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STEROLS FROM THE TOXIN-CONTAINING FAR-EAST SPONGE *Monanchora pulchra*

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It was shown earlier that toxin-containing marine invertebrates, in particular sponges (phylum Porifera) and sea cucumbers (phylum Echinodermata, class Holothurioidea), often contain unusual sterols instead of cholesterol and other Δ^5-sterols, in contrast with other animals [1]. They differ from cholesterol either by the position of the double bond in the steroid core (sometimes its absence) [2–4] or by the presence of highly alkylated side chains [5, 6]. According to a hypothesis proposed by us, the unusual sterols protect the biomembranes of toxin-producing marine invertebrates from the lytic action of their own toxins as a result of sterol–toxin biochemical coordination occurring in such instances [7].

In continuation of the search for new sources of biologically active compounds among invertebrates of Far-East seas, we recently found that EtOH extracts of the sponge *Monanchora pulchra* exhibited high cytotoxic activity. It was shown that this sponge contained a new cytotoxic polycyclic guanidine alkaloid that we called monanchocidin [8]. We investigated the sterol fraction from this sponge collected near the shores of Urup Is. (Kuril Islands, 46°07.0 N, 150°02 E) in August 2008 in order to test further this hypothesis.

The sponge for chemical investigation was stored at –20°C. Thawed ground biological material (18.9 g dry wt.) was extracted (3×) with EtOH. The combined extracts were concentrated to dryness *in vacuo* and distributed between H_2O and BuOH. The evaporated BuOH layer (2.14 g) was dissolved in EtOH (90%) and extracted (3×) with hexane. The combined hexane layers were concentrated to afford total nonpolar substances (0.69 g). A part (0.215 g) of this total was chromatographed over a column of silica gel (KSK, 50–160 μm) using a hexane→EtOAc gradient (4:1) to afford the total sterol fraction (57 mg, 0.92% of dry wt) that was separated using HPLC into three subfractions consisting of Δ^5- (1.3 mg) and Δ^7-sterols (24.3 mg), and stanols (21 mg). These were analyzed using PMR as described before [2, 3, 9, 10].

The structures of the free sterols as the acetylated derivatives were identified using GC and comparison with the relative retention times (RRT) of standard sterols and using GC–MS as before [2, 3, 9, 10]. Table 1 presents the results.

Table 1 shows that the sterol fraction from the studied sponge contained 29 previously known C_{26}, C_{27}, C_{28}, C_{29}, and C_{30} compounds that had double bonds in the Δ^7, $\Delta^{7,22}$, $\Delta^{7,24(28)}$, Δ^0, Δ^{22}, Δ^5, $\Delta^{5,22}$, and $\Delta^{5,24(28)}$ positions. Sterols of the Δ^7series were basic and made up 52.1% of the total sterol fraction. The stanol content was 45.1% whereas Δ^5-sterols were a minor fraction with a content of 2.8%. The principal components of the fraction were identified as 5-α-cholestan-3β-ol and 5α-cholest-7-en-3β-ol. Thus, yet another instance of toxin—(unusual sterols) biochemical coordination was found.

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TABLE 1. Composition of Sterol Fraction from the Sponge *Monanchora pulchra*

Sterol	RRT*	%	Mass spectrum, *m/z*
24-Nor-5α-cholesta-5,22*E*-dien-3β-ol	0.844	0.23	352, 337, 310, 282, 255, 213
24-Nor-5α-cholest-22*E*-en-3β-ol	0.854	4.73	414, 399, 344, 329, 315, 269, 257, 215, 55
24-Nor-5α-cholesta-7,22-dien-3β-ol	0.883	4.79	412, 397, 342, 313, 299, 288, 273, 255, 241, 229, 213
24-Nor-5α-cholest-5-en-3β-ol	0.891	0.004	354, 339, 255, 247, 213
24-Nor-5α-cholestan-3β-ol	0.900	0.13	416, 356, 341, 215
27-Nor-24-methyl-5α-cholesta-5,22*E*-dien-3β-ol	0.944	0.06	366, 351, 282, 281, 255, 213
27-Nor-24-methyl-5α-cholest-22*E*-en-3β-ol	0.955	1.15	428, 413, 368, 358, 344, 329, 315, 257, 55
5α-Cholesta-5,22*E*-dien-3β-ol	0.959	0.15	366, 351, 282, 255, 213
5α-Cholest-22*E*-en-3β-ol	0.968	6.10	428, 368, 353, 344, 329, 315, 274, 269, 257, 229, 215
27-Nor-24-methyl-5α-cholesta-7,22*E*-dien-3β-ol	0.995	1.57	426, 342, 313, 255, 241, 229,213
5α-Cholest-5-en-3β-ol	1.000	0.73	368, 353, 283, 260, 255, 247, 213
5α-Cholestan-3β-ol	1.010	26.51	430, 415, 370, 355, 316, 276, 262, 230, 215, 201
5α-Cholesta-7,22*E*-dien-3β-ol	1.011	0.76	426, 411, 351, 342, 313, 288, 255, 229, 213
24-Methyl-5α-cholesta-5,22*E*-dien-3β-ol	1.047	0.55	380, 365, 337, 282, 255, 228, 213
5α-Cholest-7-en-3β-ol	1.055	23.47	428, 413, 368, 353, 315, 255, 229, 213
24-Methyl-5α-cholest-22*E*-en-3β-ol	1.062	2.69	442, 382, 344, 329, 315, 285, 257, 255, 215
24-Methyl-5α-cholesta-7,22*E*-dien-3β-ol	1.110	7.31	440, 425, 365, 342, 313, 288, 255, 229, 213
24-Methyl-5α-cholesta-24(28)-dien-3β-ol	1.115	0.55	380, 365, 296, 281, 253, 229, 213
24-Methyl-5α-cholest-24(28)-en-3β-ol	1.129	1.23	358, 343, 315, 255, 215
24-Methyl-5α-cholestan-3α-ol	1.137	1.56	444, 429, 384, 369, 290, 276, 230, 215
23,24-Dimethyl-5α-cholesta-5,22*E*-dien-3β-ol	1.149	0.02	394, 282, 253, 69
24-Ethyl-5α-cholesta-5,22*E*-dien-3β-ol	1.161	0.04	394, 379, 351, 282, 255, 228, 213
24-Methyl-5α-cholesta-7,24(28)-dien-3β-ol	1.183	2.42	440, 425, 380, 356, 313, 255, 227, 213
24-Methyl-5α-cholest-7-en-3β-ol	1.193	1.18	442, 427, 315, 273, 255, 229, 213
23,24-Dimethyl-5α-cholest-5-en-3β-ol	1.244	0.23	396, 381, 288, 281, 275, 255, 213
24-Ethyl-5α-cholestan-3β-ol	1.261	2.21	458, 398, 383, 276, 230, 215
24-Propyl-5α-cholest-5,24(28)*E*-dien-3β-ol	1.328	0.01	408, 393, 296, 281, 255, 253, 228, 213
24-Ethyl-5α-cholest-7-en-3β-ol	1.331	9.35	456, 441, 315, 273, 255, 229, 213
24-Propyl-5α-cholesta-5,24(28)*Z*-dien-3β-ol	1.374	0.002	408, 296, 281, 253

*Relative retention times (RTT) are given for sterol acetates; RTT for cholesterol acetate = 1.00.

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