Polar steroidal compounds from the Far-Eastern starfish *Lethasterias nanimensis chelifera*

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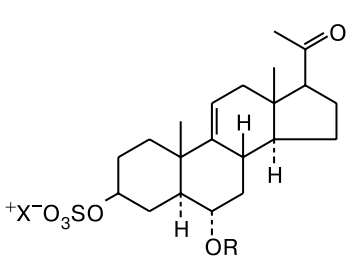
In addition to the salts described previously (with a sulfated steroid as the anion and the alkaloid salsolinol as the cation), nine other steroidal compounds including three new compounds were isolated from the Far-Eastern starfish *Lethasterias nanimensis chelifera* collected near the coast of the Onekotan island (Kuril isles). A nonsteroidal compound found in this starfish is (1*S*,3*S*)-1-methyl-1,2,3,4-tetrahydro-β-carboline-3-carboxylic acid. The structures of the isolated compounds were determined by NMR spectroscopy and mass spectrometry.

Key words: starfish, *Lethasterias nanimensis chelifera*, steroidal sulfates, glycosides, alkaloids, salsolinol, 1-methyl-1,2,3,4-tetrahydro-β-carboline-3-carboxylic acid, NMR spectra, cytostatic activity.

Recently,[1] we reported the isolation of new salts (**1**—**3**) from the starfish *Lethasterias nanimensis chelifera* collected near the coast of the Onekotan island (Kuril isles). These compounds contain a sulfated steroid as the anion and the alkaloid salsolinol as the cation.

Starfishes, unlike other Echinodermata, contain a variety of polar steroidal compounds. Usually these include polyhydroxy steroids, mono- and biosides of polyhydroxy steroids, and toxic steroidal oligoglycosides named asterosaponins.[2,3] Asterosaponins contain a 9(11) double bond and a 3β,6α-diol group in the steroid nucleus; the sulfate group is attached to C(3) of the aglycon, while the C(6) atom bears a carbohydrate chain consisting of five or six monosaccharide residues.



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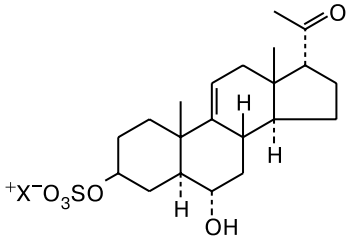

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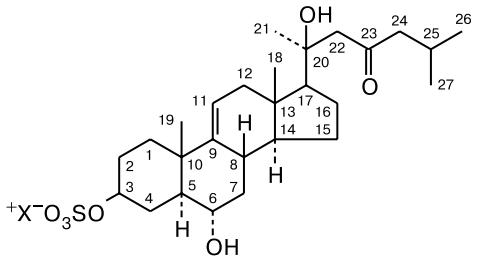

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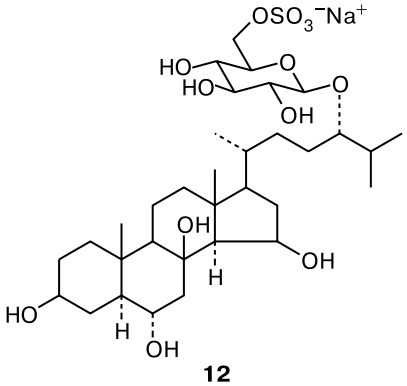

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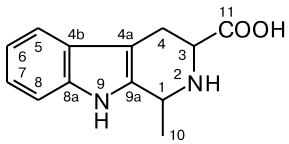

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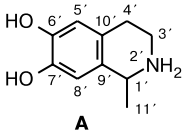
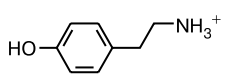


A



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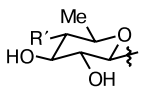

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X = **A** (**1**—**3**), Na (**4**, **6**, **8**, **10**, **11**), **B** (**5**, **7**, **9**); R = H (**1**, **4**, **5**), **C** (**10**, **11**); R′ = $OSO_3^-Na^+$ (**10**), OH (**11**)

Published in Russian in *Izvestiya Akademii Nauk. Seriya Khimicheskaya,* No. 2, pp. 427—434, February, 2004.

1066-5285/04/5302-0447

In continuation of our research into metabolites of Far-Eastern starfishes,[4,5] we isolated three new (**5—7**) and six known polar steroids (**4**, **8—12**) from the same collection of *Lethasterias nanimensis chelifera* (Verrill) (order Forcipulatida, family Asteriidae) from which steroids **1—3** had been isolated previously. The isolated compounds are mainly related to asterosaponins but are devoid of the carbohydrate chain or have only one monosaccharide residue. Glycosides **10** and **11** refer to the group of "shortened" asterosaponins, while glycoside **12**, to the group of low-molecular-weight polyhydroxysteroidal glycosides. In addition, the alkaloid (1*S*,3*S*)-1-methyl-1,2,3,4-tetrahydro-β-carboline-3-carboxylic acid (**13**) was found in *L. nanimensis chelifera*.

In this work, we describe the spectroscopic and other properties of all the compounds we isolated including steroids **1—3** and alkaloid **13**, which have been described previously in a brief communication.[1]

Results and Discussion

Repeated column chromatography of an aqueous ethanolic extract of the starfish *L. nanimensis chelifera* on Amberlite XAD-2, Sephadex LH-20, and silica gel and HPLC on Zorbax ODS and YMC-Pack ODS columns gave 13 individual compounds 12 of which were classified as polar steroids. The results of chromatographic separation of the sum of polar steroids are presented in Table 1.

Table 1. Polar steroidal compounds isolated from the starfish *Lethasterias nanimensis chelifera*[a]

Compound	*m*/mg	R_f[b]	R_t/min (HPLC)	EC_{50}[c] /μg mL^{-1}	Ref.
1	14.0	0.73	14.4[d]	—[e]	1
2	1.0	0.72	19.2[d]	—[e]	1
3	2.0	0.75	17.6[f]	>100	1
4	33.0	0.73	13.6[d]	25.3	4, 6
5	0.5	0.73	16.4[d]	—[e]	
6	1.0	0.72	17.2[d]	—[e]	
7	1.8	0.72	21.6[d]	—[e]	
8	7.0	0.75	16.8[f]	40.0	4, 11
9	7.5	0.75	19.2[f]	34.7	5
10	18.0	0.51	12.8[g]	23.1	11
11	3.8	0.62	16.4[d]	>100	12
12	3.5	0.54	13.2[h]	>100	14

[a] Isolated from 930 g of the crude weight of animals.
[b] Determined in the BuOH—EtOH—H_2O system (4 : 1 : 2).
[c] The inhibitory action on the development of the sea-urchin *Strongylocentrotus nudus* cells.
[d] 35% EtOH.
[e] Was not determined.
[f] 45% EtOH.
[g] 30% EtOH.
[h] 55% EtOH.

The structures of the obtained compounds were mainly established by ^{1}H NMR and ^{13}C NMR spectroscopy and confirmed by mass spectrometry.

The (−)-MALDI TOF mass spectrum of steroid **4** exhibits a pseudomolecular ion peak with *m*/*z* 411, $[M - Na]^-$, and its (+)-MALDI TOF mass spectrum, a pseudomolecular ion peak with *m*/*z* 457, $[M + Na]^+$. The ^{1}H NMR spectrum of compound **4** coincides with the spectrum of 3-*O*-sulfoasterone first obtained by Japanese researchers as a product of mild acid hydrolysis of asterosaponins.[6] Later,[4] this compound was detected in the free state in the Primorski population of the starfish *Aphelasterias japonica*. Direct comparison of the ^{1}H and ^{13}C NMR spectra and the chromatographic mobilities (TLC) of compound **4** (Table 2) and a sample of 3-*O*-sulfoasterone from the starfish *A. japonica*[4] showed that these were completely identical.

The ^{1}H and ^{13}C NMR spectra of sulfated compounds **1** and **5** exhibit, apart from the signals for 3-*O*-sulfoasterone, additional signals, which were assigned to organic counter-ions.

^{1}H, ^{13}C, and DEPT NMR spectra of compound **1** with recourse to HSQC, ^{1}H—^{1}H COSY, and HMBC experiments (see Table 2) allowed us to establish, in princuple, the structure of the organic cation. In particular, this was found to contain ten C atoms including those of one methyl, two methylene, and three methine groups two of which are aromatic and four aromatic C atoms two of which are linked to heteroatoms. The HSQC and ^{1}H—^{1}H COSY spectra contained cross-peaks, which made it possible to establish connectivities of the following signals: C(1′) at δ 51.1 with the quartet at δ 4.80; C(3′) at δ 39.7 with two coupled multiplets at δ 3.60 and 3.77; C(4′) at δ 25.2 with a coupled doublet of triplets at δ 2.92 and the related multiplet at δ 3.08; C(5′) at δ 116.1 with the singlet at δ 7.03; C(8′) at δ 113.7 with the singlet at δ 7.13; and C(11′) at δ 19.7 with the doublet at δ 1.81. The HMBC spectra contained the following cross-peaks: HC(1′)/C(3′), C(8′), C(9′), C(10′); H_2C(3′)/C(1′), C(4′), C(10′); H_2C(4′)/C(3′), C(5′), C(9′), C(10′); HC(5′)/C(4′), C(7′), C(9′); HC(8′)/ C(1′), C(6′), C(10′); H_3C(11′)/C(1′), and C(9′). Preirradiation of the HC(5′) proton resulted in NOE of the H_2C(4′) protons. The irradiation of HC(8′) gave NOE signals for the HC(1′) and H_3C(11′) protons. On the basis of these data, the organic cation in compound **1** was tentatively identified as 1-methyl-6,7-dihydroxy-1,2,3,4-tetrahydroisoquinoline (salsolinol). Thus, compound **1** is apparently a salt formed by the steroid 3-*O*-sulfoasterone and the alkaloid salsolinol.

Additional signals in the ^{1}H and ^{13}C NMR spectra of compound **5** corresponded to tyramine.[7] Indeed, the ^{1}H NMR spectrum (CD_3OD) contained two doublets for the aromatic protons at δ 7.08 (2 H, J = 8.5 Hz) and δ 6.77 (2 H, J = 8.5 Hz) and two triplets for the methylene

Table 2. ^{1}H and ^{13}C NMR spectra of compounds **1**, **4**, and **6** (δ, *J*/Hz)

Atom	CH_n (DEPT)	**1*** (C_5D_5N)			**4** (CD_3OD)		**6** (CD_3OD)	
		δ_C	δ_H	H—C correlation (HMBC)	δ_C	δ_H	δ_H	δ_C
1	CH_2	36.1	1.45 (m), 1.67 (m)		36.9		37.3	
2	CH_2	29.4	H_{ax}(2): 1.93 (m), H_{eq}(2): 2.57 (m)		29.6		29.6	
3	CH	77.6	4.95 (m)		79.6	4.21 (m)	79.6	4.21 (m)
4	CH_2	34.1	H_{ax}(4): 1.77 (m) H_{eq}(4): 3.45 (dm, *J* = 12.4)		31.3	H_{eq}(4): 2.45 (dm, *J* = 12.4)	31.1	H_{eq}(4): 2.45 (dm, *J* = 12.4)
5	CH	50.5	1.44 (m)		51.1		51.1	
6	CH	68.1	3.81 (td, *J* = 10.8, *J* = 4.4)		69.6	3.50 (td, *J* = 4.0, *J* = 10.9)	69.7	3.51 (td, *J* = 4.0, *J* = 10.9)
7	CH_2	43.1	H_{ax}(7): 1.27 (m) H_{eq}(7): 2.40 (m)		43.2		43.7	
8	CH	35.7	2.08 (m)		36.9		36.9	
9	C	146.5			147.3		147.3	
10	C	38.2			39.3		39.3	
11	CH	115.7	5.28 (d, *J* = 5.3)		117.3	5.41 (d, *J* = 5.3)	117.3	5.40 (br.d, *J* = 5.3)
12	CH_2	40.5	2.20 (m)		41.6		37.0	
13	C	42.4			43.7		45.3	
14	CH	53.5	1.20 (m)		54.9		50.3	
15	CH_2	25.4	1.75 (m)		26.5		25.9	
16	CH_2	22.9	1.63 (m), 2.35 (m)		23.9		28.2	
17	C	63.3	2.55 (t, *J* = 9.3)		64.5	2.73 (t, *J* = 9.3)	64.4	2.94 (dd, *J* = 3.2, *J* = 7.7)
18	CH_3	13.0	0.59 (s)	C(12), C(13), C(14), C(17)	13.4	0.54 (s)	21.1	0.89 (s)
19	CH_3	19.1	0.95 (s)	C(1), C(5), C(9), C(10)	19.5	0.97 (s)	19.4	0.97 (s)
20	C	208.1			212.2		212.2	
21	CH_3	30.8	2.10 (s)		31.1	2.13 (s)	32.8	2.13 (s)
1′	CH	51.1	4.80 (q, *J* = 6.7)	C(3′),C(8′), C(9′), C(10′)				
3′	CH_2	39.7	3.60 (m), 3.77 (m)	C(1′), C(4′), C(10′)				
4′	CH_2	25.2	2.92 (dt, *J* = 5.7, *J* = 16.8); 3.08 (m)	C(3′), C(5′), C(9′), C(10′)				
5′	CH	116.1	7.03 (s)	C(4′), C(7′), C(9′)				
6′	C	146.6						
7′	C	147.1						
8′	CH	113.7	7.13 (s)	C(1′), C(6′), C(10′)				
9′	C	125.0						
10′	C	122.3						
11′	CH_3	19.7	1.81 (d, *J* = 6.7)	C(1′), C(9′)				

* ^{1}H and ^{13}C NMR spectra were recorded at 500 and 125.8 MHz, respectively; the signals were assigned by ^{1}H—^{1}H COSY and HSQC 2D techniques.

protons at δ 3.10 (2 H, *J* = 7.5 Hz) and 2.84 (2 H, *J* = 7.5 Hz). The ^{13}C NMR spectrum (CD_3OD) contained the following signals: C(1′), δ 157.7; C(2′), C(6′), δ 130.8; C(4′), δ 128.5; C(3′), C(5′), δ 116.7; —CH_2—N<, δ 42.3; and Ar—$\underline{C}H_2$<, δ 33.8. The (+)-MALDI TOF mass spectrum of compound **5** exhibited a peak for the tyramonium cation with *m/z* 138, while the EI mass spectrum showed a tyramine peak with *m/z* 137, [cation − H]$^+$. Thus, it was shown that compound **5** is the tyramonium salt of 3-*O*-sulfoasterone. This compound has been unknown previously. Meanwhile, several cases of isolation of steroidal tyramine salts from starfishes have been reported,

for example, salts of asterosaponin P_1 from the starfish *Asterina pectinifera*,[7] 3-*O*-sulfothornasterol A from the starfish *A. japonica*,[5] and two sulfated 3α,21-diols from the starfish *Pteraster pulvillus*.[8]

Like the mass spectrum of compound **4**, the (–)-MALDI TOF mass spectrum of steroid **6** exhibits a pseudomolecular ion peak with *m/z* 411, $[M - Na]^-$. The proton chemical shifts, the corresponding spin-spin coupling constants, and the carbon chemical shifts of compound **6** virtually coincided with those in the spectra of 3-*O*-sulfoasterone (**4**), except for some signals that refer to rings C and D of the steroid nucleus (see Table 2). For example, the signals for HC(17) and $H_3C(18)$ occurred at δ 2.94 (dd, J = 3.2, 7.7 Hz) and 0.89 (s) in the ^1H NMR spectrum of compound **6** (for compound **4**, they are at δ 2.73 (t, J = 9.3 Hz) and 0.54 (s), respectively), while the ^{13}C NMR signals for the C(12), C(13), C(14), C(16), and C(18) carbon atoms of steroid **6** are at δ 37.0, 45.3, 50.3, 28.2, and 21.1 (for **4**, they are at δ 41.6, 43.7, 54.9, 23.9, and 13.4, respectively). Taking into account these spectroscopic differences and literature data,[9] we suggested that compound **6** differs from steroid **4** only in the configuration of the C(17) atom; thus, it is the sodium salt of 3-*O*-sulfoisoasterone. The parent isoasterone has been isolated previously upon acid hydrolysis of asterosaponins of some starfishes,[9] in particular, *L. nanimensis chelifera*.[10] Here, we describe the 3-*O*-sulfoisoasterone sodium salt **6** for the first time.

Apart from the signals due to 3-*O*-sulfoisoasterone, the NMR spectra of compounds **2** and **7** exhibit additional signals, indicating that the molecules contain organic counter-ions. Examination of the NMR spectra showed that these substances, like analogous derivatives of 3-*O*-sulfoasterone (**1** and **5**), contain salsolinol (**2**) and tyramine (**7**) as the organic cations. The tyramonium salt of 3-*O*-sulfoisoasterone **7** has been unknown previously.

In the (–)-MALDI TOF mass spectrum of steroid **8**, a pseudomolecular ion peak was observed at *m/z* 511, $[M - Na]^-$. By comparing the ^1H and ^{13}C NMR spectra and chromatographic behavior (TLC) of compound **8** with those for the authentic specimens that we have isolated previously from the starfishes *L. nanimensis chelifera* and *A. japonica*, this compound was identified as 3-*O*-sulfothornasterol A.[4,10] In the ^1H and ^{13}C NMR spectra of compounds **3** and **9**, apart from the 3-*O*-sulfothornasterol A signals, additional signals were present, indicating that the former is the 3-*O*-sulfothornasterol A salt with salsolinol, while the latter, with tyramine.

Thus, we isolated three series of salts, one based on 3-*O*-sulfoasterone, another one based on 3-*O*-sulfoisoasterone, and, finally, the third one based on 3-*O*-sulfothornasterol A. Each series includes a sodium salt, the salt with the alkaloid salsolinol, and the tyramonium salt.

The structures of compounds **1—3** were confirmed by a thorough mass-spectrometric investigation. The (+)-LSIMS spectra of salt **3** exhibited pseudomolecular ion peaks with *m/z* 714 $[M + Na]^+$ and 871 [M + cation]$^+$, where M is the molecular mass of this ion hybrid. The molecular formula $C_{37}H_{57}O_9NS$ was established for compound **3** using the (+)-HRMALDI TOF mass spectrum containing a peak with *m/z* 557.2331. This peak corresponds to the anionic moiety of the ion hybrid $[M - \text{cation} + 2\ Na]^+$ with the molecular formula $C_{27}H_{43}O_7SNa_2$ (calculated, 557.2528). The cation peak [salsolinol + H]$^+$ with *m/z* 180.0988 corresponds to the molecular formula $C_{10}H_{14}O_2N$ (calculated, 180.1025). The (–)-MALDI TOF mass spectrum of **3**, like the spectra of compounds **8** and **9**, exhibited an [M – cation]$^-$ peak with *m/z* 511.

The (+)-LSIMS spectra of compounds **1** and **2** contained pseudomolecular ion peaks with *m/z* 614, $[M + Na]^+$, while the spectrum of steroid **1** contained, in addition, a peak with *m/z* 771, [M + cation]$^+$. The molecular formula $C_{31}H_{45}O_8NS$ for compounds **1** and **2** was derived from analysis of the (+)-HRMALDI TOF mass spectra containing $[M - \text{cation} + 2\ Na]^+$ peaks with *m/z* 457.1517 and 457.1531 (calculated, 457.1639), which correspond to the molecular formula $C_{21}H_{31}O_6SNa_2$, and [salsolinol + H]$^+$ cation peaks with *m/z* 180.0964 and 180.0976 (calculated, 180.1025), which correspond to the molecular formula $C_{10}H_{14}O_2N$. In the (–)-MALDI TOF mass spectra of compounds **1** and **2**, the [M – cation]$^-$ peak is observed at *m/z* 411.

The electron impact mass spectra of compounds **1—3** contained peaks with *m/z* 179, [cation – H]$^+$, and 164, [cation – CH_3]$^+$, typical of salsolinol.

Steroidal glycosides were also present among the steroids we isolated. The (–)-MALDI TOF mass spectrum of glycoside **10** showed a pseudomolecular ion peak with *m/z* 659, $[M - Na]^-$, and a peak with *m/z* 557, $[M - NaSO_3 + H]^-$. Compound **10** was identified by direct comparison (^1H and ^{13}C NMR, TLC) with a cheliferoside L_1, which we have isolated previously from the same starfish and the starfish *A. japonica*.[4,11]

The (–)-MALDI TOF mass spectrum of glycoside **11** contained a pseudomolecular ion peak with *m/z* 557, $[M - Na]^-$. A pseudomolecular ion peak with *m/z* 603, $[M + Na]^+$, was observed in the (+)-MALDI TOF mass spectrum of **11**. Compound **11** was identified by direct comparison (^1H and ^{13}C NMR, TLC) with authentic forbeside E_3 that we have isolated previously from the starfish *A. japonica*.[4] Forbeside E_3 (**11**), first obtained from the starfish *Asterias forbesi*,[12] like cheliferoside L_1 (**10**), belongs to the class of asterosaponins with a shortened carbohydrate chain. Steroidal glycosides of this structural type have been found previously only in four starfish species.[4,11—13]

The (–)-MALDI TOF mass spectrum of glycoside **12** exhibited a pseudomolecular ion peak with *m/z* 693, $[M - Na]^-$. In the (+)-MALDI TOF mass spectrum of

glycoside **12**, pseudomolecular ion peaks with *m/z* 739, $[M + Na]^+$, and *m/z* 755, $[M + K]^+$, were found. The ^1H and ^{13}C NMR spectra of compound **12** were in good agreement with the literature data for pycnopodioside C from the starfish *Pycnopodia helianthoides*.[14] Thus, glycoside **12** was identified as pycnopodioside C.

Among the steroids found in this study, the salsolinol-containing salts are most unusual. The neurotoxic alkaloid salsolinol was first detected in the urine of patients with Parkinson´s disease[15] and then in some higher plants, foodstuffs, and beverages.[16,17] Salsolinol is also known as a potential marker in the alcoholism etiology.[18] This is the first time salsolinol is found in starfishes.

A comparison of the optical rotations of compounds **1—3** and the corresponding sodium salts **4**, **6**, and **8** as well as *R*- and *S*-salsolinols ([α] +30.0 and [α] −30.9, respectively)[17] suggests that ion hybrids **1—3** mainly contain *R*-salsolinol. This enantiomer usually predominates in animals, unlike plants.

Other known compounds related to salts **1—3** include phenethylammonium[19] and 2-aminoimidazolium salts of sulfated steroids from sponges[20] and tyramonium salts of sulfated steroids from starfishes.[5,7,8]

Previously,[11] in a study of the starfish *L. nanimensis chelifera* population collected near the Shiashkotan island (Kuril islands, Pacific side), we have isolated two steroids, namely, a new glycoside, cheliferoside L_1 (**10**), and a known genuine aglycon of asterosaponins, 3-*O*-sulfothornasterol A (**8**). In addition, according to NMR spectra, classical asterosaponins represented a substantial portion of polar steroids from *L. nanimensis chelifera*. In a study of the products of acid hydrolysis of the sum of these asterosaponins, we found the following known aglycons: asterone, isoasterone, isomarthasterone, (17*E*)- and (17*Z*)-3β,6α-dihydroxy-5α-cholesta-9(11),17(20)-dien-23-ones, and rare (23*S*)-5α-cholest-9(11)-ene-3β,6α,23-triol.[10]

This study has shown that, apart from compounds **8** and **10** isolated previously, the fraction of polar steroids from another specimen of this starfish contains eight sulfated compounds **1—7** and **9**, whose steroid fragments are genuine aglycons of asterosaponins, one asterosaponin with a shortened carbohydrate chain, *viz.*, forbeside E_3 (**11**), and a glycosylated polyhydroxysteroid, *viz.*, pycnopodioside C (**12**). The composition of this starfish population is unusual due to the presence of sulfated genuine aglycons of asterosaponins and of asterosaponins with a shortened carbohydrate chain and to the absence of classical asterosaponins.

When comparing different populations of the starfish *A. japonica* and other starfishes, we have already encountered differences in the steroid compositions. It is known that starfishes cannot migrate far during their lives. In some cases, animals of the same species from different habitats differ markedly from each other in biology, in particular, in the nutrition pattern and in the behavior depending thereupon.

In addition to steroid compounds, we have found several nonsteroidal metabilites in the extracts from the starfish *L. nanimensis chelifera*. L-Tryptophan and α-L-phenylalanine were identified by comparison with authentic samples using ^1H NMR spectra, amino acid analysis, and optical rotation measurement. Compound **13** was identified as (1*S*,3*S*)-1-methyl-1,2,3,4-tetrahydro-β-carboline-3-carboxylic acid (MTCA) using spectroscopic methods. The ^1H and ^{13}C NMR spectra of **13** showed a certain similarity with those of tryptophan (Table 3). According to the ^{13}C NMR and DEPT spectra, compound **13** con-

Table 3. ^1H and ^{13}C NMR spectra of 1*S*,3*S*-1-methyl-1,2,3,4-tetrahydro-β-carboline-3-carboxylic acid (**13**) and tryptophan (Try) (CD_3OD, δ, *J*/Hz)

Atom	CH_n (DEPT)	**13**			Try	
		δ_C	δ_H	H—C correlation (HMBC)	δ_C	δ_H
1	CH	51.2	4.71 (q, *J* = 6.8)			
3	CH	59.8	3.97 (dd, *J* = 5.0, *J* = 12.0)	C(4), C(11)	54.0	3.86 (dd, *J* = 4.0, *J* = 9.4)
4	CH_2	24.4	3.02 (dd, *J* = 12.1, *J* = 16.4); 3.45 (dd, *J* = 5.0, *J* = 16.4)		27.2	3.14 (dd, *J* = 9.4, *J* = 15.2); 3.52 (dd, *J* = 4.0, 15.2)
4a	C	107.9			107.9	
4b	C	127.5			128.3	
5	CH	119.2	7.48 (d, *J* = 7.6)	C(4a), C(7), C(8a)	119.9	7.69 (d, *J* = 7.5)
6	CH	120.6	7.05 (t, *J* = 7.0)	C(4b), C(8)	121.3	7.04 (t, *J* = 7.0)
7	CH	123.3	7.14 (t, *J* = 7.0)	C(5), C(8a)	123.9	7.12 (t, *J* = 7.0)
8	CH	112.3	7.35 (d, *J* = 7.6)	C(4b), C(6)	113.8	7.35 (d, *J* = 7.5)
8a	C	138.6			138.0	7.12 (t, *J* = 7.5)
9a	C	131.4			127.1	7.19 (s)
10	CH_3	17.2	1.75 (d, *J* = 6.8)	C(1), C(9a)		
11	COOH	173.7			174.7	

tains C atoms of one methyl group, one methylene group, and six methine groups as well as four C atoms bearing no H atoms. In addition, a signal for the carboxyl carbon atom was identified. The connectivities between the neighboring protons and the corresponding C atoms was determined by the $^1H—^1H$ COSY and HSQC methods. The HMBC spectra exhibited the following cross-peaks: HC(3)/C(4), C(11); HC(5)/C(4a), C(7), C(8a); HC(6)/C(4b), C(8); HC(7)/C(5), C(8a); HC(8)/C(4b), C(6); $H_3C(10)/C(1)$, C(9a).

The structure of compound **13** as 1-methyl-1,2,3,4-tetrahydro-β-carboline-3-carboxylic acid was confirmed by the electron impact mass spectrum, which displayed characteristic peaks with *m/z* 230, $[M - H_2O]^+$, 215, $[M - H_2O–CH_3]^+$, 169, and 157.

L-Tryptophan is known[21,22] to be the biogenetic precursor of MTCA; this implies the *S*-configuration for the C(3) asymmetric center in compound **13**. The position of H(1) relative to H(3) in **13** was determined in an NOE experiment. Preirradiation of the H(3) atom resulted in the enhancement of the H(1) and H(4) signals. On this basis, the absolute configuration of the C(1) and C(3) atoms in compound **13** was determined as 1*S*,3*S*.

Compound **13** is a β-carboline alkaloid. Previously,[21,22] it has been found in plants, fishes, and mammals. In starfishes, it was detected for the first time. Alkaloids are seldom found in starfishes. Only the mercaptohistidine-type alkaloid ovothiol A from *Evasterias troschelii*,[23] imbricatin from *Dermasterias imbricata* with a benzyltetrahydroisoquinoline fragment in the molecule,[24] and some guanine-containing metabolites[25] have been isolated to date.

The inhibitory action of compounds **1**, **3**, and **8—12** on the dividing eggs of the sea-urchin *Strongylocentrotus nudus* was studied using a modified procedure.[26] The results are presented in Table 1. Cheliferoside L_1 (**10**) and 3-*O*-sulfoasterone sodium salt (**4**) were found to be most active (EC_{50} = 23.1 and 25.3 μg mL^{-1}, respectively). Other compounds either exhibited slight activities or were inert. In the series of salts of 3-*O*-sulfothornasterol A, the activity varied in the following sequence: 1-methyl-6,7-dihydroxy-1,2,3,4-tetrahydroisoquinoline salt **3** < sodium salt **8** < tyramonium salt **9**, *i.e.*, the highest inhibitory activity with respect to the dividing sea-urchin eggs is found for the tyramonium salt **9**. Thus, salt **3** differed appreciably in its cytostatic action from the corresponding tyramonium (**9**) and sodium salts (**8**).

Experimental

^1H and ^{13}C NMR spectra were recorded on Bruker WM-250 (250 and 62.9 MHz), Bruker DPX 300 (300 and 75.5 MHz), and Bruker DPX 500 (500 and 125.8 MHz) spectrometers, respectively using $SiMe_4$ as the internal standard. Optical rotation was measured on a Perkin—Elmer 141 polarimeter.

Mass spectra (LSI and EI) were recorded on an AMD-604S mass spectrometer (AMD, Germany) with an accelerating voltage of 8 keV, an energy of Cs^+ ions of 10—12 keV (LSIMS), and an electron energy of 70 eV (EIMS). For recording the mass spectra, a sample was dissolved in MeOH (10 mg mL^{-1}) and an aliquot (1 μL) was analyzed using glycerol (Sigma) as the matrix. MALDI TOF mass spectra were run on a Biflex III mass spectrometer (Bruker, Germany, N_2 laser, 337 nm). The sample was dissolved in MeOH (1 mg mL^{-1}) and a 1 μL aliquot was analyzed using 2,5-dihydroxybenzoic acid as the matrix. HPLC was carried out on a DuPont Model 8800 chromatograph (refractometer as the detector), using Zorbax ODS (5 μm, 9.4×250 mm) and YMC-Pack ODS columns (5 μm, 10×250 mm).

Column chromatography was carried out using the Amberlite XAD-2 (20—80 mesh), Sephadex LH-20, silica gel L (40/100 μm), and Florisil (100—200 mesh) sorbents. Thin layer chromatography was carried out on 4.5×6.0 cm plates with a fixed Sorbfil (5—17 μm) silica gel layer.

The starfish specimens were collected in August, 1999, in the sea of Okhotsk near the Onekonan island (Kuril isles) from a depth of 70—100 m and identified by C. Sh. Dautov (Institute of Marine Biology, Far-Eastern Branch of the RAS, Vladivostok).

Isolation of compounds 1—13. The ground starfishes (930 g) were extracted twice with 70% ethanol (3 mL·g^{-1}) with heating on a water bath and the tissue was removed by centrifugation. To remove lipids, the supernatant was extracted with benzene (1 mL of benzene/3 mL of supernatant). The water—ethanol layer was concentrated *in vacuo* and the residue was dissolved in 0.5 L of water and passed through a column (7×25 cm) with Amberlite XAD-2. The column was washed with water until the eluate was free from Cl^- ions and then with ethanol, and the ethanol extract was concentrated. The resulting total fraction of steroidal compounds (5.8 g) was chromatographed on a column with Sephadex LH-20 (3.0×60 cm) in an ethanol—H_2O system (2 : 1). The fractions that contained polar steroidal compounds, according to TLC, were pooled to give three subfractions, and each of these was chromatographed on a column with silica gel (4×18 cm) in a chloroform—ethanol system (stepwise gradient, 10 : 1 → 1 : 4). The resulting fractions were purified by HPLC on a Zorbax ODS column using 40% ethanol for elution and rechromatographed on a YMC-Pack ODS column. The results of chromatographic separation of the sum of polar steroids with isolation of compounds **1—12** are listed in Table 1. Simultaneously, tryptophan (2.5 mg), α-phenylalanine (1.5 mg), and compound **13** (1.7 mg) (R_t = 11.0 min in 35% ethanol on a YMC-Pack ODS column) were isolated.

3-*O*-Sulfoasterone (3-*O*-sulfo-3β,6α-dihydroxy-5α-pregn-9(11)-en-20-one), 1-methyl-6,7-dihydroxy-1,2,3,4-tetrahydroisoquinolinium salt (1), amorphous solid; $[\alpha]_D$ +36.7 (*c* 2.1, MeOH). ^1H and ^{13}C NMR spectra are listed in Table 2. (+)-LSI MS, *m/z* (I_{rel} (%)): 614 $[M + Na]^+$ (11), 457 $[M - cation + 2 Na]^+$ (41), 337 $[M - cation - HSO_4 + Na]^+$ (10), 180 $[cation]^+$ (100); (+)-HRMALDI TOF MS, *m/z*: 457.1517 $[M - cation + 2 Na]^+$, 180.0964 $[cation]^+$; (−)-MALDI TOF MS, *m/z*: 411 $[M - cation]^-$; EI MS, *m/z* (I_{rel} (%)): 332 (1), 314 $[M - cation - HSO_4]^+$ (6), 299 (2), 296 (30), 281 (25), 263 (10), 242 (12), 211 (23), 179 $[cation - H]^+$ (14), 164 $[cation - H - CH_3]^+$ (100), 143 (17).

3-*O*-Sulfoisoasterone (3-*O*-sulfo-3β,6α-dihydroxy-5α,17α-pregn-9(11)-en-20-one), 1-methyl-6,7-dihydroxy-1,2,3,4-tetrahydroisoquinolinium salt (2), amorphous solid; $[\alpha]_D$ −6.7 (*c* 0.1, MeOH). The ^{1}H and ^{13}C NMR spectra of the steroid moiety are identical with those for compound **6**; those of the organic cation are identical with the spectra of compound **1** given in Table 2. (+)-LSI MS, *m/z* (I_{rel} (%)): 771 [M + cation]$^+$ (0.5), 614 [M + Na]$^+$ (10), 457 [M − cation + 2 Na]$^+$ (12), 337 [M − cation − HSO_4 + Na]$^+$ (5), 180 [cation]$^+$ (100); (+)-HRMALDI TOF MS, *m/z*: 457.1531 [M − cation + 2 Na]$^+$, 180.0976 [cation]$^+$; (−)-MALDI TOF MS, *m/z*: 411 [M − cation]$^-$; EI MS, *m/z* (I_{rel} (%)): 332 (1), 314 (2), 299 (2), 296 (27), 281 (23), 263 (12), 242 (11), 211 (28), 179 [cation − H]$^+$ (10), 164 [cation − H − CH_3]$^+$ (100), 143 (15).

3-*O*-Sulfothornasterol A (3-*O*-sulfo-(20*S*)-3β,6α,20-trihydroxy-5α-cholest-9(11)-en-23-one), 1-methyl-6,7-dihydroxy-1,2,3,4-tetrahydroisoquinolinium salt (3), amorphous solid; $[\alpha]_D$ −2.0 (*c* 0.4, MeOH). The ^{1}H and ^{13}C NMR spectra of the steroid moiety are identical to those reported previously;[3,5] those of the organic cation are identical to the data presented for compound **1** in Table 2. (+)-LSI MS, *m/z* (I_{rel} (%)): 871 [M + cation]$^+$ (1), 714 [M + Na]$^+$ (16), 557 [M − cation + 2 Na]$^+$ (15), 535 [M − cation + H + Na]$^+$ (8), 437 [M − cation − HSO_4 + Na$^+$ (31), 180 [cation]$^+$ (100); (+)-HRMALDI TOF MS, *m/z*: 557.2331 [M − cation + 2 Na]$^+$, 180.0988 [cation]$^+$; (−)-MALDI TOF MS, *m/z*: 511 [M − cation]$^-$; EI MS, *m/z* (I_{rel} (%)): 414 (0.5), 396 (0.5), 378 (3), 363 (2.5), 314 (3), 299 (1), 296 (10), 281 (8), 251 (12), 242 (5), 179 [cation − H]$^+$ (9), 164 [cation − H − CH_3]$^+$ (100), 143 (16).

3-*O*-Sulfoasterone, Na salt (4), amorphous solid; $[\alpha]_D$ +34.5 (*c* 0.1, MeOH). The ^{1}H and ^{13}C NMR spectra are presented in Table 2. (−)-MALDI TOF MS, *m/z*: 411 [M − Na]$^-$; (+)-MALDI TOF MS, *m/z*: 457 [M + Na]$^+$.

3-*O*-Sulfoasterone, tyramonium salt (5), amorphous solid; $[\alpha]_D$ +29.0 (*c* 0.1, MeOH). The ^{1}H and ^{13}C NMR spectra of the steroidal moiety are identical to those presented for compound **3**; those of the organic cation are identical to those reported previously.[7] (−)-MALDI TOF MS, *m/z*: 411 [M − cation]$^-$; (+)-MALDI TOF MS, *m/z*: 457 [M − cation + 2 Na]$^+$, 138 [cation]$^+$. EI MS, *m/z* (I_{rel} (%)): 332 (1), 314 [M − cation − HSO_4]$^+$ (22), 299 (16), 296 (68), 281 (62), 263 (44), 242 (50), 230 (36), 211 (66), 157 (40), 137 [cation − H]$^+$ (8), 108 (56), 107 (40), 85 (100).

3-*O*-Sulfoisoasterone, Na salt (6), amorphous solid; $[\alpha]_D$ −15.6 (*c* 0.05, MeOH). The ^{1}H and ^{13}C NMR spectra are presented in Table 2. (−)-MALDI TOF MS, *m/z*: 411 [M − Na]$^-$; (+)-MALDI TOF MS, *m/z*: 457 [M + Na]$^+$; EI MS, *m/z* (I_{rel} (%)): 332 (1), 314 [M − cation − HSO_4]$^+$ (15), 299 (5), 296 (53), 281 (47), 263 (38), 242 (45), 230 (36), 211 (95), 157 (45), 85 (100).

3-*O*-Sulfoisoasterone, tyramonium salt (7), amorphous solid; $[\alpha]_D$ −13.0 (*c* 0.2, MeOH). The ^{1}H and ^{13}C NMR spectra of the steroid moiety are identical to those presented for compound **6**; those of the organic cation are identical to those reported previously.[7] (−)-MALDI TOF MS, *m/z*: 411 [M − cation]$^-$; (+)-MALDI TOF MS, *m/z*: 457 [M − cation + 2 Na]$^+$, 138 [cation]$^+$.

3-*O*-Sulfothornasterol A, Na salt (8), amorphous solid; $[\alpha]_D$ −3.3 (*c* 0.5, MeOH). The ^{1}H and ^{13}C NMR spectra were identical to those reported previously.[4,10] (−)-MALDI TOF MS, *m/z*: 511 [M − Na]$^-$.

3-*O*-Sulfothornasterol A, tyramonium salt (9), amorphous solid; $[\alpha]_D$ −3.3 (*c* 0.3, MeOH). ^{1}H and ^{13}C NMR spectra of the steroid moiety are identical to those reported previously;[4,10] those of the organic cation are identical to published data.[7] (−)-MALDI TOF MS, *m/z*: 511 [M − cation]$^-$; (+)-MALDI TOF MS, *m/z*: 557 [M − cation + 2 Na]$^+$, 138 [cation]$^+$.

Cheliferoside L_1 (3-*O*-sulfo-6-*O*-(4-*O*-sulfo-β-D-quinovopyranosyl)-3β,6α-dihydroxy-5α-pregn-9(11)-en-20-one, disodium salt) (10), amorphous solid; $[\alpha]_D$ +38.9 (*c* 0.6, MeOH). The ^{1}H and ^{13}C NMR spectra are identical to published data.[4,11] (−)-MALDI TOF MS, *m/z*: 659 [M − Na]$^-$, 557 [M − $NaSO_3$ + H]$^-$.

Forbeside E_3 (3-*O*-sulfo-6-*O*-(β-D-quinovopyranosyl)-3β,6α-dihydroxy-5α-pregn-9(11)-en-20-one, sodium salt) (11), amorphous solid; $[\alpha]_D$ +28.3 (*c* 0.4, MeOH). The ^{1}H and ^{13}C NMR spectra are identical to published data.[4,12] (+)-MALDI TOF MS, *m/z*: 603 [M + Na]$^+$; (−)-MALDI TOF MS, *m/z*: 557 [M − Na]$^-$.

Pycnopodioside C ((24*S*)-24-*O*-(6-*O*-sulfo-β-D-glucopyranosyl)-5α-cholestan-3β,6α,8,15β,24-pentaol, sodium salt) (12), amorphous solid; $[\alpha]_D$ + 3.8 (*c* 0.3, MeOH). The ^{1}H and ^{13}C NMR spectra are identical to published data.[14] (+)-MALDI TOF MS, *m/z*: 755 [M + K]$^+$, 739 [M + Na]$^+$; (−)-MALDI TOF MS, *m/z*: 693 [M − Na]$^-$.

(1*S*,3*S*)-1-Methyl-1,2,3,4-tetrahydro-β-carboline-3-carboxylic acid (13), amorphous solid; $[\alpha]_D$ −78.2 (*c* 0.2, MeOH). The ^{1}H and ^{13}C NMR spectra are presented in Table 3. EI MS, *m/z* (I_{rel} (%)): 230 [M − H_2O]$^+$ (100), 215 [M − H_2O − CH_3]$^+$ (55), 169 (72), 157 (81).

Bioassays. The developing embryos of the sea-urchin *Strongylocentrotus nudus* were used as the cell model for determining the cytotoxicity of compounds **1**, **3**, and **8—12**.[26] The sex products were obtained by injecting a 0.5 *M* solution of KCl into the perivisceral cavity of the sea-urchin body. The collected mature eggs were washed with triply filtered sea water and the final concentration of eggs in the suspension was brought to 1000 cells · mL^{-1}. After artificial fertilization, 0.9 mL of the egg suspension was placed in each well of a 24-well microplate containing 0.1 mL of a compound to be tested. The microplate with the compounds was incubated at 18—20 °C until the embryos in the blank developed to the stage of eight blastomeres or the blastula. The number of developed embryos was estimated with an inverted microscope. All experiments were repeated three times. The effective inhibiting concentrations (EC_{50}), the average values, and the standard experimental error were calculated graphically using a SigmaPlot 3.02 program (Jandel Corporation, USA).

This work was supported by the Russian Foundation for Basic Research (Project No. 02-04-49491), the State Programs for the support of young Russian scientists (grant of RF President MK-2713.2003.03) and Leading Scientific Schools (Project NSh-725.2003.4) and by the Far-Eastern Branch and the Program for Physicochemical Biology of the Russian Academy of Sciences (Grants Nos 03-1-0-05-005, 03-3-A-05-002 and 03-3-G-05-033).

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Received June 20, 2003;
in revised form December 25, 2003