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ISOLATION AND STRUCTURE OF AAPTAMINE A NOVEL HETEROAROMATIC SUBSTANCE POSSESSING α-BLOCKING ACTIVITY FROM THE SEA SPONGE AAPTOS AAPTOS

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Summary: A novel heteroaromatic substance, aaptamine possessing an α-adrenoceptor blocking activity has been isolated from the tropical sea sponge Aaptos aaptos and its structure has been determined to be 1 on the basis of spectral data and chemical degradation.

Numerous marine natural products with biological activities have been isolated from various marine organisms[1]. It has been expected that chemical substances showing useful pharmacological actions will be obtained from marine organisms. Since the ergot alkaloids were found to be the first α-adrenoceptor blocking agents, α-adrenoceptor pharmacology have been subjected to extensive studies, indicating that these agents are theoretically and clinically important[2]. Therefore, in the course of our survey on pharmacologically active substances in marine organisms[3], much attention has been given to the occurrence of substances having such activities. As a result, a sea sponge Aaptos aaptos has been revealed to have a remarkable α-adrenoceptor blocking activity in the isolated rabbit aorta. In the present communication, we wish to report the isolation and determination of chemical structure of the new active substance named aaptamine.

Aaptos aaptos was collected at Okinawa island in July 1981. The methanol extract (86g) of the sea sponge (wet weight 986g) was fractionated by monitoring the α-adrenoceptor blocking activity[2] using isolated rabbit aorta[3]. The ethanol soluble material (30g) of the extract was chromatographed on a silica gel column with $CHCl_3$-MeOH (8:2) as the eluant to afford an active fraction (6.2g). The crude material was recrystalized three times from MeOH-acetone to give an active substance (1.7g) named aaptamine 1 as a bright yellow crystal, mp 110-113°C. Aaptamine was fluorescent (λmax 492 nm in H_2O) and showed UV absorption maxima in H_2O at 214 (ε=13700), 236 (14700), 255 (17900), 309 (3640),

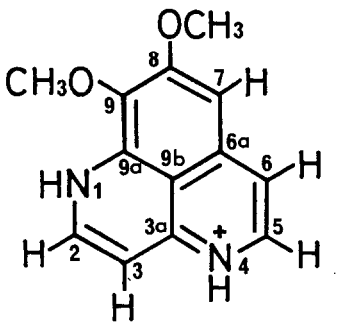

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Table 1. ^{1}H-NMR Data for 1 in DMSO-d_6 (270MHz)

atom	shift, δ in ppm	NOE
CH_3O-9	3.86 (s)	2 %
H-1	12.35 (brs)	16 %
H-2	7.90 (brd, J=6.5Hz)	12 %
H-3	6.52 (d, J=6.5Hz)	12 %
H-4	13.10 (brs)	16 %
H-5	7.45 (d, J=7.3Hz)	12 %
H-6	6.93 (d, J=7.3Hz)	6 %
H-7	7.18 (s)	19 %
CH_3O-8	4.03 (s)	

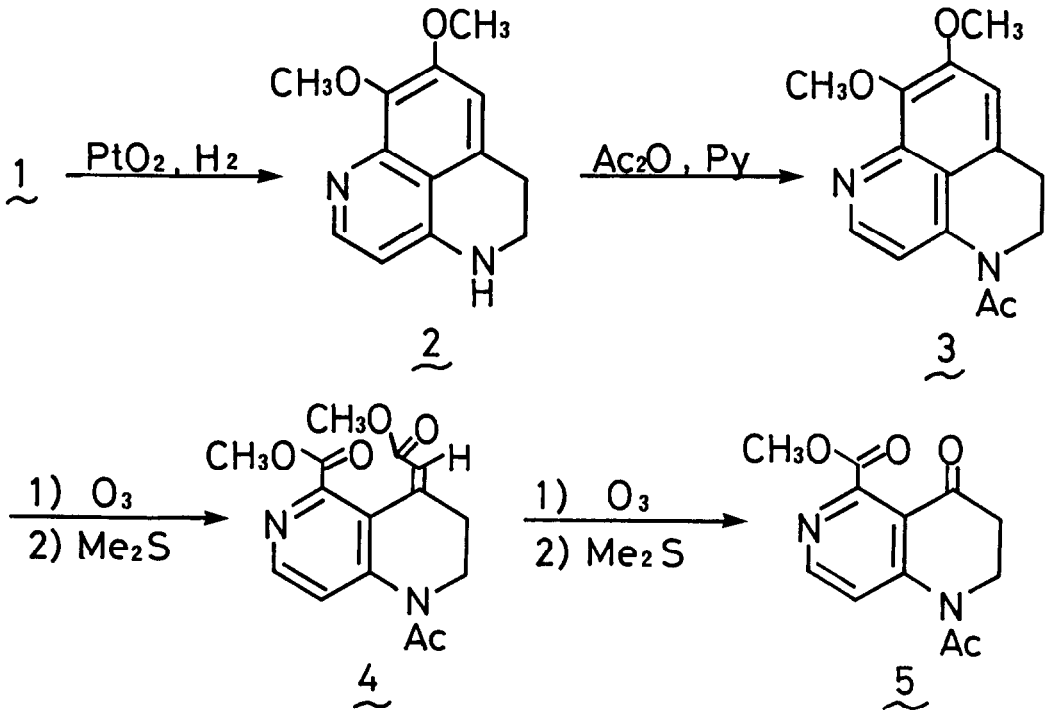
Table 2. ^{13}C-NMR Chemical Shifts[a] of 1 in D_2O (22.5MHz)

C-2	141.4 (d)	C-6a	132.6 (s)	C-9b	115.9 (s)
C-3	98.3 (d)	C-7	113.5 (d)	CH_3O-8	57.0 (q)
C-3a	149.4 (s)	C-8	157.1 (s)	CH_3O-9	61.1 (q)
C-5	129.2 (d)	C-9	131.2 (s)		
C-6	101.3 (d)	C-9a	133.2 (s)		

a: δ in ppm and dioxane was used as internal standard (δ 67.3)
(): Multiplicity in the off-resonance decoupled spectrum.

352 (3750), 381 (5000) and 394 (4570) nm. The structure 1 to aaptamine was deduced on the basis of following spectral data. The molecular formula $C_{13}H_{12}N_2O_2$ for 1 was determined by high resolution mass spectrometry (obs. m/z 228.0885, calcd mass 228.0896). The ^{1}H-NMR spectrum of 1 in DMSO-d_6 showed seven signals for eleven protons and two exchangeable signals at δ 12.35 and 13.10 ppm, indicating that aaptamine is present as a protonated form. The signals were assigned to each protons by decoupling and NOE experiments (Table 1). The proton decoupled ^{13}C-NMR spectrum of 1 in D_2O showed two signals for methoxy carbons at δ 57.0 and 61.1 ppm and eleven signals for aromatic carbons. These signals were assigned to each carbons as shown in Table 2 by proton selective decoupling experiments.

The structure 1 deduced from the spectral data was confirmed by following chemical degradation studies. Hydrogenation of 1 in AcOH-conc HCl (10:1) at 80°C using PtO_2 as a catalyst gave a dihydro compound 2[4] in 70 % yield, which was acetylated by Ac_2O/Py to afford a monoacetyl compound 3[5] in 82 % yield. The ^{1}H-NMR spectrum of 2 in DMSO-d_6 showed an exchangeable signal at δ 9.78 ppm and a couple of triplet signals due to ethylene protons at δ 3.13 and 3.64 ppm. The signal at δ 3.64 ppm coupled with the exchangeable signal. The ^{1}H-NMR



spectrum of 3 revealed two couples of coupled signals at δ 3.36 and 4.25 ppm (t, J=6.2Hz), and δ 8.27 and 8.70 ppm (d, J=7.6Hz), which were observed in that of 2 at δ 3.13 and 3.64 ppm, and δ 6.63 and 8.13 ppm, respectively. The downfield shifts of the signals by acetylation of 2 suggest that a double bond between 5 and 6 position of 1 is reduced to form $-N=CH-CH=C-NH-CH_2-CH_2-$ moiety and a nitrogen atom at 4 position is acetylated. Furthermore, the positions of substitution of two methoxy groups were established as follows. Ozonolysis of the monoacetyl compound 3 gave a dimethyl ester 4[6] in 62 % yield. In the ^{1}H-NMR spectrum of 4, signals for ethylene protons were observed as broad signals at δ 2.92 and 3.80 ppm, respectively. The former signal coupled with a signal at δ 5.99 ppm (t, J=1.1Hz) due to α-proton of α,β-unsaturated ester moiety produced by ozonolysis. Furthermore, the dimethyl ester 4 was treated with ozone to yield a monomethyl ester 5[7] in 74 % yield. These results suggest that two methoxy groups of 1 attach to a same aromatic ring and that the positions of substitution are 8 and 9, respectively.

Aaptamine is a marine natural product having a new skeleton, 1H-benzo[de]-1,6-naphthyridine. Detailed chemical and pharmacological properties will be reported elsewhere.

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References and Note

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2. F. Gross, J. Cardiovas. Pharmacol. 2 (Suppl. 3), S247 (1980).
3. J. Kobayashi, H. Nakamura, Y. Hirata and Y. Ohizumi, Toxicon, in press.
4. 2: slightly yellowish needles, mp 137-139°C (sealed tube); MS m/z 230 (M^+); UV(H_2O) λmax 236 (ε=24800), 242 (23900), 272 (7150), 282 (7550), 323 (8480), 333 (8700), 346 (7380) nm; IR (KBr) 3420, 1620, 1350, 1305, 1120, 1025, 810 cm^{-1}; ^{1}H-NMR (DMSO-d_6) δ 3.13 (2H, t, J=7.1Hz), 3.64 (2H, dt, J=1.6 and 7.1Hz), 3.88 (3H, s), 4.01 (3H, s), 6.63 (1H, d, J=7.1Hz), 7.34 (1H, s), 8.13 (1H, d, J=7.1Hz), 9.78 (brs, exchangeable); ^{13}C-NMR (CD_3OD) δ 27.0 (t), 40.8 (t), 57.3 (q), 61.8 (q), 100.4 (d), 109.2 (s), 112.0 (d), 131.3 (s), 133.7 (s), 135.3 (s), 141.4 (d), 156.0 (s), 156.8 (s).
5. 3: colorless powder, mp 122-124°C; MS m/z 272 (M^+); IR(KBr) 1665, 1610, 1595, 1505, 1405, 1290, 1115 cm^{-1}; ^{1}H-NMR (CD_3OD) δ 2.58 (3H, s), 3.36 (2H, t, J=6.2Hz), 4.06 (3H, s), 4.13 (3H, s), 4.25 (2H, t, J=6.2Hz), 7.56 (1H, s), 8.27 (1H, d, J=7.6Hz), 8.70 (1H, d, J=7.6Hz); ^{13}C-NMR ($CDCl_3$) δ 23.6 (q), 29.7 (t), 43.3 (t), 56.7 (q), 61.5 (q), 110.9 (d), 113.1 (d), 115.1 (s), 127.8 (s), 141.6 (s), 144.0 (s), 150.1 (d), 151.3 (s), 169.5 (s).
6. 4: colorless powder, mp 109-111°C; MS m/z 304 (M^+); IR (KBr) 1725, 1705, 1680, 1310, 1210 cm^{-1}; ^{1}H-NMR ($CDCl_3$) δ 2.49 (3H, s), 2.92 (2H, broad signal), 3.62 (3H, s), 3.80 (2H, broad signal), 3.89 (3H, s), 5.99 (1H, t, J=1.1Hz), 7.50 (1H, d, J=5.5Hz), 8.54 (1H, d, J=5.5Hz); ^{13}C-NMR ($CDCl_3$) δ 23.4 (q), 35.9 (t), 44.0 (t), 51.5 (q), 52.7 (q), 118.1 (d), 120.6 (d), 144.6 (s), 147.0 (s), 148.7 (d), 165.4 (s), 165.8 (s), 170.0 (s).
7. 5: colorless rods, mp 172.5-173.5°C; MS m/z 248 (M^+); UV (MeOH) λmax 235 (ε=18600), 262 (9000), 307 (3050) nm; IR($CHCl_3$) 3030, 1745, 1705, 1690, 1580, 1445, 1205 cm^{-1}; ^{1}H-NMR ($CDCl_3$) δ 2.42 (3H, s), 2.83 (2H, t, J=6.2Hz), 3.99 (3H, s), 4.21 (2H, t, J=6.2Hz), 7.74 (1H, d, J=5.7Hz), 8.57 (1H, d, J=5.7Hz).

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