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John A. Findlay, Nurettin Yayli, and Larry A. Calhoun

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NOVEL SULFATED HYDROCARBONS FROM THE SEA CUCUMBER *CUCUMARIA FRONDOSA*

JOHN A. FINDLAY,* NURETTIN YAYLI, and LARRY A. CALHOUN

Department of Chemistry, University of New Brunswick, Fredericton, New Brunswick, Canada E3B 6E2

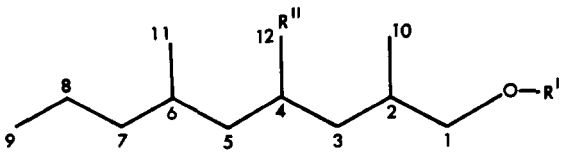
ABSTRACT.—2,6-Dimethylnonane-1-sodium sulfate and 2,4,6-trimethylnonane-1-sodium sulfate have been isolated from the sea cucumber *Cucumaria frondosa*. Structure determination by spectroscopic means was facilitated by ^{13}C chemical shift correlation tables.

Recently we reported (1) the isolation of representatives of two new classes of marine natural products from the starfish *Asterias forbesi*. One of these was the disodium salt of eicosane-1,16-disulfate. In our continuing investigations of the polar extracts of echinoderms we have now encountered similar sulfated hydrocarbons in the sea cucumber *Cucumaria frondosa* Gunnerus (Class Holothuroidea).

An MeOH extract of whole *C. frondosa* animals afforded, after Amberlite XAD-2 and Kieselgel chromatographies, a mixture of sulfated hydrocarbons. The ^{13}C-nmr spectrum of this mixture showed 22 major signals and several signals of much lower intensity attributed to minor components. Further chromatographic purification permitted the isolation of compound **1**, whose ^{13}C spectrum and DEPT analysis showed 11 signals comprising three methyls, five methylenes, and two methines in the high field region and a solitary methylene signal at δ 72.6 ppm. The high polarity of this compound, together with the particular chemical shift of the low field ^{13}C signal, suggested the presence of an Na sulfate group (1). This idea was also consistent with the presence in the ^1H nmr of a multiplet (2H) at δ 4.2 ppm (1). The ir spectrum (KBr) displayed bands at 1220, 1210, and 1110 cm^{-1} attributable to the sulfate moiety (2). The negative fabms displayed a prominent ion at *m/z* 251 (30) corresponding to $C_{11}H_{23}SO_4$, while the positive fabms showed a pseudomolecular ion at *m/z* 297 (76) ($C_{11}H_{23}SO_4Na$ + Na).

In the absence of distinctive fragmentation patterns in the mass spectra, it became necessary to find other means for distinguishing the structure **1** from various isomeric formulations, given the limitations of the DEPT analysis. We have therefore explored the use of ^{13}C chemical shift correlation tables (3) to arrive at a unique structure. Table 1 compares the observed and calculated ^{13}C chemical shifts for **1**, and it is clear that excellent agreement exists and that alternate structures can be excluded on this basis.

Initially the second major sulfated hydrocarbon **2** was not isolated in pure form, but was obtained in admixture with **1**. By subtraction of the ^{13}C chemical shifts due to **1** from those of the mix-



1 R′=SO_3Na, R″=H
2 R′=SO_3Na, R″=Me
3 R′=R″=H
4 R′=H, R″=Me

TABLE 1. ^{13}C Chemical Shifts (δ ppm) for Compounds 1 and 2.

Carbon	Compound							
	1				2			
	obs[a]	mult[b]	calcd[c]	Δ	obs[a]	mult[b]	calcd[c]	Δ
C-1	72.6	CH_2	74.2[d]	1.6	72.9	CH_2	74.2[d]	1.3
C-2	32.6	CH	31.0[d]	−1.6	29.7	CH	28.5[d]	−1.2
C-3	33.9	CH_2	32.9	−1.0	41.5	CH_2	39.8	−1.7
C-4	24.5	CH_2	24.7	0.2	27.3	CH	26.4	−0.9
C-5	37.4	CH_2	36.6	−0.8	44.9	CH_2	43.5	−1.4
C-6	33.6	CH	31.4	−2.2	30.8	CH	28.9	−1.9
C-7	39.6	CH_2	39.1	−0.5	38.8	CH_2	39.1	0.3
C-8	20.1	CH_2	20.3	0.2	20.0	CH_2	20.3	0.3
C-9	14.5	Me	13.7	−0.8	14.5	Me	13.7	−0.8
C-10	17.0	Me	15.8	−1.2	17.9	Me	15.8	−2.1
C-11	19.6	Me	19.5	−0.1	20.6	Me	19.5	1.1
C-12					21.0	Me	19.5	−1.5

[a]In pyridine-d_5.
[b]From DEPT analysis.
[c]Calculated using ^{13}C-nmr correlation tables by Brown (3).
[d]Calculated values based on substituent increments derived from observed ^{13}C chemical shifts for eicosane-1,16-disodium sulfate (1).

tures of **1** and **2**, the signals attributable to **2** could be derived without ambiguity. Repeated Si gel chromatography eventually afforded pure **2**. Table 1 shows the observed and calculated ^{13}C chemical shifts for **2**. Once again, it is apparent from the excellent agreement between observed and calculated ^{13}C chemical shifts that other formulations are excluded. Corroboration of formulation **2** is available from the negative fabms, which shows a prominent pseudomolecular ion at *m/z* 265 $[M - Na]^-$ ($C_{12}H_{25}SO_4Na - Na$).

Solvolysis of the mixture of **1** and **2** with pyridine-dioxane (1:4) gave the corresponding mixture of alcohols **3** and **4** which displayed the anticipated upfield shift (δ 3.64 ppm) for H-1. The mass spectrum displayed molecular ions *m/z* 172 and 186 for **3** and **4**, respectively.

The stereochemistry of **1** and **2** is unknown. The sulfate group is a common feature of echinoderm metabolites such as saponins, sterol glycosides, and sterol sulfates. It has also been encountered in fish metabolites (4) and from certain Pacific ophiuroids (5) in the form of sulfated sterols. Aromatic sulfates have been isolated from sponges (6). Choline sulfate has been isolated (7,8) from marine sources, but we are not aware of any other reports of sulfated hydrocarbons from marine organisms.

EXPERIMENTAL

GENERAL EXPERIMENTAL PROCEDURES.—Nmr spectra were recorded on a Varian XL-200 instrument using TMS as internal standard. Mass spectra were recorded with a Kratos MS50 instrument.

Whole specimens (30.4 kg, wet wt) of mature *C. frondosa* collected in Passamaquoddy Bay, New Brunswick, Canada in October 1988 were frozen until use. Voucher specimens are preserved at the Chemistry Department, University of New Brunswick. The animals were thawed, chopped into small pieces, placed in vats, and extracted four times with MeOH (6 liters) for 24 h. The extract obtained after removal of MeOH in vacuo at 30–35° was introduced onto an Amberlite XAD-2 column, and elution with H_2O was pursued until chloride ion was absent. Elution with MeOH (4 liters) gave, after evaporation, a crude glycoside-containing mixture (17 g) which was treated with Me_2CO (200 ml). The Me_2CO-insoluble portion was filtered off, air-dried (12 g),

and chromatographed on a Kieselgel 60 (360 g, 230–400 mesh) column with discontinuous gradient of CH_2Cl_2-MeOH-H_2O (6.5:3.8:1.1→5:5:1) to give 32 fractions (ca. 100 ml each). Evaporation of fractions 10–14 gave an amorphous material (65 mg) showing R_f 0.7–0.8 on Si gel tlc using CH_2Cl_2-MeOH-H_2O (6.5:3.8:1.1). This material was further purified on a column of Kieselgel 60 (15 g, 230–400 mesh) eluting with discontinuous gradient of CH_2Cl_2-MeOH-H_2O (7:3:1→5:3:1) to give 11 fractions. Fractions 1–7 were combined, and on partial evaporation compound **1** (12 mg, amorphous) separated and was filtered off. The filtrate after evaporation gave a mixture of **1** and **2** (21 mg). Fractions 8–11 were combined and evaporated (32 mg) and further purified on a column of Kieselgel 60 (10 g, 230–400 mesh) eluting with discontinuous gradient of CH_2Cl_2-MeOH-H_2O (8:3:1→5:4:1) to give 13 fractions. Fractions 8 and 9 were combined after tlc scrutiny to provide compound **2** (15.2 mg).

2,6-DIMETHYLNONANE-1-SODIUM SULFATE [**1**].—Amorphous; mp 180–190° dec; $[\alpha]_D$ +2.3° (c = 0.001, $CHCl_3$-MeOH (1:1)]; ir ν max (KBr) 2950, 2930, 1240, 1220, 1120 cm^{-1}; ^1H nmr (200 MHz, pyridine-d_5) δ 4.2 (2H, m, H-1), 1.8 (1H, m, H-2), 0.87 (3H, d, J = 6.7 Hz, H-10), 0.73 (3H, t, J = 6.2 Hz, H-9), 0.64 (3H, d, J = 6.3 Hz, H-11) ppm; positive fabms (magic bullet) m/z $[M + Na]^+$ 297 (76), $[M - HSO_4]^+$ 177 (100), 159 (36), 143 (71); negative fabms (Cleland) m/z $[M - Na]^-$ 251 (30).

2,4,6-TRIMETHYLNONANE-1-SODIUM SULFATE [**2**].—Amorphous; mp 180–190° dec; $[\alpha]_D$ −0.6° [c = 0.015, $CHCl_3$-MeOH (1:1)]; ^1H nmr [200 MHz, pyridine-d_5–D_2O (5:1)] δ 4.4 (2H, m, H-1), 2.0 (1H, m, H-2) ppm; positive fabms m/z $[M + Na]^+$ 311 (15).

SOLVOLYSIS OF MIXTURE OF **1** AND **2**.—The mixture of **1** and **2** (6:5, 15 mg) in dioxane (4 ml) and pyridine (1 ml) was heated at 120° for 12 h in a stoppered vial. H_2O (10 ml) was added to the cooled solution before extraction with CH_2Cl_2 (3 × 5 ml). The combined extract was washed with H_2O and evaporated in vacuo to give the mixture of alcohols **3** and **4** (8 mg): colorless oil; ^1H nmr (200 MHz, $CDCl_3$) δ 3.7 (H-1); eims m/z $[M]^+$ of **4** 186 (15), $[M]^+$ of **3** 172 (13).

LITERATURE CITED

1. J.A. Findlay, Z.-Q. He, and L.A. Calhoun, *J. Nat. Prod.*, **53**, 1015 (1990).
2. J.R. Dyer, "Application of Absorption Spectroscopy of Organic Compounds," Prentice Hall, New Jersey, 1965, p. 31.
3. D.W. Brown, *J. Chem. Educ.*, **62**, 209 (1985).
4. A.R. Tommar, *Chem. Zool.*, **8**, 595 (1974).
5. B.W. Sullivan, D.J. Faulkner, G.K. Matsumoto, C.H. He, and J. Clardy, *J. Org. Chem.*, **51**, 4568 (1986).
6. M.V. Auria, R. Riccio, L. Minale, S. La Barre, and J. Pusset, *J. Org. Chem.*, **52**, 3947 (1987).
7. B. Lindberg, *Acta Chem. Scand.*, **9**, 1323 (1955).
8. L.S. Cieresko and T.K.B. Karns, in: "Biology and Geology of Coral Reefs," Vol. II: Biology 1. Ed. by O.A. Jones and R. Endean, Academic Press, New York, 1973, p. 183.

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