Tetrahedron Letters, Vol.22, No.21, pp 1985 - 1988, 1981
Printed in Great Britain

0040-4039/81/211985-04$02.00/0

BIOACTIVE MARINE METABOLITES II.[1] HALISTANOL SULFATE, AN ANTIMICROBIAL NOVEL STEROID SULFATE FROM THE MARINE SPONGE *HALICHONDRIA* CF. *MOOREI* BERGQUIST

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Abstract: A new C_{29} steroid sulfate, 24ξ,25-dimethylcholestane-2β,3α,6α-triyl trisodium sulfate has been isolated from the Okinawan sponge *Halichondria* cf. *moorei* Bergquist as an antimicrobial constituent.

Marine sponges have been extensively studied in a search for useful drugs from the sea, and a variety of bioactive compounds have been isolated.[2-5] Our screening[6] of marine invertebrates from Japanese waters for antimicrobial substances showed that the methanol extract of the Okinawan sponge *Halichondria* cf. *moorei* Bergquist inhibited the growth of fungi, Gram-positive and Gram-negative bacteria. We have isolated from this sponge a water soluble active compound which is a sulfate ester of a novel polyhydroxylated steroid, 24ξ,25-dimethylcholestane-2β,3α,6α-triol.

The water soluble portion of the aqueous ethanol extract of the frozen sponge (500 g) collected at Ishigaki Island of the Ryukyus, Japan, was chromatographed successively on TSK G3000S, silica gel and Sephadex LH-20. Recrystallization of the active fraction from EtOH-H_2O yielded 300 mg of halistanol sulfate(1) as colorless needles, mp 159.5-160.5°; $[\alpha]_D$+17°. Besides the antimicrobial activity, the sulfate showed hemolytic and ichthyotoxic activity. Compound 1 contained three sulfate groups, which was deduced by a strong IR absorption[7] at 1250 cm^{-1}, sodium rhodizonate test[8] and colorimetric determination.[9] Atomic absorption analysis demonstrated that all three sulfates were present as sodium salts. Combustion analysis, FD and EI mass spectral data suggested a molecular formula of $C_{29}H_{52}O_{12}S_3Na_3$. The ^{13}C NMR spectrum supported this formula, which revealed 29 carbon signals including three oxygen-bearing carbons (Table 1). EI mass spectrum(m/z 412, 394, 392, 379, 275, 253, 229, 211) and ^1H NMR spectrum [(100 MHz, CD_3OD) 0.70(s,3H), 0.86(s,9H), 1.06(s,3H), 4.13(br m,1H), 4.74(m,2H)] indicated the presence of a trioxygenated steroid moiety.

Acid hydrolysis of 1 afforded halistanol(2) as colorless plates, mp 248-249°; $[\alpha]_D$ +37°, which no longer showed antimicrobial activity. The high resolution mass spectrum established a molecular formula of $C_{29}H_{52}O_3$(m/z 448.3917; calcd for 448.3914), which was supported by combustion analysis. The mass spectrum also indicated a trihydroxylated steroid skeleton as well as the presence of a saturated C_{10} side chain(e.g., peak at m/z 253 for loss of side chain + 3 H_2O, as well as ring D fission peak at 211).[10] Halistanol was easily converted to the corresponding triacetate 3 [m/z 514(M^+-AcOH); ν_{max} 1740, 1240 cm^{-1}; ^1H NMR ($CDCl_3$) 1.95(s,6H), 2.00(s,3H), 4.50(br m,1H), 4.72(m,2H); ^{13}C NMR (Table 1)].

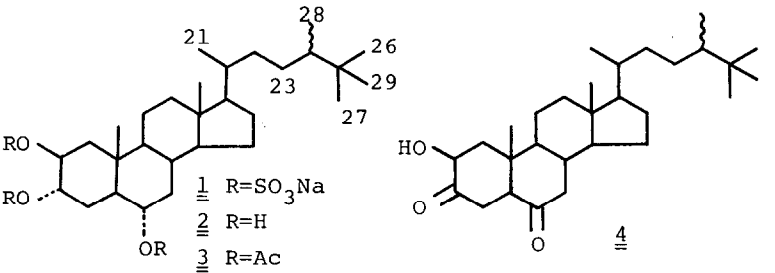

Table 1. ^{13}C NMR data of Compounds 1, 2, 3 and 4

C	1	2	2(calcd)	3	4
1	40.1	40.4 d	40.3	38.0 h	47.9
2	75.5 a	69.3 e	71.7	69.2 i	72.0
3	75.5 a	69.9 e	70.6	69.7 i	211.0 n
4	25.1	25.3	25.5	23.8 j	35.2
5	45.4	45.8	45.9	44.1 k	58.7
6	78.8 a	70.4 e	70.0	72.2 i	208.1 n
7	39.2	41.6	41.7	37.7 h	46.5
8	35.2	33.9	34.3	33.9	37.6
9	55.9	54.8	54.9	54.6	53.6
10	37.7	36.6	36.6	36.6	42.8
11	21.9	20.9	20.9	20.9	21.9
12	41.2	40.0 d	40.2	39.9	39.3
13	43.8	42.7	42.6	42.8	43.1
14	57.4 b	56.2 f	56.7	56.3	56.5
15	25.1	24.3	24.2	24.1 j	24.0
16	29.2	28.2	28.3	28.5 l	28.0 o
17	57.7 b	56.3 f	56.4	56.3	55.9
18	12.5	12.1	12.2	12.1	12.1
19	15.3 c	15.2 g	14.7	14.8 m	13.9
20	37.7	36.6		36.5	36.3
21	19.5	19.1		19.1	19.0
22	36.6	35.5		35.5	35.3
23	29.2	28.2		28.1 l	28.1 o
24	45.4	44.1		44.2 k	44.1
25	34.1	33.3		33.3	33.3
26	27.8	27.4		27.4	27.4
27	27.8	27.4		27.4	27.4
28	15.0 c	14.6 g		14.7 m	14.6
29	27.8	27.4		27.4	27.4
OAc				21.1(3C)	
				169.4(2C)	
				170.7	

* The values are in ppm down field relative to TMS. The solvents: 1, CD_3OD; 2, $CDCl_3$-CD_3OD(5:1); 3 and 4, $CDCl_3$

a-o Assignments may be interchanged.

The ^{13}C NMR spectrum of halistanol(2) [25 MHz, $CDCl_3$-CD_3OD(5:1)] exhibited five methyl signals including a three-carbon quartet at 27.4 (Table 1). This was also observed in the 400 MHz ^1H NMR spectrum ($CDCl_3$). The methyl groups at C-18 and 19 were assignable to the signals at 0.65(s) and 1.01(s), respectively. The three proton doublet at 0.93(J=7Hz) was attributed to the C-21 methyl, suggesting the 20S configuration.[11] When recorded in pyridine-d_5 the C-19 methyl signal shifted to 1.50, indicating the proximity of a hydroxyl function. Since neither isopropyl nor ethyl groups were recognized in the spectrum, a t-butyl moiety(0.85,s,9H) must be placed at the end of the side chain. The presence of a t-butyl group was also confirmed by the ^{13}C NMR spectrum(27.4,q,3C) and IR spectrum(1375,1360 cm^{-1}). The position of the remaining methyl group(0.80,d,J=7Hz) was determined by calculating the ^{13}C NMR chemical shifts for the side chain[12]; the result was consistent with a 24,25-dimethylated side chain. Furthermore, the observed carbon chemical shift values for the side chain of halistanol(2) coincided well with those of 24ξ-methylcholestane-1β,3β,5α,6β-tetrol.[13]

The position and configuration of three hydroxyl groups were deduced from the 400 MHz ^1H NMR study of triol 2 in pyridine-d_5, including double resonance experiments. There were three carbinol methine signals: 3.93(ddd,J=4,11,11Hz,6β-H), 4.57(narrow m,2α-H), 4.61(narrow m,3β-H). Irradiation of the 6β-H at δ 3.93 collapsed not only the C-7 geminal protons at 1.37(q,J=11Hz) and 1.39(br d,J=11Hz) to a triplet and a sharper doublet, respectively, but also the C-5α proton at 2.34(br t,J=11Hz) to a broad doublet. Simultaneous irradiation of the two carbinol methines at δ 4.57 and 4.61 sharpened the C-1 geminal protons at 2.02(dd,J=3,13Hz) and 2.16(br d,J=13Hz) as well as the C-4 geminal protons at 2.42(ddd,J=3,11,13Hz) and 2.90(br d,J=13Hz). Irradiation of the C-4 equatorial proton at δ 2.90 collapsed the C-5α proton at 2.34 to a broad doublet and the C-4 axial proton at 2.42 to a double doublet. Thus the 2β,3α,6α-trihydroxyl feature was fully established. This structure was further confirmed by the ^{13}C NMR spectrum of compound 2 (Table 1). When the substituent effect of hydroxyl groups on the ^{13}C NMR signals of sterols was applied as reported by Djerassi *et al.*,[14,15] the calculated values for this structure were in good agreement with the observed ones (Table 1).

Further support for structure 2 was provided by Sarett oxidation[16] of halistanol(2), which gave a diketone alcohol 4 [M^+ m/z 444; ν_{max} 3500,1720 cm^{-1}; ^1H NMR (400 MHz,$CDCl_3$) 0.69(s,3H), 0.80(d,J=7Hz,3H), 0.84(s,9H), 0.93(d,J=7Hz,3H), 1.04(s,3H), 4.26(dd,J=7,12Hz,1H); ^{13}C NMR (Table 1)], and by a ^{13}C NMR study of compound 4 using the lanthanide shift reagent ($Yb(fod)_3$).

Various steroids with unusual side chains or unconventional ring systems have been obtained from marine organisms.[17-19] Particularly polyhydroxylated sterols have recently been isolated from gorgonians[17] and soft corals.[12,20,21] However, both the side chain and the hydroxylation pattern of halistanol(2) are hitherto unknown, and biosynthetically intriguing since halistanol possesses the unusual 2β,3α,6α-trihydroxyl functions. An even more unusual feature is the t-butyl moiety at the end of the side chain. Halistanol(2) is the first example of a C-25 methylated naturally occurring sterol.

Acknowledgement: We thank Professor Paul J. Scheuer of the University of Hawaii for his reading this manuscript. We are also grateful to Dr. T. Hoshino of the Mukaishima Marine

Biological Station of Hiroshima University, for the identification of the sponge, to Mr. K. Furihata of the Institute of Applied Microbiology of this university for the measurement of 400 MHz ^{1}H NMR spectra, and to the staff of the Institute of Research and Development of Yamanouchi Pharmaceutical Co., Ltd., for the high resolution and FD mass spectral and combustion analyses. This work was partly supported by a Grant-in-Aid for Scientific Research from the Ministry of Education, Science and Culture of Japan.

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(Received in Japan 27 January 1981)