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H,K-ATPASE INHIBITORS FROM THE MARINE SPONGE SIPHONOCHALINA TRUNCATA: ABSOLUTE CONFIGURATION OF SIPHONODIOL AND TWO RELATED METABOLITES[1]

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Abstract: Siphonodiol and two related metabolites were isolated from the marine sponge Siphonochalina truncata as H,K-ATPase inhibitors, and their structures including absolute configuration were determined by spectroscopic methods.

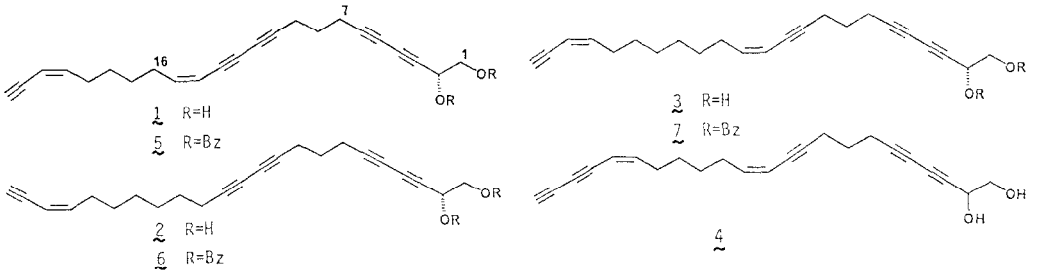
Enzyme inhibitors from marine organisms have recently been explored,[2,3] but only few such substances are known at present. In the course of our studies of bioactive metabolites from Japanese marine inverebrates,[4] we have found that the lipophilic extract of the marine sponge Siphonochalina truncata collected in the Gulf of Suruga was strongly inhibitory against the gastric H,K-ATPase. From this sponge we have isolated three active compounds: siphonodiol (**1**) which was previously isolated as an antimicrobial substance from the same sponge, and two related metabolites, dihydrosiphonodiol (**2**) and tetrahydrosiphonodiol (**3**). We have confirmed the proposed structure **1** by ^{13}C NMR studies and have determined the absolute configuration of the chiral center. The structures of **2** and **3** including absolute configuration have also been elucidated.

The EtOH extract of the frozen sponge (500 g) was partitioned between ether and water. The ether layer was fractionated on silica gel with benzene/EtOAc, and the active fractions were repeatedly purified by reversed-phase HPLC on ODS with 80% MeCN/H_2O and with 70% MeCN/H_2O to give **1** (30 mg), **2** (11 mg), and **3** (8 mg). The three compounds inhibited H,K-ATPase with IC_{50} of 1.0×10^{-5} M.

The UV and ^1H NMR data for **1** were essentially identical with those reported by the Shionogi group.[5] However, a structure represented by **4** could not be eliminated, since six-bond coupling across two acetylenic bonds can be observed in the ^1H NMR spectrum.[6] This was solved by an LSPD experiment,[7] which revealed a 4.4 Hz coupling between C-23 and H-21. Thus, structure **1** is correct, which was further supported by FABMS [m/z 438 (MH + diethanolamine)$^+$], ^{13}C NMR data,[8] and COSY spectrum. Then we attempted to determine the absolute configuration at C-2 which had not been done by the Shionogi group. The di-*p*-dimethylaminobenzoate **5** [FABMS m/z 732 (MH + diethanolamine)$^+$] prepared by reacting the diol with *p*-dimethylaminobenzoic acid in the presence of DCC gave a negative first and a positive second Cotton effect ($\Delta\varepsilon_{324}$ -25.3°, $\Delta\varepsilon_{303}$ +24.2°), which indicated the 2*R* configuration according to the CD exciton chirality method.[9,10]

Compound **2** was a dihydrosiphonodiol as judged from FABMS [m/z 440 (MH + diethanolamine)$^+$], which was supported by the ^{13}C NMR spectrum;[11] one of the disubstituted olefins of siphonodiol was replaced by two methylene groups. The ^1H NMR spectrum[11] showed that the terminal enyne, the terminal diol, and the three contiguous methylenes (C7-C9) placed between two acetylenes were intact. These features indicated that the $\Delta^{14,15}$ double bond must have been reduced and structure **2** was assigned to dihydrosiphonodiol. This structure was implied by UV spectrum [λ_{max} (EtOH) 223 nm (ε 11000)], which proved the absence of triyne or further conjugated portions in the molecule.

The third compound **3** was a tetrahydroderivative of **1** [FABMS m/z 442 (MH + diethanolamine)$^+$], which was supported by ^{13}C NMR spectrum.[12] The ^1H NMR spectrum[12] showed that **3** possesses the terminal portions identical with those of **1** and **2**, and that one of the characteristic propargyl methylene protons (δ 2.46, H_2-9) were further coupled to a *Z*-olefinic proton at δ 5.42 (1H, dtt, *J*=10.8, 2.0, 1.4 Hz; H-12) which was in turn coupled to another olefinic proton (δ 5.83; H-13) and allylic methylene



protons (H_2-14). Since no obvious coupling across two acetylenic bonds (H_2-9 and H-14) was observed for **1**, the coupling between δ 2.46 and 5.42 can be concluded to be across one acetylenic bond. As the presence of three contiguous methylene units (C7-C9) between two acetylenes was easily deduced from the ^{1}H NMR spectrum and the presence of two enyne moieties was indicated by the UV spectrum [λ_{max} (EtOH) 223 nm (ε 19600)], the structure of tetrahydrosiphonodiol was determinted as **3**.

Configuration at C-2 in **2** and **3** was also shown to be R as in the case of **1** according to the exciton chirality method applied to the di-p-dimethylaminobenzoates **6** ($\Delta\varepsilon_{323}$ -18.5°, $\Delta\varepsilon_{303}$ +18.5°) and **7**($\Delta\varepsilon_{325}$ -17.9°, $\Delta\varepsilon_{303}$ +15.3°).

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8. **1**: ^{1}H NMR ($CDCl_3$) δ 6.04 (1H, dt, 10.9, 7.5), 5.98 (1H, ddt, 10.8, 0.7, 7.6), 5.46 (1H, br d, 10.9), 5.44(1H, ddt, 10.8, 2.0, 1.4), 4.48 (1H, br dd, 3.3, 6.7), 3.74 (1H, dd, 11.4, 3.3), 3.67 (1H, dd, 11.4, 6.7), 3.07 (1H, br d, 2.0), 2.45 (2H, t, 6.8), 2.42 (2H, t, 6.8), 2.34 (4H, m), 1.77 (2H, quint, 6.9), 1.44 (4H, m); ^{13}C NMR ($CDCL_3$) δ 147.7 d, 145.7 d, 108.3 d, 108.1 d, 83.0 s, 81.4 d, 80.5 s, 78.0 s, 73.6 s, 72.5 s, 70.9 s, 66.2 t, 66.1 s, 65.0 s, 63.5 d, 30.4 t, 29.9 t, 28.2 t, 28.1 t, 26.8 t, 18.7 t, 18.4 t.
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11. **2**: ^{1}H NMR ($CDCl_3$) δ 6.00 (1H, ddt, 10.8, 1.0, 7.6), 5.45 (1H, dtt, 2.0, 1.4, 10.8), 4.49 (1H, br dd, 3.3, 6.9), 3.75 (1H, dt, 11.4, 3.3), 3.68 (1H, 11.4, 6.9), 3.07 (1H, br d, 2.0), 2.42 (2H, t, 6.9), 2.37 (2H, t, 6.9), 2.32 (2H, qd, 7.6, 1.4), 2.25 (2H, t, 7.5), 1.75 (2H, quint, 6.9), 1.55 (2H, m), 1.3-1.45 (6H, m); ^{13}C NMR δ 145.9 d, 108.2 d, 81.3 d, 80.7 s, 80.5 s, 78.0 s, 75.6 s, 73.4 s, 71.1 s, 66.3 t, 65.1 s, 65.0 s, 63.6 d, 30.1 t, 28.6 t, 28.5 t (2C), 28.2 t, 26.9 t, 19.1 t, 18.4 t (2C).
12. **3**: ^{1}H NMR ($CDCl_3$) δ 6.00 (1H, ddt, 10.7, 1.0, 6.8), 5.82 (1H, dt, 10.8, 7.4), 5.44 (1H, ddt, 10.7, 2.0, 1.4), 5.42 (1H, dtt, 10.8, 2.0, 1.4), 4.49 (1H, br dd, 3.8, 6.2), 3.75 (1H, 11.7, 3.8), 3.69 (1H, dd, 11.7, 6.2), 3.07 (1H, br d, 2.0), 2.46 (2H, dt, 2.0, 6.9), 2.44 (2H, t, 6.9), 2.33 (2H, qd, 6.4, 1.4), 2.27 (2H, qd, 6.8, 1.4), 1.77 (2H, quint, 6.9), 1.3-1.45 (8H, br m); ^{13}C NMR δ 146.1 d, 143.0 d, 108.9 d, 107.9 d, 81.0 d, 79.5 s, 73.1 s, 71.0 s, 66.2 t, 63.5 d, 30.1 t, 30.0 t, 28.8 t (2C), 28.7 t, 27.3 t, 26.9 t, 18.6 t, 18.3 t. Due to the scarcity of the material some non-protonated acetylenic carbons were not observed.

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