SUMMARY

Five minor triterpenoids (oleanolic acid and a monoside, a bioside, and two triosides of this acid) have been isolated from *Thalictrum minus* L., and have been identified by ^{13}C NMR spectroscopy and FAB mass spectrometry without chemical degradation of the glycosides.

This is the first time that any of these compounds have been detected in a plant of the genus *Thalictrum*.

LITERATURE CITED

1. M. Adinolfi, I. Mangoni, G. Marino, M. Parrilli, and R. Self, Biomed. Mass Spectrom., 11, 310 (1984).
2. H. R. Schulten, T. Komori, T. Kawasaki, T. Okuyama, and S. Shibata, Planta Med., 46, 67 (1982).
3. A. S. Gromova, V. I. Lutskii, A. A. Semenov, V. A. Denisenko, and V. V. Isakov, Khim. Prir. Soedin., 213 (1984).
4. A. S. Gromova, V. I. Lutskii, A. A. Semenov, R. B. Valeev, G. A. Kalabin, and Yu. N. El'kin, Khim. Prir. Soedin., 670 (1985).
5. A. S. Shashkov and O. S. Chizhov, Bioorg. Khim., 2, 437 (1976).
6. D. Fraisse, J. C. Tabet, M. Becchi, and J. Rainold, Biomed. Mass Spectrom., 13, 1 (1986).
7. A. S. Gromova, V. I. Lutskii, E. V. Kostyleva, N. I. Dedkova, and A. A. Semenov, Izv. Sib. Otd. Akad. Nauk SSSR, Ser. Khim. Nauk, Ser. No. 4, Issue, No. 9, 129 (1981).

STEROID COMPOUNDS OF MARINE SPONGES.

VIII. 24-ISOPROPYL-5α-CHOLEST-22-ENE-2β,3α,6α-TRIOL TRISULFATE – A NEW STEROID IDENTIFIED IN THE SPONGE *Trachyopsis halichondrioides*

T. N. Makar'eva, L. K. Shubina, and V. A. Stonik

UDC 547.92:639.29

A new sulfated steroid triol has been identified in extracts of the sponge *Trachyopsis halichondrioides* and its structure has been established as 24-isopropyl-5α-cholest-22-ene-2β,3α,6α-triol trisulfate. The possibility has been shown for the first time of the existence of identical side chains for free sterols and trisulfated steroids.

Continuing a study of the steroid compounds of sponges [1], we have established the structure of a new trisulfated steroid triol detected in the sponge of *Trachyopsis halichondrioides* (Halichondriidae).

Fractions of free sterols and of sulfated steroids giving a single spot on TLC in suitable systems were isolated from an ethanolic extract of the sponge by chromatography on Polychrome [2].

GLC and GLC-MS showed that the components of the free sterol fraction of this sponge were alcohols known previously [2, 3] – 24-isopropylcholesta-5,22-dien-3β-ol and its 22,23-dihydro analog.

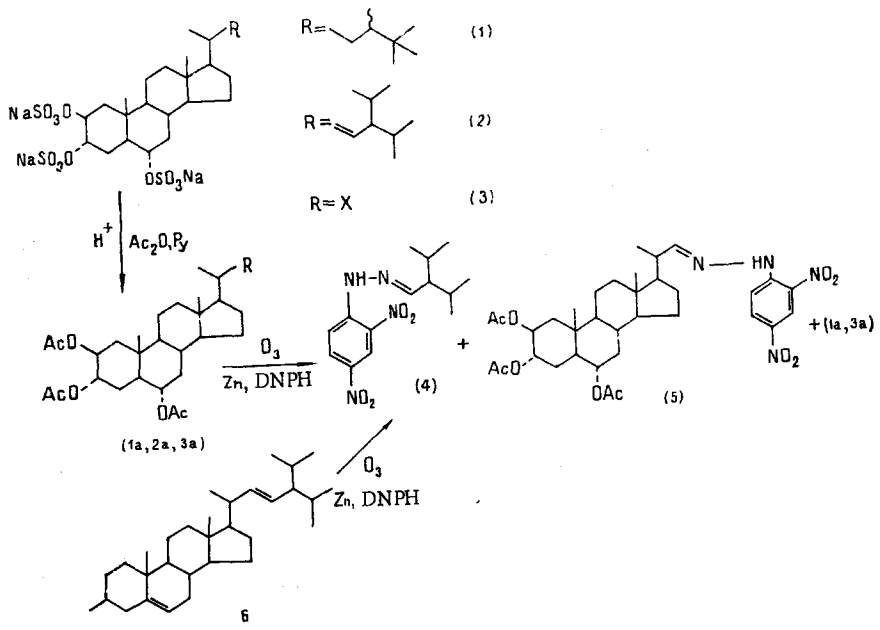
Analysis of the mass and ^1H NMR spectra of the sulfated fraction revealed the presence in it not only of halistanol sulfate (1), but also of substances related to it (2, 3). However, we did not succeed in separating the isolated mixture of sulfated steroid triols with the aid of high-pressure liquid chromatography on a ODS column in ethanol–water systems.

Acid hydrolysis of the combined sulfates (1 + 2 + 3) and subsequent acetylation of the triols obtained led to a fraction of triacetates (1a, 2a, and 3a). According to GLC and GLC-

---

Pacific Ocean Institute of Bioorganic Chemistry, Far Eastern Scientific Center, Academy of Sciences of the USSR, Vladivostok. Translated from Khimiya Prirodnykh Soedinenii, No. 1, pp. 111-115, January February, 1986. Original article submitted July 25, 1986.

0009-3130/87/2301-0093$12.50



MS, this fraction contained halistanol triacetate (64%) and two previously unknown C_{30}-steroids, one of which had a double bond.

Attempts to separate the C_{30} derivatives from the halistanol triacetate by chromatography on silica gel impregnated with $AgNO_3$, and also with the aid of CC on hydrophobic sorbents of the ODS type were unsuccessful. In view of the fact that only one of the components had a double bond, we considered it possible to determine its structure without isolating it from the mixture.

The ozonolysis of the mixture of (1a, 2a, and 3a) led to the degradation only of component (2a), as was shown by the GLC-MS method. After the ozonides had been treated with zinc dust and an excess of 2,4-dinitrophenylhydrazine (DNPH), acetylation and column chromatography on silica gel gave the hydrazones (4) and (5).

In the ^{1}H NMR spectrum of (4) (Table 1), there were two doublets at 0.94 and 1.00 ppm which we assigned to the protons of isopropyl groups. A broadened multiplet at ~2.01 ppm corresponded to methine protons at C-24, C-25, and C-28. In the weak field, in addition to the signals characteristic for aromatic protons, there was a doublet of the vinyl C-23 protons. The mass spectrum of hydrazone (4) contained the peak of the molecular ion at m/z 308 and an intense signal at m/z 265 due to the allyl elimination of the isopropyl group.

We obtained hydrazone (4) by independent synthesis from the known sterol (6) after its ozonolysis and appropriate working up.

In the ^{1}H NMR spectrum of hydrazone (5), in addition to the signals of the aromatic and vinyl protons, in the strong field region there were two singlet signals at 0.76 and 1.00 ppm which we assigned to CH_3-18 and CH_3-19. A doublet at 1.23 ppm related to the protons of the CH_3-21 group. The agreement of the chemical shifts and multiplicities of the carbinyl protons at C-2, C-3, and C-6 in the triacetates (5) and (1a, 2a) showed the identities of the positions and configurations of the functional groups in these derivatives. In its mass spectrum, hydrazone (5) gave a molecular peak with m/z 670.

A determination of the structures of compounds (4) and (5) showed that extracts of the sponge contained 24-isopropyl-5α-cholest-22-ene-2β,3α,6α-triol trisulfate (2).

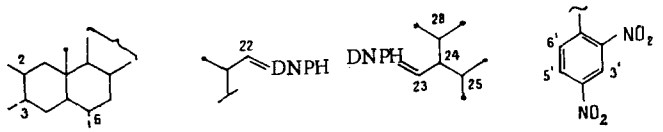
Thus, it has been found that one and the same sponge contains a free sterol and a steroid triol trisulfate analogous to it with respect to its carbon skeleton. The sterol (6) is obviously the biogenetic precursor of compound (2).

EXPERIMENTAL

The sponge was collected at a depth of 2-3 m in the north-western littoral of the island of Madagascar during the twelfth voyage of the Scientific Research Ship "Professor Bogorov" in December, 1981.

TABLE 1. ^{1}H NMR Spectra of the Compounds (chemical shifts, δ, $CDCl_3$)

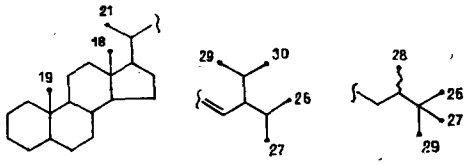
Protons of CH groups



	2 m	3 m	6 td	22 d	23 d	24, 25, 28 m	3′ d	5′ dd	6′ d
Mixture of 1a + 2a	4,90	4,94	4,73	—	—	—	—	—	—
4	—	—	—	—	7,37	2,01	9,13	8,31	7,96
5	4,91	4,96	4,72	7,37	—	—	9,13	8,31	7,91

TABLE 1 (Continued)

Protons of CH_3 groups



	18 s	19 s	21 d	26, 27 d	29, 30 d	28 d	26, 27, 29 s
Mixture of 1a + 2a + 3a	0,64	0,97	0,91	—	—	0,80	0,84
4	—	—	—	0,94	1,00	—	—
5	0,76	1,00	1,23	—	—	—	—

The melting point determinations, GLC analyses and the chromato-mass spectrometric (GLC-MS) study, and the recording of the ^{1}H NMR spectra were carried out as described previously [2]. A Du Pont 8800 chromatograph was used for the high-performance LC.

Isolation of the Steroid Fractions. The comminuted sponge (dry weight 100 g) was extracted with ethanol. The extract was concentrated in vacuum to dryness. Column chromatography on Polychrome-1 with elution by 50% ethanol yielded 1.1 g (1.1% on the dry weight of the animal) of a mixture of sulfated steroid triols (1, 2, 3); mp 170-172°C; mass spectrum (m/z, %), 408 (M^+C_{30}—$NaHSO_4$, 13); 406 (M^+C_{30}—$NaHSO_4$, 14); 394 (M^+C_{29}—$NaHSO_4$, 100).

Elution by ethanol gave 10 mg (0.01% on the dry weight of the animal) of combined sterols (6 and 7); composition: (6) — 40%; (7) — 60% [2].

Preparation of the Triacetates from the Sulfated Steroid Fraction. A mixture of 733 mg of the sulfates (1, 2, and 3) and 10 ml of 9% HCl was heated at 90°C for 2 h. Then the mixture was cooled and extracted with butanol (SO_4^{2-} ions were detected in the aqueous layer by the usual method). The butanolic extract was washed with water, the organic layer was concentrated to dryness in vacuum, and the residue obtained was chromatographed on silica gel (40/100 μm) in ethyl acetate. This gave 328 mg (yield 75%) of a white amorphous substance consisting of the sum of the desulfated natural steroid derivatives. The acetylation of this sum was carried out in 12 ml of acetic anhydride—pyridine (1:1) at 25°C for 16 h. After the solution had been concentrated in vacuum, 320 mg (yield 80%) of the triacetates (1a, 2a, and 3a) was obtained.

GLC-MS analysis showed that the fraction consisted of:

halistanol triacetate (1a, 64%); RRT — 3.5 (relative retention time with respect to cholesterol as 1.00); mass spectrum (m/z, %): M^+ — absent, 514 (M^+ — AcOH, 3); 454 (M^+ — 2AcOH, 46); 412 (M^+ — 2AcOH — 42, 100), 394 (M^+ — 3AcOH, 60); 253, 211;

the triacetate of a monounsaturated C_{30}-steroid (2a, 28%); RRT – 3.8; mass spectrum (m/z, %): M^+ – absent, 526 (M^+ – AcOH, 3); 466 (M^+ – 2AcOH, 50); 424 (M^+ – 2AcOH – 42; 100); 406 (M^+ – 3AcOH, 70), 253, 211;

the triacetate of a saturated C_{30}-steroid (3a, 8%); RRT – 4.32; mass spectrum (m/z, %): M^+ – absent, 528 (M^+ – AcOH, 3); 468 (M^+ – 2AcOH, 52); 426 (M^+ – 2AcOH – 42,100); 408 (M^+ – 3AcOH, 65); 271, 253, 211.

Ozonolysis of the Triacetates. A current of ozone was passed through a solution of 141 mg of the mixture of compounds (Ia and IIIa) in 5 ml of methylene chloride and 1 ml of methanol at –70°C until the mixture had acquired a permanent blue coloration (3 h). The solution was worked up as we have described previously [2]. The hydrazones were chromatographed on silica gel. Elution by hexane–benzene (3:1) yielded 20.6 mg of hydrazone (4); mp 149-151°C (from ethanol); mass spectrum (m/z, %), 308 (M^+, 27), 265 (M^+ – 43,100); the ^{1}H NMR spectrum is given in Table 1.

Elution with benzene gave 111 mg of product, which was acetylated with a mixture of acetic anhydride and pyridine (1:1) at 25°C for 16 h, and the product was chromatographed on a column of silica gel in the hexane–ethyl acetate (2:1) system. This gave 68.3 mg of halistanol triacetate with mp 116-117°C (according to the literature [4]: 116-117°C) which, according to GLC-MS, contained as an impurity 8% of the triacetate of a saturated C_{30} steroid (3a). In addition, 22.6 mg of hydrazone (5) with mp 150-152°C (from ethanol) was obtained; mass spectrum (m/z, %): 670 (M^+, 100); 610 (M^+ – AcOH, 100); 608, (M^+ – $2HNO_2$, 100); 550 (M^+ – 2AcOH, 40); 508 (M^+ – 2AcOH – 42.55); 490 (M^+ – 3AcOH, 80), 253 (25); the ^{1}H NMR spectrum is given in Table 1.

Preparation of 2-Isopropyl-3-methylbutanol 2,4-Dinitrophenylhydrazone (4). A current of ozone was passed through a solution of 40 mg of sterol (6) in a mixture of 3 ml of methylene chloride and 0.5 ml of methanol at –70°C until the reaction mixture had acquired a permanent blue coloration (2 h). The ozonides were worked up as described previously [2]. Column chromatography on silica gel led to the isolation of 30 mg of hydrazone (4), identical with that described above according to a comparison of the constants of the mass and ^{1}H NMR spectra.

SUMMARY

A new polysulfated steroid triol has been identified in the sponge *Trachyopsis halichondrioides*, and its structure has been established as 24-isopropyl-5α-cholest-22-ene-2β,3α,6α-triol trisulfate.

LITERATURE CITED

1. L. K. Shubina, T. N. Makar'eva, and V. A. Stonik, Khim. Prir. Soedin., 715 (1985).
2. T. N. Makarieva, L. K. Shubina, A. I. Kalinovsky, V. A. Stonik, and G. B. Elyakow, Steroids, 42, 267 (1983).
3. W. Hofheinz and G. Oesterhelt, Helv. Chim. Acta, 62, 1307 (1979).
4. N. Fusetani, S. Matsunaga, and S. Konusu, Tetrahedron Lett., 22, 1985 (1981).