STEROID COMPOUNDS OF MARINE SPONGES.

X. 26-NORSOKOTRASTEROL SULFATE — A NEW STEROID IDENTIFIED IN THE SPONGE Trachyopsis halichondroides

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A new sulfated steroid triol — 26-norsokotrasterol sulfate — has been identified in extracts of the sponge (Trachyopsis halichondroides). Its structure has been shown as 24,25,26-trimethyl-5α-cholest-23-ene-2β,3α,6α-triol trisulfate. No steroid substances with side chains having the structure of that in 26-norsokotrasterol sulfate have previously been found.

Continuing a study of steroid compounds from sponges of the family Halichondriidae [1], we have found in Trachyopsis halichondroides (determined by V. M. Koltun) a new unsual sulfated steroid triol (1) that is a homolog of the sokotrasterol sulfate (2) that we have described previously [2].

From an ethanol-chloroform extract, using known methods [2], we obtained two steroid fractions: free steroids and sulfated steroid triols. In the first fraction, with the aid of GLC and GLC-MS we identified as the main components 24-isopropylcholesta-5,22-diene-3β-ol and its 22,23-dihydro derivative [3]. The second fraction was analyzed after the conversion of the steroid trisulfates into the corresponding triacetates [1]. In it we detected halistanol trisulfate (3a), a previously unknown steroid (1a) (36.5 and 34.0% of the weight of the fraction, respectively), and six minor steroid triacetates (totaling 23%) each having a Δ^{22}-double bond in the side chain. In view of the fact that one of the two main components of the fraction, namely (3a), was a saturated compound, while the other (1a) was a monounsaturated compound (molecular weight of the triacetate 586 according to its mass spectrum), we attempted to determine the structure of (1) on the basis of the results of the ozonolysis of the corresponding fraction, as described in [1].

With this aim, after the ozonolysis of the combined triacetates and the treatment of the ozonides with zinc dust and with 2,4-dinitrophenylhydrazine, we obtained unchanged (3a) and a number of products, including the hydrazones (4—6).

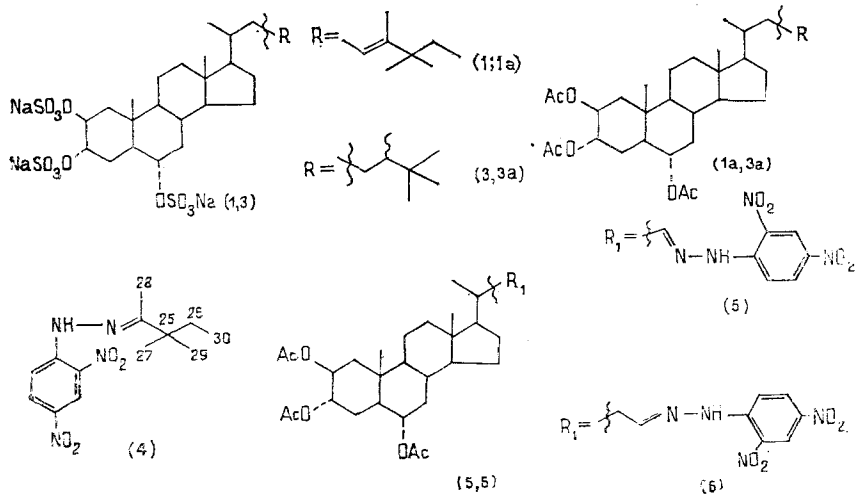

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TABLE 1. ^1H NMR Spectra (chemical shifts, δ, $CDCl_3$)

Compound	Protons of CH groups							
	2 m	3 m	6 td	22 d	23 t	3′ d	5′ dd	6′ d
Combined acetates	4,90	4,94	4,73	—	—	—	—	—
4	—	—	—	—	—	9,16	8,34	7,99
5	4,92	4,95	4,74	7,37	—	9,13	8,31	7,91
6	4,92	4,95	4,74	—	7,53	9,13	8,31	7,95

Compound	Protons of CH_2 and CH_3 groups								
	18 s	19 s	21 d	28 s	27, 29 s	30 t	26 q	28 d	26, 27, 29 s
Combined acetates	0,64	0,97	0,91	—	—	—	—	0,80	0,84
4	—	—	—	2,02	1,23	0,83	1,62	—	—
5	0,76	1,01	1,23	—	—	—	—	—	—
6	0,72	0,99	1,07	—	—	—	—	—	—

The ^1H NMR spectrum of (4) (Table 1) contained two signals at 2.02 ppm (3 H) and 1.23 ppm (6 H), which we assigned to the protons of the olefinic CH_3-28 and the gem-dimethyl (CH_3-27, 29) groups, respectively. A three-proton signal in the form of a triplet at 0.83 ppm with a spin-spin constant of 7 Hz and a quartet (2 H) at 1.62 ppm (J = 7 Hz) showed the presence of an ethyl fragment at a quaternary carbon atom in compound (4).

The mass spectrum was also in agreement with structure (4). It contained a signal with m/z 294 (M^+) and a strong peak with m/z 265 corresponding to the allyl splitting out of this fragment.

In the ^1H NMR spectrum of the hydrazones (5 and 6), obtained in the form of a mixture in a ratio of 2:3, we identified all the signals relating to the protons of the known hydrazone (5) [1]. The remaining signals were assigned to the protons of the related substance (6) (see Table 1). The presence of a triplet at 7.53 ppm and of a doublet at 1.07 ppm corresponded to the CH-23 and CH_3-21 groups of hydrazone (6).

The agreement of the chemical shifts and the multiplicities of the protons at the signals C-2, C-3, and C-6 in the triacetates (5 and 6) and in the spectrum of the initial mixture of acetates showed the identity of the positions and configurations of the functional groups in these derivatives.

The mass spectrum of the mixture of (5) and (6) showed peaks with m/z 684 (M^+, C_{23}-steroid) and 670 (M^+, C_{22}-steroid). The identification of the hydrazones of C_{22}- and C_{23}-steroid aldehydes showed the existence of Δ^{22}- and Δ^{23}-derivatives in the triacetate fraction of the sponge under investigation.

In order to determine the structure of (1) from the results obtained, we also analyzed the mass spectrum of (1a) (GLC-MS).

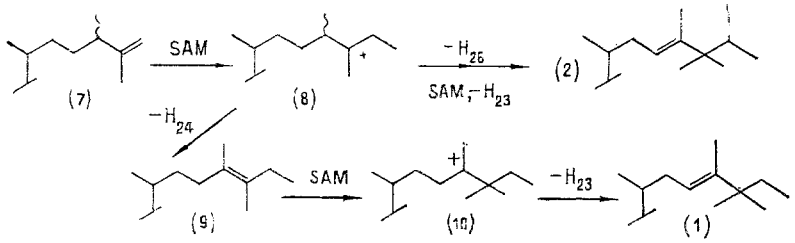
In the mass spectrum of (1a), in addition to the signals of the molecular ion with m/z 586 and of other fragmentary ions, there were strong signals probably arising as the result of the cleavage of the $C_{20}-C_{22}$ bond (m/z 474, 414, 372, and 294) and of the $C_{22}-C_{23}$ bond (m/z 460, 401, 341, and 281) with the subsequent elimination of a molecule of acetic acid. These results do not contradict features of the breakdown of Δ^{23}-steroids under the action of electron impact [4]. Thus, in the mass spectrum of a $\Delta^{5,7,23}$-sterol, a signal with m/z 281 is observed, which is characteristic for $C_{20}-C_{22}$ cleavage in the side chain and the elimination of a molecule of water [4].

The determination of the structure of the hydrazone (4), the identification of the C_{23}-steroid hydrazone (6), and analysis of the mass spectrum of (1a) showed that the sponge extracts contained 24,25,26-trimethyl-5α-cholest-23-ene-2β,3α,6α-tril trisulfate (1). which we have called 26-norsokotrasterol sulfate.

At the present time, steroid (1) is the fifth representative of trisulfated steroids of type (2—3) and is a very close analog of sokostrasterol sulfate (2). The new trisulfate (1) differs from (2) by the absence of one methyl group at C-26 [2]. No steroid substances with the same structure of the side chain as for 26-norsokotrasterol sulfate have been

identified previously [5]. However, in the steroid fraction of the sponge Xestospongia muta a minor component has been found with a similar side chain but with a different position of the duouble bond [6].

On the basis of modern biogenetic ideas concerning the biosynthesis of sponge steroids [5], the formation of steroid (1), biogenetically related to sokotrasterol suflate (2) can be represented by the following scheme:



Apparently, substances (1) and (2) have the common precursor (8) with a side chain containing an additional methyl group at C-26. The splitting out of the proton at C-26 in the carbocation (8) leads to the side chain of sokotrasterol sulfate (2) and the splitting out of a proton from C-24 leads to 26-norsokotrasterol sulfate (I).

The further study of the steroid composition of sponges of the family Halichondriidae and the isolation of new compounds will probably permit the routes of biosynthesis of such unique substances as sokotrasterol to be determined in more detail.

EXPERIMENTAL

The sponge was collected at a depth of 45 m in the central part of the Indian Ocean (Saiya de Malia Bank) during the third cruise of the Scientific Research Ship Akademik Oparin (October, 1986). Melting points and mass and ^1H NMR spectra were obtained as described previously [2]. GLC was performed on a Perkin-Elmer Sigma 2000 chromatograph with a 0.2 mm × 25 m capillary column containing OV-101 at 290°C with argon as the carrier gas, and the chromato-mass-spectrometric study on an LKB-9021 instrument with a 0.32 mm × 25 m capillary column containing OV-101 at 290°C with an ionizing voltage of 70 V, the carrier gas being helium.

Isolation of the Steroid Fractions. The comminuted sponge material (dry weight 300 g) was extracted successively with ethanol and with ethanol–chloroform (1:1). The combined extracts were concentrated in vacuum to dryness. The residue was distributed between aqueous ethanol (10%) and hexane. The hexane layer, after evaporation in vacuum and crystallization from ethanol, yielded 100 mg (0.03% on the dry weight of sponge) of total sterols, with the composition 24-isopropylcholesta-5,22-dien-3β-ol (66%) and 24-isopropylcholest-5-en-3β-ol (34%).

The aqueous ethanolic layer was evaporated to an aqueous residue, and this was extracted with butanol. The butanol layer was concentrated and chromatographed on Polikhrom. Elution with 50% ethanol gave 1.15 g (0.4% on the dry weight of sponge) of a mixture of sulfated steriod triols. Mass spectrum (m/z, %): 406 (C_{30} M^+–3 $NaHSO_4$, 42): 394 (C_{29} M^+–3 $NaHSO_4$, 70).

Preparation of Triacetates from the Sulfated Steroid Fraction. From 498 mg of the sulfates after acid hydrolysis and acetylation, 218 mg of triacetates (yield 60%) was isolated by the procedure of [1].

GLC and GLC-MS analysis on a capillary column with OV-101 showed that the fraction consisted of a combination of saturated and unsaturated components.

Mass Spectrum of the Triacetate of the $C_{29}\Delta^0$-Steroid (3a) (m/z, %): 574 (M^+, 1); 514 (M^+–AcOH, 5); 499(M^+–AcOH–15.1); 454(M^+–2AcOH, 61); 439(M^+–2AcOH–15.5); 412(M^+–2AcOH–42.100); 394(M^+–3AcOH, 81); 313(M^+–S.ch.,3); 271(10); 253(15); 244(17); 226(17); 211(32).

Mass Spectrum of the Triacetate of the $C_{30}\Delta^{23}$-Steroid (1a) (m/z, %): 586(M^+, 13); 571 (M^+–15.10); 557(M^+–29,4); 526(M^+–AcOH, 4) 511(M^+–15–AcOH, 46); 497(M^+–29–AcOH, 13); 474(M^+–112.7); 466(M^+–2AcOH, 6); 460(M^+–126,19); 437(M^+–29–2AcOH, 28); 431(M^+–S. ch.–2H, 70); 414(M^+–112–AcOH, 7); 401(M^+–125–AcOH, 19); 395(M^+–29–2AcOH–42.6); 377(M^+–29–3AcOH, 28); 372(M^+–112–2AcOH–42.19); 371(M^+–s. ch.–2H–AcOH, 19); 359(M^+–125–AcOH–42.10); 354 (M^+–112–2AcOH, 19); 341(M^+–125–2AcOH, 88); 313(M^+–S. ch.–2AcOH, 17); 299(M^+–125–2AcOH–42.23); 294(M^+–112–3AcOH, 20); 281(M^+–125–3AcOH, 100); 217(10); 251(15); 231(20); 213(20); 211(20).

Ozonolysis of the Triacetates. Using the method of [1, 2], 145 mg of the resulting mixture of triacetates yielded:

3,3-Dimethylpentan-2-one 2,4-dinitrophenylhydrazone (4), 7.1 mg (yield 70%, mp 105–106°C) from ethanol. Mass spectrum (m/z, %): 294 (M^+, 100); 265 (M^+–29.44). ^{1}H NMR spectrum (δ, $CDCl_3$): 11.08 (s, NH): $J_{26,30}$ = 7 Hz; the chemical shifts of the CH, CH_2, and CH_3 protons are given in Table 1.

Mixture of the 2,4-dinitrophenylhydrazones of the C_{22}-(5) and C_{23}-(6) steroid aldehydes, 20 mg, mp 133-136°C (amorphous powder from ethanol). Mass spectrum (m/z, %): 684($C_{23}M^+$, 0.2); 670($C_{22}M^+$, 1); 624($C_{23}M^+$–AcOH, 2); 622($C_{23}M^+$–15–47.2); 610($C_{22}M^+$–AcOH, 3); 608($C_{22}M^+$–15–47.3); 460(15); 400(10); 340(40); 332(100); 271(30); 253(20); 244(50); 241(50); 226(60); 211(80). ^{1}H NMR spectrum (δ, $CDCl_3$): 10.92 (s, NH (5)); 11.50 (s, NH(6)); 2.05 (s, 6H, CH_3COO–); 2.10 (s, 3H, CH_3COO–); $J_{20,21}$ = 6.5 Hz, $J_{20,22}$ (5) = 7 Hz, $J_{22,22}$ (6) = 6 Hz. The chemical shifts of the CH, CH_2, and CH_3 groups are given in Table 1.

SUMMARY

A new polysulfated steroid triol — 26-norsokotrasterol sulfate — has been identified in the sponge Trachyopsis halichondroides. Its structure has been established as 24,25,26-trimethyl-5α-cholest-23-ene-2β,3α,6α-triol trisulfate.

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