Notes

Isolation of 1-Methylherbipoline Salts of Halisulfate-1 and of Suvanine as Serine Protease Inhibitors from a Marine Sponge, *Coscinoderma mathewsi*

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Bioassay-guided isolation of serine protease inhibitors from a marine sponge, *Coscinoderma mathewsi*, has yielded 1-methylherbipoline salts of halisulfate-1 and of suvanine. Structures of these compounds were identified by spectroscopic analyses and literature data. Antithrombin and antitrypsin activity was determined.

While screening marine sponges for thrombin and trypsin inhibitors, we detected activity in the MeOH extract of the sponge *Coscinoderma mathewsi* Lendenfield (order Dictyoceratida, family Spongiidae) collected at Pohnpei, Micronesia. Bioassay-guided isolation resulted in the characterization of 1-methylherbipoline salts of halisulfate-1 (**1a**) and of sulvanine (**2a**) as active substances.

The freeze-dried sponge was soaked in MeOH, and the $CHCl_3$-soluble portion of the MeOH extract was chromatographed on an ODS column with a stepwise gradient solvent system of MeOH and water. These steps were monitored by inhibitory activity against thrombin. The active fraction was concentrated under reduced pressure, furnishing a precipitate of the 1-methylherbipoline salt of halisulfate-1 (**1a**). The filtrate was separated by repetitive ODS HPLC (aqueous MeOH followed by aqueous MeCN), which yielded 1-methylherbipoline (**2a**), sodium (**2b**), and *N*,*N*-dimethylguanidium salts of suvanine (**2c**) (Chart 1).

The NMR data of **1a** and **2a** indicated salts that shared the same cation but differed in their anions. Anions of **1a** and **2a** were recognized as halisulfate-1 and suvanine.[1,2] The structure of the counterion appeared to be a methylated purine base, but its structure could not be confirmed by NMR spectra of the intact salts. Hence, separation of this base from the anions was carried out by treatment of **1a** with TFA. The resulting TFA salt (**3a**) was submitted to NMR spectral analysis. In the HMBC spectrum of **3a** in D_2O, correlations of N-1 CH_3 with C-2 and C-6, N-7 CH_3 with C-5 and C-8, and N-9 CH_3 with C-4 and C-8 matched those of 1-methylherbipoline. For confirmation of the structure, **1a** was passed through an anion-exchange column (Dowex Cl^- type) to obtain 1-methylherbipoline chloride whose spectral data were in agreement with those reported by Fusetani and co-workers.[4]

Identification of **2b** and **2c** as the sodium and *N*,*N*-dimethylguanidium salts of suvanine (**2**) was made by comparison of MS and NMR data with those in the literature.[2]

Inhibitory activity against thrombin and trypsin was determined for compounds **1a**, **2a**, **2b**, and **2c**. The sodium salt of halisulfate-1 (**1b**) was also prepared by base exchange.[1] As thrombin is known to recognize the guanidine moiety of Arg in the substrate, the TFA salt of 1-methylherbipoline (**3a**), which contains the guanidine group, was also tested.[4] The results are shown in Table 1. Almost all compounds showed inhibition against both enzymes. The nature of the cation appears to be inconsequential. The only anomaly seems to be the activity of **1a** against thrombin, which is weak. It is interesting to note that the TFA salt of 1-methylherbipoline (**3a**) showed no inhibition against thrombin or trypsin. Other sulfate compounds have been reported as thrombin inhibitors with comparable IC_{50} values.[5]

Both sesterterpene sulfates, halisulfate-1 (**1**) and suvanine (**2**), are known compounds. Sulvanine (**2**) was reported from a sponge of the same genus as reported here, *Coscinoderma* sp., order Dictyoceratida.[2] Halisulfate-1 (**1**), on the other hand, is one of six compounds bearing that name.[1] Four of these, halisulfate-3 to -6, differ from each other in minor stereo or regio details. The remaining two, halisulfate-1 and -2, represent two separate structural types that vary significantly from the dominant type. Moreover, the halisulfate-bearing sponge was identified only at the family (Halichondriidae) level, placing it in the order of Halichondria.

The rare cation, 1-methylherbipoline (**a**), has been reported only once with an unidentified anion.[3] It

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Chart 1

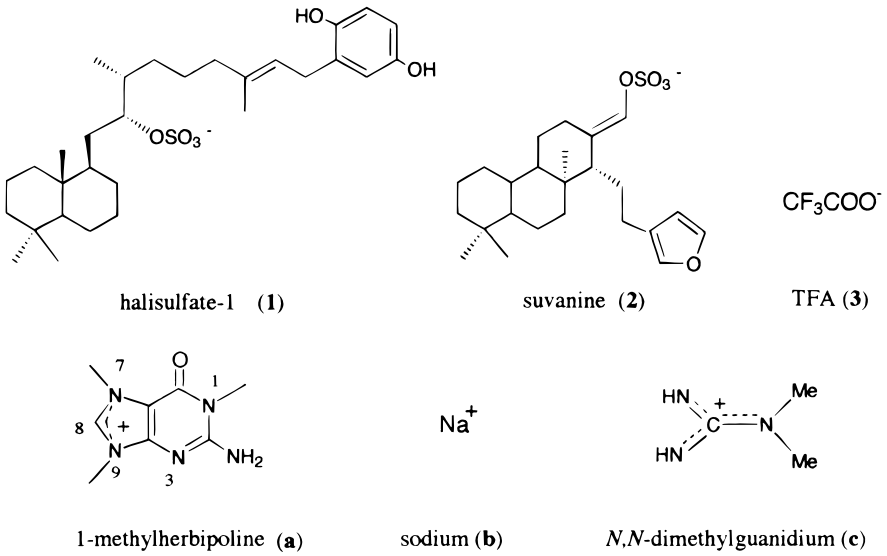

Table 1. Inhibition Against Thrombin and Trypsin (IC_{50}, μg/mL)

compd	thrombin	trypsin
1a	>100	25
2a	27	12
3a	inactive	inactive
1b	35	2
2b	9	27
2c	25	23

was isolated from a sponge *Jaspis* sp., order Choristida. The parent compound herbipolin, lacking the 1-methyl substituent, was first reported in 1957 from a sponge *Geodia* sp.[6] and the correct structure followed in 1960.[7] *Geodia* sp. and *Jaspis* sp. belong to the order Choristida.

Experimental Section

Sponge Material. The sponge, collected from Paliker Pass, Pohnpei, on June 19, 1990, is massive and semispherical, with numerous exhalant oscules in a depressed concave upper surface. Color in life is deep grayish black with a pale gold interior. Texture is soft and very elastic, and the surface has a very distinct star-shaped pattern. The skeleton is a dense mass of whorled secondary fibers, and the primary fibers are cored and complex. The sponge is *Coscinoderma mathewsi* (Lendenfeld), order Dictyoceratida, family Spongiidae. A voucher specimen has been deposited at the Natural History Museum, London, U.K. (BMNH 1996:6:6:1).

Bioassay. Thrombin inhibition assay was performed essentially according to the method of Sevendsen et al.,[8] and trypsin inhibition assay was also performed by a modification of the method of Cannell et al.[9] Experimental details were described previously.[10]

Extraction and Isolation. The freeze-dried sponge (179 g) was soaked in MeOH (3 L), and the MeOH extract (48 g) was partitioned by a modification of the method of Bligh and Dyer[11] using the solvent system of $CHCl_3-H_2O-MeOH$ (7:5:1, 1.3 L). The upper layer was further extracted with 1-BuOH, and the 1-BuOH solution was combined with the lower phase which, after evaporation, was subjected to flash ODS column chromatography with a stepwise gradient solvent system [200 mL each: $MeOH-H_2O$ (6:4), (8:2), (10:0), and $CHCl_3-MeOH-H_2O$ (8:2:0.1)] to yield four fractions. These fractions were monitored for thrombin inhibition; the 80% and 100% MeOH fractions were active. The 80% methanol fraction was concentrated under reduced pressure until the 1-methylherbipoline salt of halisulfate (**1a**, 360 mg, 2×10^{-1}% based on dry weight) was precipitated. The filtrate was separated by ODS HPLC using $MeOH-H_2O$ (85:15), and four fractions were obtained. The third fraction was further purified by ODS HPLC again with $MeCN-H_2O$(6:4) to give the 1-methylherbipoline salt of sulvanine (**2a**, 140 mg, 8×10^{-2}%) together with the known sodium and *N*,*N*-dimethylguanidium salts(**2b**, 9 mg, 5×10^{-3}%, and **2c**, 900 mg, 5×10^{-1}%).

Compound 1a: colorless crystals; mp 165−170 °C; FABMS *m/z* 837 $(M + \mathbf{a})^+$, $(C_{33}H_{49}N_5O_6S + C_8H_{12}N_5O)^+$; IR(KBr) 1718, 1618, 1553, 1456, 1240, 1196 cm^{-1}, UV (MeOH) λ_{max}(log ϵ) 287 (3.96), 260 (4.04) nm; *anal.* C 62.24%, H 7.88%, N 9.02%, calcd for $C_{38}H_{57}O_6N_5S \cdot H_2O$, C 61.62%, H 8.10%, N 9.23%. (Elemental analyses were carried out at the Institute of Physical Chemical Research, Wako-shi, Saitama, 351 Japan.)

Compound 2a: FABMS *m/z* 935 $(M + \mathbf{a})^+$, $(C_{33}H_{49}N_5O_6S + C_8H_{12}N_5O)^+$; IR (KBr) 1703, 1653, 1526, 1458, 1238, 1053 cm^{-1}; UV (MeOH) λ_{max} (log ϵ) 280 (sh), 257 (3.89) nm.

Preparation of 1b and 2b from 1a and 2c. Each vial containing 10 mg each of **1a** or **2c** with 10% $NaClO_4$ (0.5 mL) was kept standing for 10 min, and the sodium salt (**1b** and **2b**) was obtained after ODS HPLC using $MeCN-H_2O$ (7:3) containing 0.1 M $NaClO_4$ as solvent.

Isolation of 1-Methylherbipoline Trifluoroacetate (3a). A vial containing 4 mg of **1a** with TFA (0.1 mL) was kept standing for 5 min and then was evaporated. The residue was passed through ODS HPLC using $MeOH-H_2O-TFA$ (10:90:0.05) to give **3a** (1 mg): ^1H NMR (D_2O, 500 MHz) δ 8.84 (1H, s, H-8), 4.11 (3H, s, N-7 CH_3), 3.81 (3H, s, N-9 CH_3), 3.49 (3H, s, N-1 CH_3); ^{13}C NMR δ 157.4 (s, C-6), 155.9 (s, C-2), 149.3 (s, C-4), 140.0 (s, C-8), 108.4 (s, C-5), 36.3 (q, N-7 CH_3), 32.0 (N-9 CH_3), 29.6 (N-1 CH_3).

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