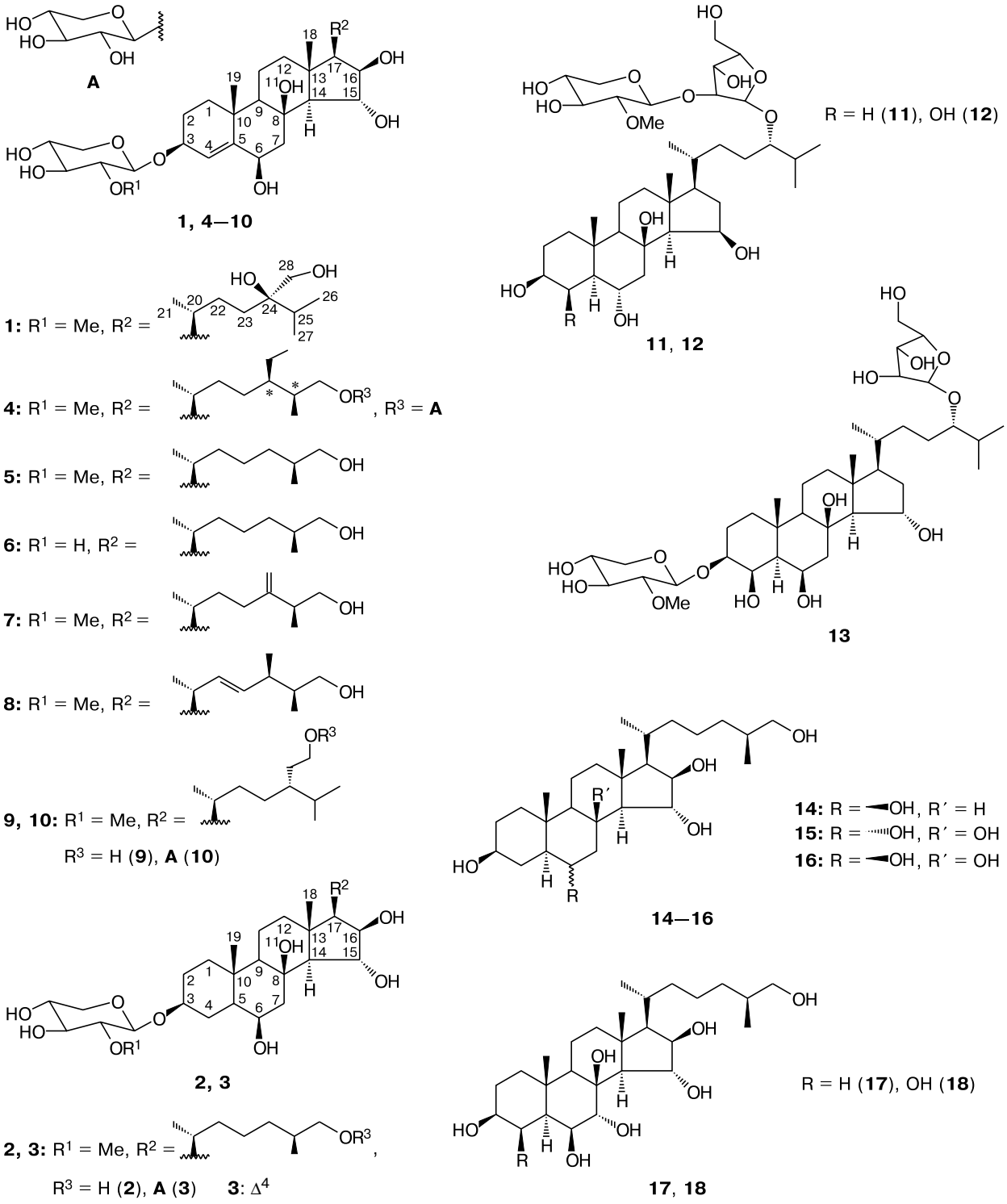
Results and Discussion

Fractions of steroid compounds were isolated from an ethanolic extract of the starfish *L. laevigata* by column chromatography on Polychrom 1, silica gel, and Florisil. Four new glycosides, which were called linckosides L3—L6 (**1—4**), nine previously known glycosides (**5—13**), and five previously known free polyhydroxysteroids (**14—18**) were isolated from these fractions by HPLC on semipreparative and analytical Diasfer-110-C18 columns. The results of chromatographic separation of extracted polar steroids are given in Table 1. The known compounds were identified by comparing the ^{1}H and ^{13}C NMR spectra and mass spectra with the data published in the literature.

The high-resolution (+)-ESI-TOF mass spectrum of linckoside L3 (**1**) contains a peak of a pseudomolecular ion $[M + Na]^+$ at m/z 665.3898, which corresponds to the molecular formula $C_{34}H_{58}O_{11}$. The ^{13}C NMR and DEPT spectra show signals for 34 carbon atoms, among which are signals of six methyl groups, including one methoxy group, nine methylene groups, thirteen methine groups, two quaternary carbon atoms, two carbon atoms that are not bound to protons, and two olefinic carbon atoms, including one carbon atom bound to a proton. The chemical shifts of the anomeric proton at δ 4.41 (J = 7.6 Hz), the anomeric carbon atom at δ 104.6, and the carbon atoms bound to the oxygen atoms at δ 77.5, 76.4, 76.2, 80.5, 83.0, 77.3, 66.1, 84.9, 77.5, 71.2, and 66.8 evidenced that the molecule contains the β-pentoside fragment and the heptasubstituted steroidal core. An analysis of the NMR spectroscopic data for compound **1** showed that linckoside L3 is structurally similar to echinasteroside C (**5**), which was isolated for the first time from the starfish *Echinaster brasiliensis*[4] and contains the analogous Δ^4-3β,6β,8,15α,16β-pentahydroxycholestane aglycone with the 2-*O*-methyl-β-D-xylopyranosyl residue at the C(3) atom. The ^{1}H—^{1}H COSY 90, HSQC, and HMBC experiments allowed us to assign the signals for all protons and carbon atoms (Table 2). The attachment of the monosaccharide residue to the C(3) atom was confirmed by the correlation between C(3) and the anomeric proton HC(1′) in the HMBC spectrum. A comparison of

Published in Russian in *Izvestiya Akademii Nauk. Seriya Khimicheskaya,* No. 4, pp. 792—799, April, 2007.

1066-5285/07/5604-0823



Note. The relative configurations of the asymmetric centers C(24) and C(25) are marked with asterisks.

the NMR spectra of compound **1** with the corresponding data for certonardosterol B_4 from the starfish *Certonardoa semiregularis*[16] confirmed that compound **1** contains the identical 24-hydroxymethyl-24-hydroxycholestane side chain. The 20*R* configuration of the asymmetric center was determined based on the chemical shift of the proton HC(21) at δ 0.94 (δ 0.90—0.96 for 20*R* steroids[1]). Earlier, it has been reported that there are small but important differences in the signals for the HC(28), H′C(28), and C(28) atoms in the NMR spectra of the synthetic 24*R* and

Table 1. Polyhydroxylated steroid compounds isolated from the Vietnamese starfish *L. laevigata*[a]

Compound	Amount (mg)	R_f[b]	(+) MALDI-TOF mass spectrum $[M + Na]^+$	Starfish[c]
Linckoside L3 (**1**)	3.8	0.42	665[d]	
Linckoside L4 (**2**)	2.0	0.41	637	
Linckoside L5 (**3**)	2.2	0.35	767	
Linckoside L6 (**4**)	4.3	0.36	795[d]	
Echinasteroside C (**5**)	45.2	0.56	635	*Echinaster brasiliensis*[4]
Linckoside J (**6**)	5.5	0.42	621	*L. laevigata*[e,5]
Linckoside F (**7**)	3.0	0.55	647[d]	*L. laevigata*[e,5]
Desulfated echinasteroside A (**8**)	2.4	0.62	647	*Henricia downeyae*[6]
Desulfated echinasteroside B (**9**)	7.0	0.61	663	*E. sepositus*[7]
Linckoside B (**10**)	27.0	0.37	795	*L. laevigata*[e,8]
Halityloside E (**11**)	4.2	0.56	767	*Halityle regularis*[9]
Culcitoside C1 (halityloside D) (**12**)	5.0	0.48	783	*Culcita novaeguineae*[10]
Granulatoside A (**13**)	1.2	0.50	769	*Choriaster granulatus*[11]
(25*S*)-5α-Cholestane-3β,6β,15α,16β,26-pentol (**14**)	1.2	0.57	475	*Hacelia attenuate*[12]
(25*S*)-5α-Cholestane-3β,6α,8,15α,16β,26-hexol (**15**)	0.8	0.49	491	*Protoreaster nodosus*[13]
(25*S*)-5α-Cholestane-3β,6β,8,15α,16β,26-hexol (**16**)	4.2	0.60	491	*Sphaerodiscus placenta*[14]
(25*S*)-5α-Cholestane-3β,6β,7α,8,15α,16β,26-heptol (**17**)	8.7	0.62	507	род *Rosaster*[15]
(25*S*)-5α-Cholestane-3β,4β,6β,7α,8,15α,16β,26-octol (**18**)	4.1	0.66	523	род *Rosaster*[15]

[a] Isolated from 3.6 kg of the crude mass of animals.
[b] In the 9 : 5 toluene—ethanol system.
[c] The starfish from which the compound was isolated for the first time.
[d] The (+)-ESI-TOF mass spectrum.
[e] The starfish was collected near the Okinawa Island (Japan).

Table 2. ^1H and ^{13}C NMR spectra of compounds **1** and **2** (CD_3OD, δ, *J*/Hz)[a]

Atom	**1**			**2**		
	δ_H	HMBC	δ_C[b]	δ_H	HMBC	δ_C[b]
1	1.79 (m) 1.27 (m)		39.7 (CH_2)	1.73 (m) 0.97 (m)		41.4 (CH_2)
2	1.97 (m) 1.76 (m)		27.9 (CH_2)	1.85 (m) 1.57 (m)		30.3 (CH_2)
3	4.18 (m)		77.5 (CH)	3.66 (m)		80.4 (CH)
4	5.63 (br.s)	C(2), C(6), C(10)	126.9 (CH)	1.83 (m) 1.74 (m)		33.3 (CH_2)
5			148.5 (C)	1.22 (m)		50.5 (CH)[c]
6	4.30 (t, *J* = 3.0)		76.4 (CH)	3.87 (q, *J* = 2.5)		74.1 (CH)
7	2.57 (dd, *J* = 3.0, *J* = 14.8); 1.49 (dd, *J* = 3.2, *J* = 14.8)		44.4 (CH_2)	2.42 (dd, *J* = 3.0, *J* = 14.7) 1.56 (m)	C(6), C(8), C(9)	45.4 (CH_2)
8			76.2 (C)			76.9 (C)
9	1.03 (m)		57.8 (CH)	0.97 (m)		57.2 (CH)
10			37.7 (C)			36.8 (C)

(*to be continued*)

Table 2 (*continued*)

Atom	**1**			**2**		
	δ_H	HMBC	δ_C[b]	δ_H	HMBC	δ_C[b]
11	1.85 (m) 1.47 (m)		19.5 (CH_2)	1.84 (m) 1.52 (m)		19.6 (CH_2)
12	1.97 (m) 1.18 (m)		43.0 (CH_2)	1.96 (m) 1.20 (m)		43.1 (CH_2)
13			45.1 (C)			45.3 (C)
14	1.01 (d, J = 10.6)	C(15), C(18)	63.6 (CH)	1.04 (d, J = 10.4)	C(15), C(17)	63.8 (CH)
15	4.15 (dd, J = 2.6, J = 10.8)		80.5 (CH)	4.13 (dd, J = 2.5, J = 10.8)	C(16)	80.9 (CH)
16	4.01 (dd, J = 2.6, J = 7.5)	C(13)	83.0 (CH)	3.96 (dd, J = 2.5, J = 7.5)	C(13), C(15)	82.9 (CH)
17	1.18 (m)		60.9 (CH)	1.19 (m)		60.7 (CH)
18	1.12 (s)	C(12), C(13), C(14), C(17)	16.7 (CH_3)	1.11 (s)	C(12), C(13), C(14), C(17)	16.8 (CH_3)
19	1.36 (s)	C(1), C(5), C(9), C(10)	22.7 (CH_3)	1.16 (s)	C(1), C(5), C(9), C(10)	15.9 (CH_3)
20	1.77 (m)		31.7 (CH)	1.85 (m)		30.7 (CH)
21	0.94 (d, J = 7.0)	C(17), C(20), C(22)	18.6 (CH_3)	0.92 (d, J = 7.5)	C(17), C(20), C(22)	18.5 (CH_3)
22	1.60 (m)		30.0 (CH_2)	1.57 (m) 1.04 (m)		37.2 (CH_2)
23	1.61 (m) 1.42 (m)		31.6 (CH_2)	1.22 (m) 1.47 (m)		24.8 (CH_2)
24			77.3 (C)	1.43 (m) 1.05 (m)		34.9 (CH_2)
25	1.85 (m)	C(24), C(26), C(27)	33.8 (CH)	1.57 (m)		37.0 (CH)
26	0.91 (d, J = 7.0)	C(24), C(25), C(27)	17.1 (CH_3)	3.30[c] 3.42 (dd, J = 5.6, J = 10.3)		68.4 (CH_2)
27	0.91 (d, J = 7.0)	C(24), C(25), C(26)	17.4 (CH_3)	0.90 (d, J = 7.0)	C(24), C(25), C(26)	17.3 (CH_3)
28	3.47 (m)		66.1 (CH_2)			
1′	4.41 (d, J = 7.6)	C(3)	104.6 (CH)	4.41 (d, J = 7.5)	C(3)	103.6 (CH)
2′	2.81 (dd, J = 7.6, J = 9.1)	C(1′)	84.9 (CH)	2.81 (dd, J = 7.5, J = 9.1)	C(1′), C(3′)	84.9 (CH)
3′	3.30[c]		77.5 (CH)	3.30[c]		77.5 (CH)
4′	3.45 (m)		71.2 (CH)	3.47 (m)		71.3 (CH)
5′	3.80 (dd, J = 5.2, J = 11.4); 3.16 (t, J = 11.2)		66.8 (CH_2)	3.79 (dd, J = 5.2, J = 11.0); 3.14 (t, J = 11.0)	C(3′), C(4′)	66.8 (CH_2)
2′-OMe	3.57 (s)	C(2′)	61.2 (CH_3)	3.57 (s)	C(2′)	61.0 (CH_3)

[a] The ^1H and ^{13}C NMR spectra were recorded at 500 and 125.8 MHz, respectively; the assignment of the signals was made using two-dimensional NMR spectroscopy (^1H—^1H COSY and HSQC).
[b] The multiplicities of the signals were determined from the DEPT spectrum.
[c] The signals overlap with the signals of the solvent.

24*S* epimers of 24-hydroxymethyl-24-hydroxycholesterol (24*R* epimer: δ_H 3.52 and 3.48, $\Delta\delta_H$ = 0.04, δ_C 66.0; 24*S* epimer: δ_H 3.53 and 3.47, $\Delta\delta_H$ = 0.06, δ_C 66.3).[17] The NMR spectroscopic data for glycoside **1** ($\Delta\delta_H$ = 0.00, δ_C 66.1) better correspond to the 24*R* epimer. An analysis of the ^1H NMR spectra of 28-(*R*)- and 28-(*S*)-MTPA esters, which were prepared by the reaction of **1** with *S*-(+)- and *R*-(−)-MTPA chlorides, respectively (see Experimental), confirmed the *R* configuration of the asymmetric center C(24). For example, the signals for HC(28) and H′C(28) are observed at δ 4.24 and 4.19 ($\Delta\delta_H$ = 0.05) in the ^1H NMR spectrum of (*R*)-MTPA ester and are more closely spaced (δ 4.24 and 4.21, $\Delta\delta_H$ = 0.03) in the spectrum of (*S*)-MTPA ester of compound **1**, which is

consistent with the spectroscopic data for the (*R*)- and (*S*)-MTPA derivatives of (24*R*)-24-hydroxymethyl-24-hydroxycholesterol.[16,17] Based on the available data, the structure of linckoside L3 was identified as (24*R*)-3-*O*-(2-*O*-methyl-β-D-xylopyranosyl)-24-methylcholest-4-ene-3β,6β,8,15α,16β,24,28-heptol.

Linckoside L4 (**2**) has the molecular formula $C_{33}H_{58}O_{10}$, as evidenced from the high-resolution (+)-MALDI-TOF mass spectrum containing a peak of a pseudomolecular ion $[M + Na]^+$ at *m/z* 637.3986. An examination of its NMR spectra showed that compound **2** is the 4,5-dihydro derivative of echinasteroside C (**5**).[4] The $^1H—^1H$ COSY 90, HSQC, and HMBC correlations supported the structure of glycoside **2** (see Table 2). The *S* configuration was assigned to the asymmetric center C(25) in compound **2** based on the identity of the chemical shifts of the protons and carbon atoms in the NMR spectra of glycosides **2** and **5**. As a result, linckoside L4 was identified as (25*S*)-3-*O*-(2-*O*-methyl-β-D-xylopyranosyl)-5α-cholestane-3β,6β,8,15α,16β,26-hexol.

The high-resolution (+)-MALDI-TOF mass spectrum of linckoside L5 (**3**) contains a peak of a pseudomolecular ion $[M + Na]^+$ at *m/z* 767.4125 corresponding to the molecular formula $C_{38}H_{64}O_{14}$. The ^1H and ^{13}C NMR spectroscopic data for compound **3** (Table 3) are similar to those of echinasteroside C (**5**),[4] which confirms the presence of identical Δ^4-3β,6β,8,15α,16β,26-hexahydroxycholestane aglycone containing the 2-*O*-methyl-β-xylopyranosyl residue at the C(3) atom. However, unlike the spectra of compound **5**, the NMR spectra of glycoside **3** show signals of an additional monosaccharide residue. These signals virtually coincided with the spectroscopic data for the unsubstituted β-D-xylopyranosyl residue, which is common in glycosides of polyhydroxysteroids of starfishes.[1,8] The signals for C(26) and HC(26) in the side chain of glycoside **3** are shifted downfield relative to those in the spectra of compound **5** from δ 68.4 to 76.3 and from δ 3.42 to 3.68, respectively. This indicates that the xylose residue is bound to the side-chain C(26) atom of aglycone. The presence of 2-*O*-methyl-β-xylopyranose at the C(3) atom and of β-xylopyranose at the C(26) atom was also supported by the HMBC correlations between the anomeric protons and the carbon atoms: from HC(1′) to C(3) and from H(1″) to C(26). By analogy with accompanying echinasteroside C (**5**), the *S* configuration was assigned to the asymmetric center C(25). Therefore, the structure of linckoside L5 was established as (25*S*)-3-*O*-(2-*O*-methyl-β-D-xylopyranosyl)-26-*O*-(β-D-xylopyranosyl)-cholest-4-ene-3β,6β,8,15α,16β,26-hexol.

The high-resolution (+)-ESI-TOF mass spectrum of linckoside L6 (**4**) contains a peak of a pseudomolecular ion $[M + Na]^+$ at *m/z* 795.4492 corresponding to the molecular formula $C_{40}H_{68}O_{14}$. The ^1H and ^{13}C NMR spectra of glycoside **4** (see Table 3) provide evidence that this compound and glycoside **3** contain the identical Δ^4-3β,6β,8,15α,16β-pentahydroxysteroid aglycone with the 2-*O*-methyl-β-D-xylopyranosyl residue at C(3) and the β-D-xylopyranosyl residue at the side-chain C(26) atom. However, the chemical shifts of the side-chain atoms of

Table 3. ^1H and ^{13}C NMR spectra of compounds **3** and **4** (CD_3OD, δ, *J*/Hz)[a]

Atom	**3**			**4**		
	δ_H	HMBC	δ_C[b]	δ_H	HMBC	δ_C[b]
1	1.78 (m) 1.26 (m)		39.7 (CH_2)	1.79 (m) 1.27 (m)		39.7 (CH_2)
2	1.97 (m) 1.75 (m)		27.9 (CH_2)	1.97 (m) 1.75 (m)		27.9 (CH_2)
3	4.17 (m)		77.5 (CH)	4.18 (m)	C(1′), C(4), C(5)	77.5 (CH)
4	5.63 (br.s)	C(6), C(10)	126.9 (CH)	5.64 (br.s)	C(2), C(6), C(10)	126.9 (CH)
5			148.5 (C)			148.5 (C)
6	4.30 (t, *J* = 3.0)	C(8)	76.4 (CH)	4.30 (t, *J* = 3.0)	C(4), C(8), C(10)	76.4 (CH)
7	2.57 (dd, *J* = 3.0, *J* = 14.7); 1.49 (dd, *J* = 3.2, *J* = 14.8)	C(8), C(9)	44.4 (CH_2)	2.57 (dd, *J* = 3.0, *J* = 14.8); 1.49 (dd, *J* = 3.3, *J* = 14.8)	C(5), C(8), C(9)	44.4 (CH_2)
8			76.2 (C)			76.1 (C)
9	1.03 (m)		57.8 (CH)	1.03 (m)		57.8 (CH)
10			37.7 (C)			37.7 (C)

(*to be continued*)

Table 3 (*continued*)

Atom	**3**			**4**		
	δ_H	HMBC	δ_C[b]	δ_H	HMBC	δ_C[b]
11	1.86 (m) 1.45 (m)		19.5 (CH_2)	1.86 (m) 1.47 (m)		19.5 (CH_2)
12	1.97 (m) 1.18 (m)		43.0 (CH_2)	1.97 (m) 1.17 (m)		43.0 (CH_2)
13			45.1 (C)			45.1 (C)
14	1.01 (d, J = 10.6)		63.6 (CH)	1.01 (d, J = 10.8)	C(13), C(15), C(18)	63.7 (CH)
15	4.14 (dd, J = 2.6, J = 10.8)		80.9 (CH)	4.14 (dd, J = 2.6, J = 10.8)	C(16)	81.0 (CH)
16	3.96 (dd, J = 2.6, J = 7.5)	C(13)	82.9 (CH)	3.96 (dd, J = 2.6, J = 7.6)	C(13), C(15)	82.9 (CH)
17	1.17 (m)		60.6 (CH)	1.20 (m)		60.4 (CH)
18	1.12 (s)	C(12), C(13), C(14), C(17)	16.8 (CH_3)	1.12 (s)	C(12), C(13), C(14), C(17)	16.8 (CH_3)
19	1.36 (s)	C(1), C(5), C(9), C(10)	22.7 (CH_3)	1.36 (s)	C(1), C(5), C(9), C(10)	22.7 (CH_3)
20	1.85 (m)		30.7 (CH)	1.83 (m)		31.0 (CH)
21	0.92 (d, J = 7.0)	C(17), C(20), C(22)	18.5 (CH_3)	0.93 (d, J = 6.5)	C(17), C(20), C(22), C(23)	18.6 (CH_3)
22	1.53 (m) 1.03 (m)		37.1 (CH_2)	1.56 (m) 1.05 (m)		34.8 (CH_2)
23	1.44 (m) 1.19 (m)		24.7 (CH_2)	1.33 (m) 1.23 (m)		28.1 (CH_2)
24	1.43 (m) 1.07 (m)		35.1 (CH_2)	1.23 (m)		43.2 (CH)
25	1.73 (m)		34.8 (CH)	1.87 (m)		36.5 (CH)
26	3.68 (dd, J = 6.0, J = 9.5); 3.27 (dd, J = 7.6, J = 9.5)		76.3 (CH_2)	3.80 (m) 3.30 (m)	C(24), C(25), C(27)	74.8 (CH_2)
27	0.93 (d, J = 6.5)	C(24), C(25), C(26)	17.6 (CH_3)	0.89 (d, J = 7.0)	C(24), C(25), C(26)	14.2 (CH_3)
28				1.20 (m)		23.6 (CH_2)
29				0.88 (t, J = 7.5)	C(24), C(28)	12.4 (CH_3)
1′	4.41 (d, J = 7.6)	C(3)	104.6 (CH)	4.41 (d, J = 7.6)	C(3)	104.6 (CH)
2′	2.81 (dd, J = 7.6, J = 9.1)	C(1′), C(3′), OMe	84.9 (CH)	2.81 (dd, J = 7.6, J = 9.1)	C(1′), C(3′), OMe	84.9 (CH)
3′	3.30[c]		77.5 (CH)	3.30[b]		77.5 (CH)
4′	3.46 (m)		71.2 (CH)	3.46 (m)	C(2′)	71.2 (CH)
5′	3.80 (dd, J = 5.2, J = 11.0) 3.16 (t, J = 11.5)	C(4′)	66.8 (CH_2)	3.80 (dd, J = 5.2, J = 11.0) 3.16 (t, J = 11.5)	C(1′), C(3′), C(4′)	66.8 (CH_2)
2′-OMe	3.58 (s)	C(2′)	61.1 (CH_3)	3.57 (s)	C(2′)	61.2 (CH_3)
1″	4.16 (d, J = 7.5)	C(26)	105.4 (CH)	4.16 (d, J = 7.6)	C(26)	105.5 (CH)
2″	3.16 (dd, J = 7.5, J = 9.1)		75.0 (CH)	3.15 (dd, J = 7.4, J = 9.1)		75.0 (CH)
3″	3.29 (t, J = 8.8)		77.9 (CH)	3.29 (t, J = 8.9)		77.9 (CH)
4″	3.46 (m)		71.2 (CH)	3.46 (m)		71.2 (CH)
5″	3.83 (dd, J = 5.2, J = 11.5); 3.17 (t, J = 10.8)	C(4′)	66.9 (CH_2)	3.83 (dd, J = 5.2, J = 11.2); 3.17 (t, J = 11.0)	C(1′), C(3′)	66.9 (CH_2)

[a] The ^1H and ^{13}C NMR spectra were recorded at 500 and 125.8 MHz, respectively; the assignment of the signals was made using two-dimensional NMR spectroscopy (^1H—^1H COSY and HSQC).
[b] The multiplicities of the signals were determined from the DEPT spectrum.

glycoside **4** differ from the corresponding shifts in the spectrum of glycoside **3**. According to the ^{13}C NMR and DEPT spectroscopic data, the side chain of glycoside **4** contains three methyl groups, four methylene groups, including one carbon atom bound to the oxygen atom (δ 74.8), and three methine groups. The ^{1}H NMR spectrum of glycoside **4** shows the following characteristic signals for the side-chain protons: two methyl doublets of $H_3C(27)$ at δ 0.89 and $H_3C(21)$ at δ 0.93, one methyl triplet of $H_3C(29)$ at δ 0.88, and two multiplets of HC(26) and H´C(26) at δ 3.80 and 3.30. All signals for the side-chain protons and carbon atoms were identified based on $^{1}H—^{1}H$ COSY 90, HSQC, and HMBC experiments (see Table 3), which showed that glycoside **4** contains the rarely observed side chain, which has been found only in attenuatoside S-III from the starfish *Hacelia attenuata*.[18] The relative configurations of the C(24) and C(25) atoms were established by comparing the ^{13}C NMR spectrum of glycoside **4** with the spectra of model synthetic compounds, *viz.*, the enantiomeric pairs (24*R*/25*S*)/(24*S*/25*R*)- and (24*R*/25*R*)/(24*S*/25*S*)-24-ethyl-26-hydroxysteroids, which differ in the chemical shifts of the C(23), C(24), and C(28) atoms.[1] The signals for the C(23), C(24), and C(28) atoms in the ^{13}C NMR spectrum of glycoside **4** at δ 28.1, 43.2, and 23.6 are in good agreement with the corresponding signals at δ 28.3, 43.1, and 23.6 and at δ 28.4, 42.8, and 23.7 in the spectra of (24*R*/25*S*)-24-ethyl-26-hydroxysteroid and (24*S*/25*R*)-24-ethyl-26-hydroxysteroid, respectively.[1] Thus, only two structures of the side chain having the (24*R*/25*S*) or (24*S*/25*R*) configurations of the asymmetric centers are possible for glycoside **4**. Unfortunately, compound **4** was isolated in a small amount, and it was impossible to prepare the 26-(*R*)- and 26-(*S*)-MTPA derivatives, which are necessary for the determination of the absolute configurations of the asymmetric centers C(24) and C(25). Based on the available data, the structure of 3-*O*-(2-*O*-methyl-β-D-xylopyranosyl)-26-*O*-(β-D-xylopyranosyl)-24-ethylcholest-4-ene-3β,6β,8,15α,16β,26-hexol was assigned to linckoside L6 (**4**).

Earlier, as a result of investigations of the population of the starfish *L. laevigata* collected near the Okinawa Island (Japan), monosides and biosides containing the Δ^4-3β,6β,8,15α,16β-pentahydroxysteroidal aglycone with the 2-*O*-methyl-β-D-xylopyranosyl or β-D-xylopyranosyl residue at the C(3) atom were isolated.[5,8] The present study demonstrated that the fraction of steroid compounds isolated from the Vietnamese collection of the starfish *L. laevigata* contains structurally identical or similar compounds, including new glycosides **1—4**. The unusual composition of this starfish population is associated with the presence of steroid biosides **11** and **12** glycosylated only at the side chain and free polyhydroxylated steroids **14—18**.

The inhibitory action of new compounds **1** and **4** on the eggs of the sea urchin *Strongylocentrotus intermedius* was studied in the step of eight blastomers. Linckoside L3 (**1**) exhibited weak cytotoxic activity with the effective inhibitory concentration $EC_{100} = 100$ μg mL^{-1}. Linckoside L6 (**4**) containing an additional monosaccharide residue in the side chain of the aglycone appeared to be more active with $EC_{100} = 50$ μg mL^{-1}.

Experimental

The ^{1}H and ^{13}C NMR spectra were recorded in CD_3OD on a Bruker DPX 300 spectrometer at 300 and 75.5 MHz and on a Bruker DRX 500 spectrometer at 500 and 125.8 MHz, respectively, with $SiMe_4$ as the internal standard. The optical rotation was measured on a Perkin—Elmer 343 polarimeter.

The high-resolution ESI-TOF (electrospray ionization time-of-flight) mass spectra were obtained on a Micromass Q-TOF MICRO spectrometer (Micromass, UK). The instrument was calibrated for high-resolution measurements using a mixture of polyethylene glycols with molecular weights from 200 to 1000 (instrumental resolution was 5000, the deviation <5 ppm).

The MALDI-TOF mass spectra were obtained on a Biflex III mass spectrometer (Bruker, Germany, N_2 laser, 337 nm). Samples were dissolved in MeOH (1 mg mL^{-1}), and an aliquot (1 μL) was analyzed with 2,5-dihydroxybenzoic acid as the matrix. High-performance liquid chromatography was performed on an Agilent 1100 Series chromatograph equipped with a refractometer as the detector and with Diasfer-110-C18 columns ((1) 10 μm, 15×250 mm, and (2) 5 μm, 4×250 mm).

Polychrom 1 (Teflon powder, Biolar, Latvia), silica gel KSK (50—160 μm, Sorbpolimer, Krasnodar, Russia), and Florisil (200—300 mesh, Aldrich Chemical Co.) were used for column chromatography. Thin-layer chromatography was carried out on Sorbfil plates (4.5×6.0 cm, Sorbpolimer, Krasnodar, Russia) with a layer of silica gel STKh-1A (5—17 μm) fixed on a foil. Compounds were visualized by spraying the chromatograms with concentrated H_2SO_4 followed by heating at 110 °C for 10 min.

Samples of the starfishes were collected from a depth of 5—10 m in the South China Sea (Van Phong Bay, the Vietnamese coast) during the expedition No. 30 of the research ship "Akademik Oparin" in January 2005. The species determination was carried out by V. B. Krasokhin (Pacific Institute of Bioorganic Chemistry of the Far Eastern Branch of the Russian Academy of Sciences).

Isolation of compounds 1—18. Starfishes (3.6 kg) were milled and twice extracted with ethanol (3 L kg^{-1}) at room temperature. Ethanolic extracts were concentrated *in vacuo*. The residue was dissolved in water (1.5 L) and passed through a chromatographic column (8×62 cm) packed with Polychrom 1. The column was washed with water until Cl^- ions disappeared in the eluate and then with 50% ethanol. The ethanolic eluate was concentrated. The resulting total fraction of steroid compounds (5.5 g) was successively chromatographed on columns with silica gel (4×18 cm) using a chloroform—ethanol system (stepwise gradient, 4 : 1 → 1 : 6) and with Florisil (2.5×15 cm) using a chloroform—ethanol system (stepwise gradient, 4 : 1 → 1 : 2). The resulting fractions were separated by HPLC on a Diasfer-110-C18 column (10 μm, 15×250 mm) in 65% ethanol and rechromatographed on a Diasfer-110-C18 column (5 μm, 4×250 mm). The results of the chromatographic separation of

the total steroids and the isolation of compounds **1—18** are given in Table 1.

Linckoside L3 (1). Amorphous compound, $[\alpha]_D$ ±0 (*c* 0.4, MeOH). The ^1H and ^{13}C NMR spectra are given in Table 2. High-resolution mass spectrum (+)-ESI-TOF, *m/z*: 665.3898 $[M + Na]^+$ (calculated for $C_{34}H_{58}O_{11}Na$, 665.3877).

Linckoside L4 (2). Amorphous compound, $[\alpha]_D$ −12.0 (*c* 0.1, MeOH). The ^1H and ^{13}C NMR spectra are given in Table 2. High-resolution mass spectrum (+)-MALDI-TOF, *m/z*: 637.3986 $[M + Na]^+$ (calculated for $C_{33}H_{58}O_{10}Na$, 637.3928).

Linckoside L5 (3). Amorphous compound, $[\alpha]_D$ −8.8 (*c* 0.2, MeOH). The ^1H and ^{13}C NMR spectra are given in Table 3. High-resolution mass spectrum (+)-MALDI-TOF, *m/z*: 767.4125 $[M + Na]^+$ (calculated for $C_{38}H_{64}O_{14}Na$, 767.4194).

Linckoside L6 (4). Amorphous compound, $[\alpha]_D$ −17.2 (*c* 0.2, MeOH). The ^1H and ^{13}C NMR spectra are given in Table 3. High-resolution mass spectrum (+)-ESI-TOF, *m/z*: 795.4492 $[M + Na]^+$ (calculated for $C_{40}H_{68}O_{14}Na$, 795.4507).

Synthesis of MTPA esters of compound 1. Compound **1** (1.5 mg) was dissolved in dry pyridine (200 μL). Then (*R*)- or (*S*)-MTPA chloride (8 μL) was added. The reaction mixture was kept at room temperature for 2 h and then concentrated *in vacuo*. The dry residue was purified on a silica gel column (1×5 cm) using a 1 : 4 hexane—chloroform system as the eluent, and (*R*)-MTPA ester and (*S*)-MTPA ester of compound **1** were isolated in a yield of 1 mg each.

3´,4´,15,28-Tetra-(*R*)-MTPA ester of compound 1. ^1H NMR* (CD_3OD), δ, selected signals: 0.85 (d, 3 H, Me(26)C, *J* = 7.0 Hz); 0.88 (d, 3 H, Me(27)C, *J* = 7.0 Hz); 0.90 (d, 3 H, Me(21), *J* = 6.0 Hz); 1.16 (s, 3 H, Me(18)C); 1.29 (s, 3 H, Me(19)C); 4.19 (d, 1 H, H(28), *J* = 11.4 Hz); 4.24 (d, 1 H, H´(28), *J* = 11.4 Hz); 4.61 (d, 1 H, H(1´), *J* = 7.5 Hz); 5.25 (m, 2 H, H(15), H(4´)); 5.45 (t, 1 H, H(3´), *J* = 9.0 Hz).

3´,4´,15,28-Tetra-(*S*)-MTPA ester of compound 1. ^1H NMR* (CD_3OD), δ, selected signals: 0.86 (d, 6 H, Me(26)C, Me(27)C, *J* = 7.0 Hz); 0.90 (d, 3 H, Me(21)C, *J* = 6.0 Hz); 1.18 (s, 3 H, Me(18)C); 1.29 (s, 3 H, Me(19)C); 4.21 (d, 1 H, H(28), *J* = 11.4 Hz); 4.24 (d, 1 H, H´(28), *J* = 11.4 Hz); 4.76 (d, 1 H, H(1´), *J* = 7.5 Hz); 5.10 (m, 1 H, H(4´)); 5.28 (dd, 1 H, H(15), *J* = 2.0 Hz, *J* = 11.5 Hz); 5.47 (t, 1 H, H(3´), *J* = 9.0 Hz).

Bioassays. The cytotoxicity of compounds **1** and **4** was determined with the use of eggs of the sea urchin *Strongylocentrotus intermedius* as the cell model according to a procedure described earlier.[19] The effective inhibitory concentration EC_{100} was calculated graphically using the SigmaPlot 3.02 computer program (Jandel Corporation, USA).

We thank the Directory of the Institute of Oceanography of the Vietnamese Academy of Science and Technology (Nha Trang, Vietnam) for help in collecting starfishes. This study was financially supported by the Russian Academy of Sciences (Program of the Presidium of the Russian Academy of Sciences "Molecular and Cellular Biology," Grants of the Far Eastern Branch of the Russian Academy of Sciences Nos 06-II-CM-05-001 and 06-III-B-05-128), the Russian Foundation for Basic Research (Project No. 05-04-48246), and the Council on Grants of the President of the Russian Federation (Program for State Support of Leading Scientific Schools, Grant NSh-6491.2006.4).

* The spectra were recorded at 23 (±0.1) °C.

References

1. L. Minale, R. Riccio, and F. Zollo, in *Progress in the Chemistry of Organic Natural Products*, Eds W. Herz, G. W. Kirby, R. E. Moore, W. Steglich, and Ch. Tamm, Springer-Verlag, Wien—New York, 1993, **62**, 234 pp.
2. V. A. Stonik, *Usp. Khim.*, 2001, **70**, 763 [*Russ. Chem. Rev.*, 2001, **70**, 673 (Engl. Transl.)].
3. N. V. Ivanchina, A. A. Kicha, A. I. Kalinovsky, P. S. Dmitrenok, E. L. Chaikina, V. A. Stonik, M. Gavagnin, and G. Cimino, *J. Nat. Prod.*, 2006, **69**, 224.
4. M. Iorizzi, F. de Riccardis, L. Minale, and R. Riccio, *J. Nat. Prod.*, 1993, **56**, 2149.
5. C. Han, J. Qi, and M. Ojika, *Bioorg. Med. Chem.*, 2006, **14**, 4458.
6. E. Palagiano, F. Zollo, L. Minale, M. Iorizzi, P. Bryan, J. McClintock, and T. Hopkins, *J. Nat. Prod.*, 1996, **59**, 348.
7. F. Zollo, E. Finamore, and L. Minale, *Gazz. Chim. Ital.*, 1985, **115**, 303.
8. J. Qi, M. Ojika, and Y. Sakagami, *Bioorg. Med. Chem.*, 2002, **10**, 1961.
9. M. Iorizzi, L. Minale, R. Riccio, M. Debray, and J. L. Menou, *J. Nat. Prod.*, 1986, **49**, 67.
10. A. A. Kicha, A. I. Kalinovskii, E. V. Levina, and P. V. Andriyashchenko, *Khim. Prirod. Soedin.*, 1985, 801 [*Chem. Nat. Compd.*, 1985 (Engl. Transl.)].
11. C. Pizza, L. Minale, D. Laurent, and J. L. Menou, *Gazz. Chim. Ital.*, 1985, **115**, 585.
12. L. Minale, C. Pizza, F. Zollo, and R. Riccio, *Tetrahedron Lett.*, 1982, **23**, 1841.
13. R. Riccio, L. Minale, S. Pagonis, C. Pizza, F. Zollo, and J. Pusset, *Tetrahedron*, 1982, **38**, 3615.
14. F. Zollo, E. Finamore, and L. Minale, *J. Nat. Prod.*, 1987, **50**, 794.
15. I. Bruno, L. Minale, R. Riccio, S. La Barre, and D. Laurent, *Gazz. Chim. Ital.*, 1990, **120**, 449.
16. W. Wang, H. Jang, J. Hong, C. O. Lee, K. S. Im, S. J. Bae, and J. H. Jung, *J. Nat. Prod.*, 2004, **67**, 1654.
17. M. Iorizzi, P. Bryan, J. McClintock, L. Minale, E. Palagiano, S. Maurelli, R. Riccio, and F. Zollo, *J. Nat. Prod.*, 1995, **58**, 653.
18. L. Minale, C. Pizza, A. Plomitallo, R. Riccio, F. Zollo, and F. A. Mellon, *Gazz. Chim. Ital.*, 1984, **114**, 151.
19. A. A. Kicha, N. V. Ivanchina, A. I. Kalinovskii, P. S. Dmitrenok, S. O. Kainara, D. L. Aminin, I. G. Agafonova, and V. A. Stonik, *Izv. Akad. Nauk, Ser. Khim.*, 2004, 427 [*Russ. Chem. Bull., Int. Ed.*, 2004, **53**, 447].

Received October 24, 2006;
in revised form April 6, 2007