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Alkaloidosteroids from the starfish *Lethasterias nanimensis chelifera*

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Abstract—New ionic compounds containing an alkaloidal cation and a steroidal anion have been isolated by reverse-phase liquid chromatography from the extracts of the starfish *Lethasterias nanimensis chelifera*. Their structures have been elucidated by NMR and mass spectroscopy as 1-methyl-6,7-dihydroxy-1,2,3,4-tetrahydroisoquinolinium salts of 3-*O*-sulfoasterone **1**, 3-*O*-sulfo*iso*asterone **2** and 3-*O*-sulfothornasterol A **3**. In addition, the alkaloid 1-methyl-1,2,3,4-tetrahydro-β-carboline-3-carboxylic acid (MTCA) **4** was found in this starfish.

Starfishes are an extremely rich source of new, highly polar natural products. For instance, these invertebrates contain steroidal oligoglycosides and related natural products having a sulfate group attached to C-3 of the aglycone.[1,2]

In the course of our continuing interest on polar marine natural products from starfish,[3] we have studied polar metabolites from the far-eastern starfish *Lethasterias nanimensis chelifera* (order Forcipulatida, family Asteriidae), collected from a depth of about 100 m by trawling near Onekotan Island (Kuril Islands) in August of 1999 (the r/v 'Akademik Oparin'). The starfish specimens (0.93 kg) were chopped and twice extracted with 70% ethanol. Unusual salts of sulfated steroids and 1-methyl-6,7-dihydroxy-1,2,3,4-tetrahydroisoquinoline (salsolinol) named by us as alkaloidosteroids **1**–**3** along with MTCA **4** were obtained from aqueous ethanol-soluble materials by column chromatography on Amberlite XAD-2 (50% ethanol), Sephadex LH-20 (EtOH–H_2O, 2:1), on a short column with Silica gel L ($CHCl_3$–EtOH, 10:1→1:4) followed by HPLC on Zorbax ODS (40% EtOH) and YMC-Pack ODS columns (35% EtOH). Each alkaloidosteroid had a specific retention time on reverse phase HPLC.

Two of the isolated alkaloidosteroids **1**, **2** are isomeric amorphous solids with $[\alpha]_D^{25}$ +36.7 (*c* 0.3, MeOH) and $[\alpha]_D^{25}$ −6.7 (*c* 0.1, MeOH), respectively. These compounds were identified as salts of an organic cation and the known asterone sulfate[4] and the previously undescribed *iso*asterone sulfate. Quasi-molecular ion peaks at *m*/*z* 614 $(M+Na)^+$, 771 (M+organic cation)$^+$, where M is the molecular weight of the intact salt, were detected by LSIMS (positive mode) of both the salts. The molecular formula $C_{31}H_{45}O_8NS$ was established for **1** and **2** by combined MS and ^{13}C NMR analyses. In fact, derived from the anion peaks (M−organic cation+2Na)$^+$ at *m*/*z* 457.1517 and 457.1531 in the (+)-MALDI-TOF mass spectra of **1** and **2** corresponded to the molecular formula $C_{21}H_{31}O_6SNa_2$ (calcd 457.1639), while cation peaks (salsolinol+H)$^+$ in the same spectra at *m*/*z* 180.0964 and 180.0976 corresponded to the molecular formula $C_{10}H_{14}O_2N$ (calcd 180.1025). The (−)-MALDI-TOF spectra gave peaks at *m*/*z* 411 similar to the spectra of 3-*O*-sulfoasterone.[4] The EIMS of **1**, **2** (*m*/*z* 179, 164 and other fragmentary ion peaks) were identical to that of the neurotoxic alkaloid salsolinol, which had been found in the urine of Parkinsonian patients[5] and later in some higher plants and different types of foodstuffs and beverages,[6,7] but never found in starfish. Salsolinol is also known as a potential marker involved in the ethiology of alcoholism.[8] NMR spectra of **1**, including ^1H–^1H COSY, HSQC and HMBC experiments confirmed the structures of both the anionic and cationic moieties as 3-*O*-sulfoasterone and salsolinol, respectively (Table 1).

Keywords: starfish metabolites; steroids; alkaloids; salsolinol; MTCA.

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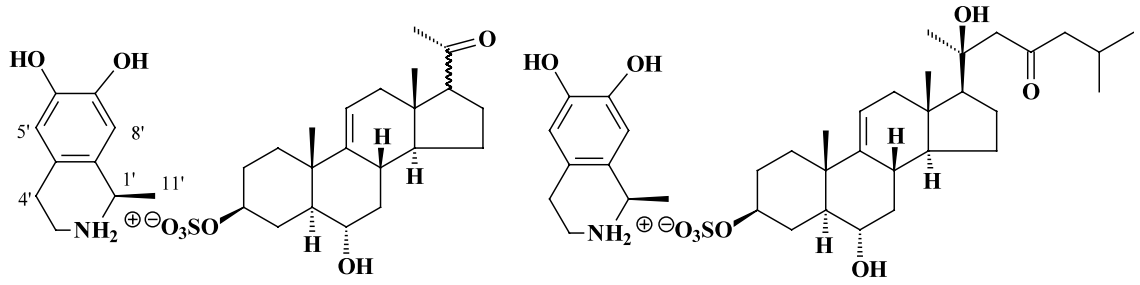
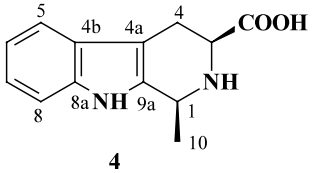


1,2

1 (17β)
2 (17α)

3



4

The NMR data of **1** and **2** (CD_3OD) were almost identical, except the signals of H-17 and H_3-18. In the spectrum of **2**, these signals were observed at δ 2.94 (dd, 3.2, 7.7 Hz) and 0.89 (s) ppm compared to δ 2.73 (t, 9.3 Hz) and 0.54 (s) ppm in the spectrum of **1**. In the ^{13}C NMR spectrum of **2**, the signals for C-12, C-13, C-14, C-16 and C-18 were observed at δ 37.0, 45.3, 50.0, 28.2, and 21.1 ppm, respectively, compared to δ 41.6, 43.7, 54.9, 23.9, and 13.4 ppm in **1**. These data indicated that **1** and **2** differ from each other in the stereochemistry at C-17 in the steroidal moiety. Comparison of the NMR spectra of **2** with those of *iso*asterone[9] confirmed that **2** was formed from salsolinol and *iso*asterone sulfated at position 3.

Alkaloidosteroid **3**, an amorphous solid, $[\alpha]_D^{25}$ –2.0 (*c* 0.3, MeOH) was found to possess the molecular formula $C_{37}H_{57}O_9NS$ by combined MALDI-TOF, LSIMS and ^{13}C NMR analyses. In fact, quasi-molecular ion peaks at *m/z* 714 $(M+Na)^+$ and *m/z* 871 (M+organic cation)$^+$ were detected in the (+)-LSIMS. The peak, derived from the anion (M-organic cation+2Na)$^+$ at *m/z* 557.2331 in the (+)-MALDI-TOF spectrum of **3** corresponded to molecular formula $C_{27}H_{43}O_7SNa_2$ (calcd 557.2528) and the cation peak (salsolinol+H)$^+$ at *m/z* 180.0988 corresponded to the molecular formula $C_{10}H_{14}O_2N$ (calcd 180.1025). In the (–)-MALDI-TOF spectrum, the anion peak at *m/z* 511 corresponded to that in the spectrum of the sodium salt of 3-*O*-sulfothornasterol A.[4] The NMR spectra of **3** confirmed the suggested structures of both the cationic and anionic moieties as salsolinol and 3-*O*-sulfothornasterol A, respectively. The comparison of optical rotations of **1**–**3** with those of the corresponding steroid sulfates and *R*- and *S*-salsolinols[7] allowed us to suggest that the *R*-isomer of salsolinol is predominantly incorporated into our salts. This enantiomer of salsolinol has often been found in animals.

Table 1. ^1H and ^{13}C NMR data (C_5D_5N) of compound **1**[a] [*J* (Hz) values are shown in parentheses]

No.	DEPT	δC	δH[b]	No.	DEPT	δC	δH[b]	HMBC correlations
1	CH_2	36.1	1.45 m, 1.67 m	15	CH_2	25.4	1.75 m	
2	CH_2	29.4	2a: 1.93 m	16	CH_2	22.9	1.63 m, 2.35 m	
			2e: 2.57 m	17	CH	63.3	2.55 t (9.3)	
3	CH	77.6	4.95 m	18	CH_3	13.0	0.59 s	C12, C13, C14, C17
4	CH_2	34.1	4a: 1.77 m	19	CH_3	19.1	0.95 s	C1, C5, C9, C10
			4e: 3.45 dm (12.4)	20	C	208.1		
5	CH	50.5	1.44 m	21	CH_3	30.8	2.10 s	
6	CH	68.1	3.81 td (4.4; 10.8)	1′	CH	51.1	4.80 q (6.7)	C8′, C9′, C10
7	CH_2	43.1	7a: 1.27 m	3′	CH_2	39.7	3.60 m, 3.77 m	C1′, C4′, C10′
			7e: 2.40 m	4′	CH_2	25.2	2.92 dt (5.7; 16.8), 3.08m	C3′, C5′, C9′, C10′
8	CH	35.7	2.08 m	5′	CH	116.1	7.03 s	C4′, C7′, C9′
9	C	146.5		6′	C	146.6		
10	C	38.2		7′	C	147.1		
11	CH	115.7	5.28 d (5.3)	8′	CH	113.7	7.13 s	C1′, C6′, C10′
12	CH_2	40.5	2.20 m	9′	C	125.0		
13	C	42.4		10′	C	122.3		
14	CH	53.5	1.20 m	11′	CH_3	19.7	1.81 d (6.7)	C1′, C9′

[a] In ppm at 500 MHz for ^1H NMR and 125.8 MHz for ^{13}C NMR.
[b] Assignments were aided by HSQC and ^1H–^1H COSY.

These salts are unique in as much as they are ionic hybrids of marine sulfated steroids and an isoquinoline alkaloid. To the best our knowledge, these types of hybrids have not been previously found in natural sources. Only the rare phenethylammonium or tyrammonium salts of sulfated steroids from some sponges[10] and starfish[11–13] resemble this group of compounds.

(1*S*,3*S*)-1-Methyl-1,2,3,4-tetrahydro-β-carboline-3-carboxylic acid **4** was isolated as an amorphous solid, $[\alpha]_D^{25}$ −78.2 (*c* 0.2, MeOH); ^{1}H NMR (300 MHz, CD_3OD): δ 1.75 (3H, d, CH_3-10), 3.02 (1H, dd, 12.1, 16.4, H-4), 3.45 (1H, dd, 5.0, 16.4, H′-4), 3.97 (1H, dd, 5.0, 12.0, H-3), 4.71 (1H, q, 6.8, H-1), 7.05 (1H, t, 7.0, H-6), 7.14 (1H, t, 7.0, H-7), 7.35 (1H, d, 7.6, H-8), 7.48 (1H, d, 7.6, H-5); ^{13}C NMR (CD_3OD): δ 51.2 (C-1), 59.8 (C-3), 24.4 (C-4), 107.9 (C-4a), 127.5 (C-4b), 119.2 (C-5), 120.6 (C-6), 123.3 (C-7), 112.3 (C-8), 138.6 (C-8a), 131.4 (C-9a), 17.2 (C-10), 173.7 (C-11); EIMS, 70 eV, *m*/*z* (I, %): 230 $[M-H_2O]^+$ (100), 215 $[M-H_2O-CH_3]^+$ (55), 169 (72), 157 (81). The following cross-peaks confirming the structure of **4** were detected in the HMBC spectrum: H-3/C-4, C-11; H-5/C-4a, C-7, C-8a; H-6/C-4b, C-8; H-8/C-4b, C-6; H-10/C-1, C-9a. The relative stereochemistry of **4** was established by NOE enhancements of H-1 and H-4 after irradiation of H-3. The *S*-configuration at C-3 followed from the corresponding configuration of L-tryptophan, a biogenetic precursor of **4**. Diastereoisomers of **4** differing from each other in the configuration at C-1 have been previously found in higher plants and some animals.[14,15] This alkaloid was found by us for the first time in marine invertebrates. Earlier the alkaloids ovothiol A,[16] imbricatine[17] and several guanidine containing metabolites[18] were known from starfish.

The alkaloidosteroids raise questions concerning their biological activities. What kind will take place: additivity of the activities of both ions, synergism, reciprocal inhibition or peculiar properties? We have found some anomalies in the comparison of the action of alkaloidosteroid **3** and the sodium salt of 3-*O*-sulfothornasterol A on the development of the embryos of the sea urchin *Strongylocentrotus nudus*. The sodium salt was toxic in this test with an EC_{50} value of about 40 μg/mL in contrast with **3** which was non-toxic up to a concentration of 100 μg/mL.

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