Isolation of an Unusual 2-Aminoimidazolium Salt of Steroid Trisulfate from a Marine Sponge *Topsentia* sp.

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(Received August 12, 1996)

An unusual salt comprised of three 2-aminoimidazolium cations and halistanol sulfate anion was isolated from a marine sponge *Topsentia* sp. along with known halistanol trisodium salt. Their structures were determined by spectroscopic methods. The number of 2-aminoimidazolium ions in the molecule was estimated from the signal intensity in the ¹H NMR spectrum, and that of sodium ions was from the atomic absorption spectrometry. This is the first report of a unique salt consisted of 2-aminoimidazolium cations in natural source. Both halistanol sulfate salts showed antimicrobial activities against *Flavobacterium marinotypicum* and *Mortierella ramaniana* at 10 µg/disk.

Key words: 2-aminoimidazole, antibacterial activity, antifungal activity, halistanol sulfate, marine sponge, *Topsentia* sp.

Marine sponges of the families Halichondriidae, Adociidae, and Axinellidae often contain steroid sulfates which possess a variety of bioactivities, such as antimicrobial,^{1,2} anti-thrombin,³ HIV-inhibitory,⁴ guanosine diphosphate/G-protein RAS exchange inhibitory,⁵ and tyrosine kinase inhibitory⁶ activities.

During our studies on antimicrobial substances in marine invertebrates,^{7,8} we found that the MeOH extract of a marine sponge *Topsentia* sp. inhibited growth of the bacterium *Flavobacterium marinotypicum* and the fungus *Mortierella ramaniana*. Bioassay-guided isolation afforded a known trisodium salt, halistanol sulfate 1, and an unusual tri-2-aminoimidazolium halistanol sulfate 2 (Fig. 1).

Materials and Methods

Infrared spectrum was measured with a JASCO IR-700 spectrometer. ¹H and ¹³C NMR spectra were recorded on a Bruker ARX-500 NMR spectrometer at 27°C in MeOH-*d*₄ or DMSO-*d*₆. Carbon multiplicities were determined by a DEPT spectrum. Mass spectra were measured on a JEOL SX-102A mass spectrometer. Atomic absorption spectrum was obtained with a Hitachi Z-8100 polarized Zeeman atomic spectrophotometer.

Collection and Extraction

The marine sponge was collected by scuba at a depth of 3 m off Hachijo-jima Island and identified as *Topsentia* sp. (family Halichondriidae) by Dr. Rob van Soest. A voucher specimen (ZMA POR. 11062) was deposited at the Institute for Systematics and Population Biology, University of Amsterdam. The frozen sponge (1.1 kg, wet weight) was extracted with MeOH (1 l × 3). The concentrated aque-

ous residue was successively extracted with Et₂O (1 l × 3) and *n*-BuOH (1 l × 3).

Isolation of Tri-2-aminoimidazolium Halistanol Sulfate

The *n*-BuOH layer (2.90 g) was fractionated by reverse-phase column chromatography on YMC-GEL ODS-A 60-400/230 (Yamamura Chem. Co., Ltd., Kyoto, 2.2 × 30 cm) with 30, 60, and 80% MeOH-H₂O and MeOH (each 300 ml). The second and third fractions were separately purified on Sephadex LH-20 (Pharmacia, 2.2 × 70 cm, MeOH, 2.0 ml/min) followed by reverse-phase column chromatography (1.1 × 25 cm, 25% CH₃CN-H₂O containing 0.01% TFA, 1.0 ml/min) to afford tri-2-aminoimidazolium halistanol sulfate (2, 70.9 mg, 0.025% based on wet weight) and the trisodium salt, halistanol sulfate (1, 67.1 mg, 0.024%), respectively.

Halistanol Sulfate 1: FABMS (negative) see Fig. 2.

Tri-2-aminoimidazolium Halistanol Sulfate 2: IR (film) $\nu_{\max}$ 3300, 3160, 2940, 1680, 1220, and 1060 cm⁻¹. ¹H NMR (DMSO-*d*₆) δ 3.80 (1 H, dt, *J* = 11 and 4 Hz, H-6), 4.26 (1 H, br s, H-3), 4.42 (1 H, br s, H-2), 6.85 (6 H, s, olefin protons of 2-aminoimidazolium units), 7.30 (6 H, br s, NH of 2-aminoimidazolium units), and 11.72 (6 H, br s, NH₂ of 2-aminoimidazolium units). ¹³C NMR (MeOH-*d*₄) δ 12.5 (q), 15.0 (q), 15.4 (q), 19.6 (q), 21.8 (t), 25.1 (2 C, t), 27.8 (3 C, q), 29.1 (t), 29.3 (t), 34.0 (s), 35.1 (d), 36.6 (t), 37.7 (d), 37.7 (s), 39.0 (t), 40.0 (t), 41.1 (t), 43.8 (s), 45.4 (2 C, d), 55.8 (d), 57.3 (d), 57.5 (d), 75.7 (d), 75.6 (d), 78.9 (d), 114.4 (6 C, d), and 148.7 (3 C, s). FABMS (positive, glycerol matrix) *m/z* 84 (M as 2-aminoimidazolium ion)⁺. FABMS (negative) see Fig. 2.

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1 (Halistanol sulfate): $R^+ = Na^+$

Fig. 1. Structures of antimicrobial substances.

Separation of the Steroidal and 2-Aminoimidazole Units of 2

Compound 2 (12.7 mg) was applied onto a cation exchange HPLC column (YMC-Pack IES-AX, 4.6×250 mm, Yamamura Chem. Co., Ltd., Kyoto) and eluted with 50 mM triethylamine-formic acid (pH 5.49) (1.0 ml/min) to afford an 2-aminoimidazole unit (3.0 mg). Then, the steroidal unit (7.3 mg) was eluted with 50% MeOH-H₂O containing 1 M NaCl followed by desalting.

Determination of Antimicrobial Activity

Antimicrobial activity was determined by the paper disk method. Paper disks (thick, 8 mm, Toyo Roshi Kaisha, Ltd., Tokyo) impregnated with each 10 μ g of samples were placed on agar plate containing *F. maritimum* ATCC 19260 or *M. ramaniana*, and the plate was incubated for 24 h at 28°C.

Results

Two antimicrobial compounds 1 and 2 were isolated from the MeOH extract of a marine sponge *Topsentia* sp. Compound 1 was readily identified as halistanol sulfate (Fig. 1) by comparison of spectral data with those in literature,¹⁾ and the number of sodium ions was determined by atomic absorption spectrometry; composition of sodium, potassium, and magnesium ions in 1 was 7.66, 0.39, and 0.02%, respectively, indicating that more than two and half molecules of the cation in 1 consisted of sodium ions.

¹³C NMR data clearly revealed that 2 consisted of a steroidal moiety of halistanol sulfate 1, supported by an intense IR band at 1220 cm⁻¹ and pseudomolecular ion peaks at m/z 731 and 709 in the negative FAB mass spectrum (Fig. 2). The additional ¹³C signals at δ 114.4 (6 C, d) and 148.7 (3 C, s) and ¹H signals at δ 6.85 (6 H, s, olefin protons), 7.30 (6 H, br s, NH), and 11.72 (6 H, br s, NH₂)

Fig. 2. FAB mass spectra (negative mode) of trisodium halistanol sulfate 1 and tri-2-aminoimidazolium halistanol sulfate 2.

1: (glycerol matrix) m/z 731 (M as steroidal part + $2 \times Na$)⁻, 709 ($M + Na + H$)⁻, and 629 ($709 - SO_3$)⁻, 2: (NBA matrix) m/z 853 (M as steroidal part + 2×2 -aminoimidazolium ion)⁻, 792 ($M + 2$ -aminoimidazolium ion + Na)⁻, 770 ($M + 2$ -aminoimidazolium ion + H)⁻, 731 ($M + 2 \times Na$)⁻, 709 ($M + Na + H$)⁻, 629 ($709 - SO_3$)⁻, 607 ($629 - Na + H$)⁻, and 527 ($607 - SO_3$)⁻.

suggested the presence of three moles of 2-aminoimidazole units in 2 (Fig. 1), which was also supported by IR bands at 3300 (N-H stretch), 3160 (N-H stretch), 1680 (N-H bend), and 1060 cm⁻¹ (C-N stretch) and a molecular ion peak at m/z 84, coinciding with 2-aminoimidazolium ion, in positive FAB mass spectrum. The ¹H NMR spectra of the steroidal and 2-aminoimidazole units of 2 which had been separated on an ion exchange column were superimposable on those of 1 and the commercially available 2-aminoimidazole sulfate (Aldrich Chem. Co., Inc.), respectively. Thus, the gross structure of 2 was established (Fig. 1). Interestingly, the negative FAB mass spectra of 1 and 2 implied their ion composition (Fig. 2); 1 showed prominent pseudo- and desulfonated-molecular ion peaks at m/z 731 and 629, respectively, while 2 revealed additional peaks comprising one or two 2-aminoimidazole unit(s) at

m/z 770, 792, and 853.

Compounds 1 and 2 showed the similar antimicrobial activities: 9 and 12 mm of growth inhibitory zones against *F. marinothicum* and *M. ramaniana* at 10 μ g/disk, respectively. Incidentally, 2-aminoimidazole sulfate (Aldrich Chem. Co., Inc.) was inactive.

Discussion

It is noteworthy that tri-2-aminoimidazolium salt 2 and trisodium salt 1 of halistanol sulfate co-occurred in the present marine sponge. Similar examples have been reported: β -carbolinium salts, reticulatines A and B⁹⁾ composed of different anions; and (*E*)- and (*Z*)-narains, *N,N*-dimethylguanidinium styryl sulfates.¹⁰⁾

Steroid sulfates including halistanol sulfate¹⁾ and halistanol sulfates A–H^{3,4)} have been isolated as trisodium salts except for echinocasterol sulfate phenethylammonium salt.²⁾ 2-Aminoimidazole-containing metabolites have been reported from a variety of marine invertebrates.^{11–13)} Tri-2-aminoimidazolium halistanol sulfate 2, however, is the first report of a unique 2-aminoimidazolium salt of natural product.

Acknowledgments We are indebted to Prof. I. Kitagawa of Kinki University for valuable discussions, to Dr. R. van Soest of the University of Amsterdam for identification of the sponge, and to Dr. H. Onuki of Nippon Suisan Kaisha, Ltd. for the measurement of the atomic absorption spectrometry.

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