The further elution of the column with the same solvent mixture led to 50 mg of 2-deoxy-α-ecdysone 22-monoacetate (III) [4], $C_{29}H_{46}O_6$, mp 146-148° (aqueous methanol), $[\alpha]_D^{20}$ +50.2 ± 2° (c 0.15; methanol); $\nu_{max}^{KBr}$ ($cm^{-1}$): 3445, 1665, 1730, 1255.

Mass spectrum, m/z (%): 490 ($M^+$; 21), 472 (11), 462 (14), 457 (31), 454 (42), 444 (20), 430 (14), 412 (22), 403 (13), 397 (36), 394 (35), 384 (36), 379 (16), 331 (14), 314 (14), 313 (13), 303 (14), 302 (14), 285 (46), 284 (100), 269 (25), 251 (25), 234 (91), 233 (90), 109 (46), 99 (46), 81 (48), 69 (46).

PMR spectrum (δ, ppm): 0.62 (3H at C-18, s); 0.90 (3H at C-19, s); 1.01 (3H at C-21, d, J = 6 Hz); 1.20 (6H at C-26 and C-27, s); 1.95 (3H, $OCOCH_3$, s); 3.30 (H at C-9, m); 3.93 (H at C-3, m); 5.14 (H at C-22, m); 6.02 (H at C-7, broadened singlet).

Subsequent elution with the same mixture of solvents gave 82 mg of the initial 2-deoxy-α-ecdysone (I).

## SUMMARY

A new ecdysteroid, 2-deoxy-α-ecdysone 3-acetate, has been isolated from the epigeal organs of Silene scabrifolia Kom.

## LITERATURE CITED

1. Z. Saatov, M. B. Gorovich, S. Melibaev, and N. K. Abubakirov, Khim. Prir. Soedin., 77 (1986).
2. L. L. Waller, F. H. Stodola, and R. W. Jackson, Type Reactions in Fermentation Chemistry, US Dept. of Agriculture, ARS 71-13 (1959).
3. Z. Saatov, B. Z. Usmanov, and N. K. Abubakirov, Khim. Prir. Soedin., 793 (1979).
4. Ya. V. Rashkes and N. K. Abubakirov, Khim. Prir. Soedin., 518 (1980).
5. Z. Saatov, M. B. Gorovich, N. D. Abdullaev, B. S. Usmanov, and N. K. Abubakirov, Khim. Prir. Soedin., 738 (1981).

# STEROID COMPOUNDS OF MARINE SPONGES.

# VII. PREPARATION OF DERIVATIVES OF SOKOTRASTEROL AND HALISTANOL SULFATES AND STRUCTURE-ACTIVITY INTERRELATIONSHIPS AMONG THESE COMPOUNDS

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UDC 547.92:639.29

Five new sulfated derivatives of sokotrasterol and halistanol have been obtained: 24-nor-5α-cholane-2β,3α,6α,23-tetraol 2β,3α,6α-tri(sodium sulfate); 24-nor-5α-cholane-2β,3α,6α,23-tetraol 2β,3α,6α-tri(sodium sulfate) 23-palmitate; 24ε,25-dimethyl-5α-cholestane-2β,3α,6α-triol 3α-(sodium sulfate); 24ε,25-dimethyl-5α-cholestane-2β,3α,6α-triol 6α-(sodium sulfate); and 24ε,25-dimethyl-5α-cholestane-2β,3α,6α-triol 2β,6α-di(sodium sulfate). The inhibiting and membranolytic properties of the polysulfated steroids from sponges and their derivatives have been studied. It has been shown that physiological activity in this series of compounds depends on biphilicity.

Sulfated steroid polyols from sponges of the family Halichondriidae – halistanol sulfate (1) [1] and sokotrasterol sulfate (2) [2] – possess cytotoxic properties and disturb membrane permeability.

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TABLE 1. Chemical Shfits in the $^1H$ NMR Spectra of Compounds (1, 5, 8) ($C_5D_5N$)

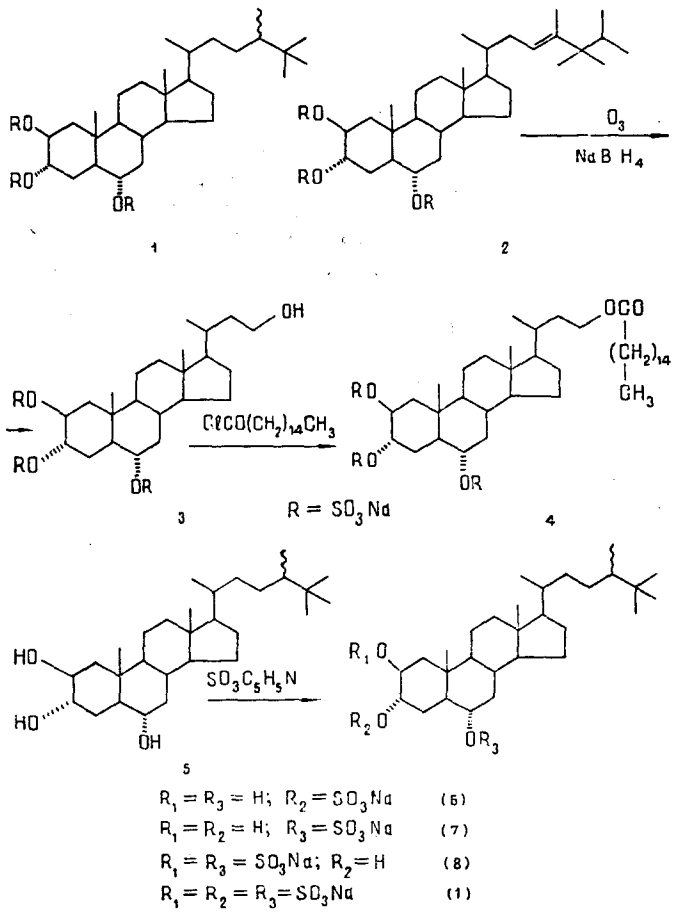
| Comp. | Proton | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|
| | 1e | 1a | 2e | 3e | 4e | 4a | 5a | 6a | 7e | 7a |
| 1 | 2,23 | 1,84 | 5,62* | 5,92* | 3,21* | 2,40* | 2,05 | 4,78 | 2,78 | |
| 5 | 2,15 | 2,00 | 4,58 | 4,62 | 2,90 | 2,41 | 2,33 | 3,94 | 2,35 | 1,34 |
| 6 | 2,05 | 1,77 | 4,97 | 5,59 | 3,27 | 2,38 | 2,12 | 3,86 | 2,29 | 1,20 |
| 7 | 2,14 | 2,00 | 4,53 | 4,56 | 3,10 | 2,50 | 2,67 | 5,06 | 3,02 | |
| 8 | 2,51 | 1,93 | 5,45 | 4,94 | 2,96 | 2,48 | 2,40 | 4,90 | 2,90 | 1,31 |

| Comp. | $CH_3$ group | | | | |
|---|---|---|---|---|---|
| | $CH_3$-18 s, 3H | $CH_3$-19 s, 3H | $CH_3$-21 d, 3H, 6,8 Hz | $CH_3$-28 d, 3H, 6,5 Hz | $CH_3$-26, 27, 29 s, 9H |
| 1 | 0,49 | 1,13 | 0,93 | 0,85 | 0,87 |
| 5 | 0,70 | 1,51 | 0,99 | 0,87 | 0,89 |
| 6 | 0,66 | 1,41 | 1,00 | 0,87 | 0,89 |
| 7 | 0,60 | 1,46 | 0,95 | 0,86 | 0,88 |
| 8 | 0,52 | 1,28 | 0,93 | 0,85 | 0,87 |

*Assignment of the signals ambiguous.

In order to find with what structure features the membranolytic properties of these compounds are connected, we have obtained a number of derivatives and have studied their physiological activities, comparing them with the activities of natural compounds.

The sulfated polyol (2) was ozonized and, after reductive treatment, the tetraol trisulfate (3) was obtained which lacked the hydrophobic side chain of sokotrasterol sulfate. The acylation of (3) with palmitoyl chloride gave the ester (4) having a hydrophobic side chain similar to that of the initial metabolite (2).




$R_1 = R_3 = H;\ R_2 = SO_3Na$ (6)

$R_1 = R_2 = H;\ R_3 = SO_3Na$ (7)

$R_1 = R_3 = SO_3Na;\ R_2 = H$ (8)

$R_1 = R_2 = R_3 = SO_3Na$ (1)

TABLE 2. Physiological Activity of the Sulfated Polyhydroxy Steroids

| Activity | Compound | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|
| | 1 | 2 | 3 | 4 | 6 | 7 | 8 | 9† | 10‡ |
| $T_{50}$, μM 5 μg of enzyme*/ml 100% efflux of potassium, μg/ml | 9 | 18 | 100 | 32 | — | 55 | — | — | — |
| | 1,5 | 5,0 | >25 | 1,25 | 5,0 | 10 | 1,0 | 1,0 | >20 |

*Enzyme – $Na^+,K^+$-ATPase, $I_{50}$ – 50% inhibition.
†24ε,25-Dimethyl-5-cholesten-2β,3α-diol 2β,3α-di(ammonium sulfate) was obtained as described in [6].
‡5-cholesten-3β-ol 3β-(sodium sulfate) was obtained as in [7].

Derivatives of halistanol sulfate with different numbers and positions of O-sulfate groups in the steroid nucleus (6-8) were obtained by treating halistanol (5) [2] with pyridine/sulfur trioxide in pyridine.

It is known that a sulfate group shifts the signals of equatorial protons in the β position downfield by approximately 0.5 ppm, and for α protons such a shift is usually even more considerable. This made it possible to determine the numbers and positions of the O-sulfate groups in compounds (6-8) with the aid of high-resolution $^1H$ NMR spectroscopy (Table 1).

We studied the inhibiting effect of the substances obtained in relation to $Na^+,K^+$-ATPase (ATP phosphohydrolase, EC 3.6.1.3) – an enzyme performing the active transport of univalent cations through biomembranes that is also capable of causing the passive efflux of potassium ions from cells through a disturbance of the integrity of the membrane. The enzyme was isolated from rat brain by the method of Kodos et al. [4], and to determine the concentrations of potassium ions in the intercellular medium a potassium-selective electrode and a 0.05% suspension of erythrocytes were used as test system [5].

As can be seen from Table 2, the greatest inhibiting effect on the active transport of cations was possessed by the natural metabolites (1) and (2). A decrease in biphilicity due to the oxidation of the side chain (in compound (3)) led to a sharp fall in inhibiting properties. The restoration of biphilicity (compound 4) again led to a highly active derivative. Similar results were obtained in a study of the effect on the efflux of potassium from cells. In this case, derivative (4) proved to be even more active than (1) and (2). Some decrease in polarity in the polycyclic moiety through desulfation likewise had a slight effect on the membranolytic properties. With respect to this activity, the monosulfate (7) and the disulfate (8) were not inferior to the natural metabolites (1) and (2). At the same time, cholesterol sulfate (10) containing only one sulfate group and lacking a hydroxy group, does not possess such properties.

Thus, for the manifestation of a physiological action in this series of compounds the optimum biphilicity created by polar substituents in the polycyclic nucleus and by the presence of a hydrophobic side chain is of great importance. The presence of one sulfate and two hydroxy groups in rings A and B is sufficient for the steroid derivatives to manifest strong membranolytic properties.

## EXPERIMENTAL

Melting points were measured on a Boëtius stage, and optical rotations ($[\alpha]_D$) on a Perkin-Elmer 141 polarimeter.

$^1H$ NMR spectra were determined on a Bruker WM-250 instrument in deuteropyridine with tetramethylsilane as internal standard. The chromato-mass-spectrometric study was performed on a LKB 9000S spectrometer at an ionizing voltage of 70 V. Halistanol and sokotrasterol sulfates were isolated as described in [2].

24-Nor-5α-cholane-2β,3α,6α,23-tetraol 2β,3α,6α-tri(sodium sulfate) (3). At −30°C, ozone was passed through a solution of 100 mg of sokotrasterol sulfate (2) in 2 ml of $H_2O$ and 8 ml of MeOH for 2 h, and then the temperature of the solution was brought to that of the room and it was treated with $NaBH_4$. The passage of ozone and the addition of $NaBH_4$ were repeated several times, until the initial sulfate (2) had disappeared (TLC analysis on silanized silica gel in 60% aqueous ethanol). The total time of ozonolysis was 12 h. Then the reaction mixture was acidified with AcOH to neutrality, the solution was evaporated to dryness several times with the addition of methanol, and the residue was dissolved in a small volume of water and was chromatographed on a column of Polikhrom-1 (water–50% ethanol). The aqueous ethanolic eluates were evaporated, and then part of the substance (79 mg) was chromatographed on a column of silica gel in the $CHCl_3$–$C_2H_5$–$H_2O$ (30:20:1) → (20:40:1) system. This gave 19.4 mg of substance (3) [22% on the initial (2)], mp 220-222°C (from ethanol, $[\alpha]_D^{20}$ +30° (c 0.07; ethanol). $C_{23}H_{37}O_{13}S_3Na_3$. Mass spectrum (m/z, %): 326 (21, $M^+ - 3NaHSO_4$); 308 (100, $M^+ - 3NaHSO_4 - H_2O$); 251 (77), 211 (92). $^1H$ NMR spectrum ($C_5D_5N$, δ); 0.71 ($CH_3$-18, s, 3H); 1.06 ($CH_3$-19, s, 3H); 0.95 ($CH_3$–21, d, J = 6.8 Hz, 3H); 5.64 (C-2, m, 1H); 5.92 (C-3, m, 1H); 4.78 (C-6, td, 1H); 3.92 (C-23, m, 2H).

24-Nor-5α-cholane-2β,3α,6α,23-tetraol 2β,3α,6α-tri(sodium sulfate) 23-Palmitate (4). A solution of 11.3 mg of (3) in 5 ml of dry pyridine was evaporated in vacuum to a volume of 2 ml, and 0.5 ml of a 10% solution of palmitoyl chloride in dry benzene was added. The mixture was stirred at room temperature for 3 days (until the spot of the initial compound had disappeared on TLC). The reaction mixture was diluted with water and the excess of palmitic acid was extracted with chloroform. The aqueous solution after evaporation was evaporated and chromatographed on a column of Polychrome-1 with elution by aqueous ethanol. The eluate was concentrated to an aqueous residue which was treated with the resin Amberlite CG-120 in the $Na^+$ form and was then evaporated to dryness. Part of the substance (9 mg) was chromatographed on preparative plates coated with silica gel in the $CHCl_3$–$C_2H_5OH$–$H_2O$ (10:10:1) system. This gave 3.5 g of an amorphous substance [23% on the initial substance (3)] which decomposed without melting above 240°C, $[\alpha]_D^{20}$ +10° (c 0.08, ethanol). $C_{39}H_{67}O_{14}S_3Na_3$. Mass spectrum (m/z, %): 564 (100, $M^+ - 3NaHSO_4$); 326 [24, $M^+ - 3NaHSO_4 - 238$); 308 (83, $M^+ - 3NaHSO_4 - 238 - 30$); 251 (52); 211 (93). $^1H$ NMR spectrum ($C_5D_5N$, δ): 0.71 ($CH_3$-18, s, 3H); 1.04 ($CH_3$-19, s, 3H); 0.98 ($CH_3$-21, d, J = 6.8 Hz, 3H); 2.3 ($-CH_2-C=O$, t, J = 6.8 Hz, 3H); 1.4 ($-(CH_2)_{13}-$, s, 26H); 0.90 ($-CH_3$, t, J = 6.8 Hz, 3H); 5.76 (C-2, M, 1H); 5.88 (C-3, M, 1H); 4.81 (C-6, td, 1H); 4.27 (C-23, m, 2h).

Sulfation of Halistanol (5). A solution of 400 mg of (5) in 10 ml of dry pyridine was treated with 456 mg of $SO_3 \cdot C_5H_5N$, and the mixture was stirred at room temperature for 24 h and was left to stand for 10 days, after which hexane was added for the more complete precipitation of the product. The solvent was decanted off (300 mg of the initial (5) was isolated from it by column chromatography). The precipitate obtained was dissolved in a small amount of water and the solution was neutralized with a 10% aqueous solution of NaOH. According to TLC, four compounds were formed. The mixture of substances obtained was chromatographed repeatedly on columns of silica gel in the $CHCl_3$–$C_2H_5$–$H_2O$ (20:5:0.3) → (20:20:0.6) systems. The following compounds were obtained:

1. 24ε,25-Dimethyl-5α cholestane-2β,3α,6α-triol-3α-(sodium sulfate) (6). Yield 8 mg (2% on the initial (5)), mp 239-241°C (from ethanol), $[\alpha]_D^{20}$ +31.3° (c 0.16; pyridine). $C_{29}H_{49}O_6SNa$. $R_f$ 0.59 (silica gel, $CHCl_3$–$C_5H_5OH$–$H_2O$ (3:2:0.2)). Mass spectrum (m/z, %): 430 (1, $M^+ - NaSO_4$); 412 (32, $M^+ - NaHSO_4 - H_2O$); 394 (100, $M^+ - NaHSO_4 - 2H_2O$); 379 (13); 275 (14); 253 (13); 286 (14); 211 (100). Details of the $^1H$ NMR spectrum are given in Table 1.

2. 24ε,25-Dimethyl-5α-cholestane-2β,3α,6α-triol-6α-(sodium sulfate) (7). Yield 38 mg (8%), mp 245-247°C (from ethanol) $[\alpha]_D^{20}$ +25° (c 0.14; pyridine); $C_{29}H_{49}O_6SNa$; $R_f$ 0.57. Mass spectrum (m/z, %): 430 (1, $M^+ - 2H_2O$); 379 (12); 275 (12); 253 (13); 226 (10); 211 (80). Details of the $^1H$ NMR spectrum are given in Table 1. $^{13}C$ NMR spectrum: ($C_5D_5N$, δ): 40.4 (C-L); 70.7 (C-2); 71.4 (C-3); 27.0 (C-4); 45.2 (C-5); 77.3 (C-6); 40.7 (C-7); 34.3 (C-8); 55.2 (C-9), 37.7 (C-10); 21.3 (C-11); 40.4 (C-12); 43.0 (C-13); 56.7 (C-14); 24.5 (C-15); 28.9 (C-16); 56.7 (C-17); 12.4 (C-18); 15.9 (C-19); 36.8 (C-20); 19.3 (C-21); 35.8 (C-22); 28.5 (C-23); 44.5 (C-24); 33.3 (C-25); 27.5 (C-26); 27.5 (C-27); 14.9 (C-28); 27.5 (C-29).

3. 24ε,25-Dimethyl-5α-cholestane-2β,3α,6α-triol-2β,6α-di(sodium suflate) (8). Yield 11 mg (2%); mp 225-227°C (from ethanol), $[\alpha]_D^{20}$ +19° (c 0.1; pyridine; $C_{19}H_{50}O_9S_2Na_2$; $R_f$ 0.41. Mass spectrum (m/z, %): 394 (75, $M^+ - 2NaHSO_4 - H_2O$); 379 (25); 275 (15); 253 (25); 226 (35); 211 (100). For details of the $^1H$ NMR spectrum, see Table 1.

4. 24ξ,25-Dimethyl-5α-cholestane-2β,3α,6α-triol-2β,3α,6α-tri(sodium sulfate) (I). Yield 3.5 mg (0.5%), $R_f$ 0.27. $^1$H NMR spectrum ($CD_3OD$, δ): 0.49 ($CH_3$-18, s, 3H); 1.13 ($CH_3$-19, s, 3H); 0.93 ($CH_3$-21, d, J = 6.8 Hz, 3H); 0.85 ($CH_3$-28, d, J = 6.8 Hz, 3H); 0.87 ($CH_3$-26, -27, -29; s, 9H); 4.78 (C-6, td, 1H); 5.64 (C-2, m, 1H); 5.89 (C-3, m, 1H).


## SUMMARY

The inhibiting and membranolytic properties of polysulfated steroids from sponges and of derivatives obtained from them have been studied. It has been shown that in this series of compounds physiological acivity depends on biphilicity.


## LITERATURE CITED


1. N. Fusetani, S. Matsunaga, and S. Konosu, Tetrahedron Lett., 22, 1985 (1981).
2. T. N. Makarieva [Makar'eva], L. K. Shubina, A. I. Kalinovsky, V. A. Stonik, and G. B. Elyakov, Steroids, 42, 267 (1983).
3. A. A. Kicha, A. I. Kalinovskii, and E. V. Levina, Khim. Prir. Soedin., 738 (1984).
4. I. Klodos, P. Ottolenghi, and A. Boldyrev, Anal. Biochem., 67, 397 (1975).
5. A. S. Ivanov, V. V. Petrov, and V. F. Antionov, Biofizika, 23, 253 (1978).
6. T. N. Makar'eva, L. K. Shubina, A. I. Kalinovskii, and V. A. Stonik, Khim. Prir. Soedin., 272 (1985).
7. A. E. Sobel, P. E. Spaerri, J. Am. Chem. Soc., 63, 1259 (1941).


# GLYCOSYLATION OF TRITERPENOIDS OF THE DAMMARANE SERIES. V. β-D-GLUCOPYRANOSIDES OF 12β-ACETOXY-20(S),24(R)-EPOXYDAMMARANE-3α,25-DIOL AND OF 3-EPIOCOTILLOL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UDC 547.917+547.918+547.597



The synthesis of glucosides from the 12-O-acetyl derivatives of betulafolienetriol oxide and of 3-epiocotillol has been carried out under the conditions of the Koenigs-Knorr reaction and of Helferich's modification. It has been established that glycosylation in the presence of silver zeolite and mercury cyanide takes place nonregioselectively and gives a mixture of the corresponding 3-O- and 25-O-mono- and 3,25-di-O-β-D-glucopyranosides. The structures of all the newly obtained glucosides have been established on the basis of IR and $^{13}$C NMR spectroscopy.


The main component of the unsaponifiable fraction of extracts of the leaves of Betula nana L. and B. exilis Sukacz [1] and 3-epiocotillol [20(S),24(R)-epoxydammarane-3α,25-diol (1)], which, as compared with betulafolienetriol oxide (2), lacks a hydroxy group at $C^{12}$. As reported previously, a decisive influence on the regiochemistry of the glycosylation of the triol (2) and its 3-epimer is exerted by a strong intramolecular hydrogen bond (intraHB) between the proton of the 12β-OH group and the oxygen atom of the tetrahydrofuran ring [2]. A study of the glycosylation of the diol (1) is therefore of interest not only because it is the main component of extracts of these birches but also because it is a compound in which there is no strong intraHB and, consequently, no factor which could appreciably raise the nucleophilicity of the oxygen atom of one of the hydroxy groups.

In the same connection we have studied the glycosylation of the 12-O-acetyl derivative of betulafolienetriol (3), one of the components of extracts of the leaves of B. platyphilla


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