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Annasterol sulfate[1], a Novel Marine Sulfated Steroid, Inhibitor of Glucanase Activity from the Deep Water Sponge *Poecillastra laminaris.*

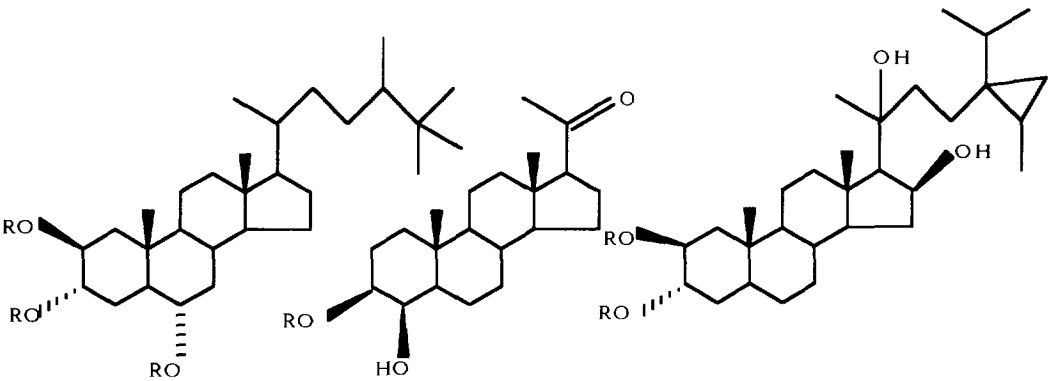
Tatyana N.Makarieva, Valentin A.Stonik*, Olesya G.D'yachuk, and Andrei S.Dmitrenok

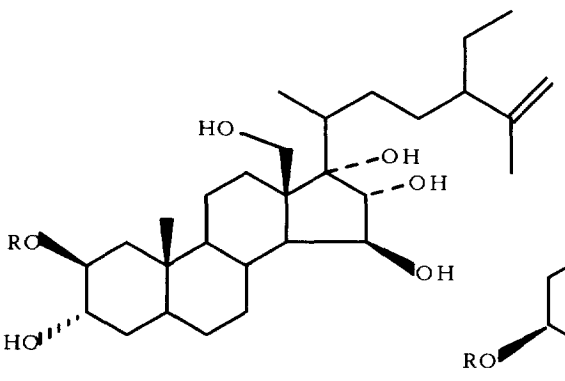
Laboratory of the Chemistry of Marine Natural Products, Pacific Institute of Bioorganic Chemistry of the Russian Academy of Sciences, Vladivostok-22, Russia

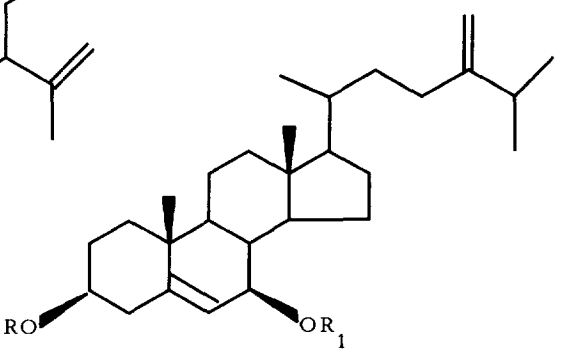
Abstract. ***A novel sulfated steroid, annasterol sulfate (5) has been isolated from the deep water sponge Poecillastra laminaris. Its structure has been determined on the basis of chemical transformations and spectral data. This steroid was shown to be a potent inhibitor of glucanases.***

Among the many unusual steroid metabolites from sponges, sulfated steroid polyols have attracted a great attention by their unusual chemical structures[2], potential activity against HIV[2,3] and as inhibitors of some enzymes[4]. Four structural types of these compounds have been reported so far, namely: halistanol sulfate (**1**) and related steroids[5-7] from several sponge species; 3β−sulfoxy−4β−hydroxypregnane (**2**) from the sponge *Stylopus australis*[8]; weinbersterol disulfate A (**3**) and other antiviral steroids from the sponge *Petrosia weinbergi*[9]; and echinoclasterol (**4**) from the sponge *Echinoclathria subhispida*[10]. In the course of our continuing studies on biologically active marine natural products, we have found that extracts from the Pacific deep water sponge *Poecillastra laminaris* were highly antimicrobial against *Bacillus subtilus* and *Proteus vulgaris.* Bioassay−guided isolation afforded several active compounds, of which annasterol sulfate (**5**) was a first representative of new structural series of sponge steroid sulfates. In this report we describe the isolation and structure elucidation of **5.**

The EtOH extract of the fresh sponge, collected from an underwater mountain (the depth of 750 m) in Philippine Sea[11] was partitioned between aqueous EtOH and $CHCl_3$. $CHCl_3$−soluble materials were further separated by column chromatography on Silica gel in the system $CHCl_3$:EtOH, 7:3 and by repeated HPLC on Silasorb C−18 (10 mm x 25 cm, 70 % aqueous acetone) to afford annasterol sulfate (**5**)[12] (10^{-2} %, based on dry weight of sponge). The FAB MS and EIMS [m/z 535 $(M-Na)^-$ and m/z 378 ($M^+ - NaHSO_4$ − AcOH), respectivly] correspond to a molecular formula $C_{30}H_{47}O_6SNa$, as confirmed







by other spectral data. The presence of an O−sulfate group was supported by a strong IR absorption at 1236 cm^{-1}. Atomic absorption analysis demonstrated that cation at sulfate group is sodium. Desulfatation of **5** by solvolysis in pyridine at 100° C, 1h gave **5a**[13] which was converted into the diol **5b**[14] by the treatment with MeONa in dry MeOH. EIMS and ^1H NMR spectra of **5**, **5a,b** indicated the disubstituted monounsaturated steroidal skeleton of **5** with O−sulfate and acetyl groups as substituents and the presence of an unsaturated C_9 side chain. Positions of these groups and structure of the side chain were established by NOE and INDOR experiments in NMR spectra of **5a** (see Table 1 and figure 1). ^1H NMR spectra of polycyclic moieties of **5b** and related $\Delta^5-3\beta,7\beta-$steroid diols[15] were practically identical, confirming 3β− and 7β−positions of functional groups in 5.

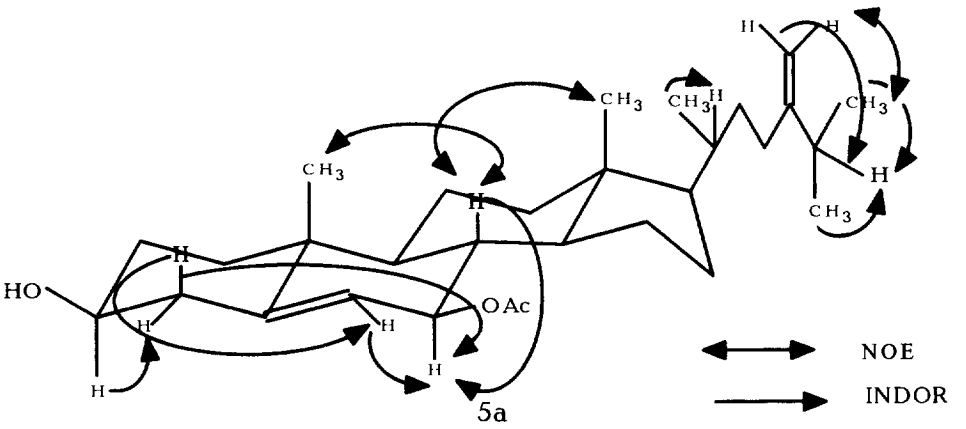


Figure 1

Table 1. NMR data for compounds **5** (CD_3OD) and **5a,b** ($CDCl_3$); J(Hz); δ.

H−atom	Compound		
	5	5a	5b
H−3, 1H, m	4.18	3.54	3.56
H−4a, 1H, m	2.28	−	−
H−4e, 1H, m	2.28	−	−
H−6, 1H, t	5.27, J=2.0; 2.0	5.22, J=2.0; 2.0	5.29, J=2.0; 2.0
H−7, 1H, dt	5.00, J=8.0; 2.0;	5.03, J=8.5; 2.0;2.0	3.85, J=7.5; 2.0
H−8, 1H, m	1.67	−	−
H−18, 3H, s	0.72	0.70	0.72
H−19, 3H, s	1.09	1.08	1.06
H−21, 3H, d	0.98, J=6.5	0.94, J=6.5	0.97, J=6.5
H−25, 1H, m	2.22	−	−
H−26, 3H, d	1.02, J=6.8	1.02, J=6.8	1.04, J=6.8
H−27, 3H, d	1.03, J=6.8	1.03, J=6.8	1.05, J=6.8
H−28, 1H, d	4.64, J=1.5	4.66, J=1.5	4.67, J=1.5
H−28, 1H, brs	4.72	4.72	4.73

Annasterol sulfate (**5**) is a first naturally occurring derivative of 3β−O−sulfated steroid diols of the ergostane series. This compound inhibits endo−β−1,3−glucanases L_0 and L_{IV}[4] (1.0 and 2.8 μg/0.02 unit of activity, respectively).

It is of interest that we have isolated this steroid using probably the most deep water collection of sponges from all those which have been earlier studied with the purpose to isolate secondary metabolites.

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11. The sponge was collected by dredging during 13−th scientific cruise of R/V "Academik Oparin", April, 1991, 25°07'5"N, 135°,40'7"E, −750m.
12. Compound **5**: m.p. 149−150° C, $[\alpha]_{578}$ +28.6° (C 0.35 MeOH); IR (KBr): 2920, 1726, 1234 cm^{-1}; EIMS (rel.int.,%): 378 (100), 363 (14), 312 (10), 271 (10), 253 (40), 211 (20).
13. Compound **5a** : m.p. 65−67°C, $[\alpha]_{578}$+51° (C 0.78 $CHCl_3$); EIMS (rel.int.,%): 396 (100), 378 (22), 381 (11), 363 (19), 312 (33), 271 (55), 253 (33), 211 (20).
14. Compound **5b**: $[\alpha]_{578}$ −26.6° (C 0.30 MeOH); EIMS (rel.int.,%): 414 (10); 396 (100); 312 (12); 271 (13); 253 (16); 211 (23)
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