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KERAMADINE, A NOVEL ANTAGONIST OF SEROTONERGIC RECEPTORS ISOLATED FROM THE OKINAWAN SEA SPONGE AGELAS SP.[1]

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Summary: A new bromine-containing alkaloid, keramadine, has been isolated from the Okinawan sea sponge Agelas sp. as a novel antagonist of serotonergic receptors and its structure has been elucidated by n.m.r. spectrometry.

During our study on physiologically active substances of marine organisms, we have examined the pharmacological actions of 70% ethanolic extracts of numerous sea sponges collected at Okinawa using the isolated vascular smooth muscle. As a result, it was found that a brown sea sponge Agelas sp. had antagonistic activities[2] on serotonergic receptors in the rabbit aorta[3]. In this communication we report the isolation and structure determination of a new antagonist of serotonergic receptors, named keramadine 1 from the sea sponge.

The methanolic extract of the sea sponge Agelas sp., which was collected at Kerama Rettō, Okinawa, was suspended in water and extracted with n-butanol. The n-butanol soluble portion of the extract was chromatographed on columns of silica gel (chloroform-methanol 1:1, chloroform-n-butanol-acetic acid-water 25:60:16:10, and isoamylalcohol-acetic acid-water 30:13:10) and Sephadex LH-20 (methanol and chloroform-methanol 1:1) by monitoring the antagonistic activity on serotonergic receptors in the isolated aorta to give an active fraction. The fraction was chromatographed on a Develosil ODS column by using 6:4 water-methanol containing 0.2% trifluoroacetic acid as eluant to obtain an active substance, keramadine 1, as colorless powder (mp. 183-187°C, 0.0014% yield from the fresh sponge).

The field desorption mass spectrum of 1 showed intense M+H ions at m/z 324 and 326, indicating that 1 is a monobromo compound ($C_{12}H_{14}N_5OBr$). The i.r. spectrum of 1 (KBr) showed an amide carbonyl absorption at 1680cm^{-1}. A detailed analysis of the ^{1}H n.m.r. spectrum of 1 revealed a partial structure

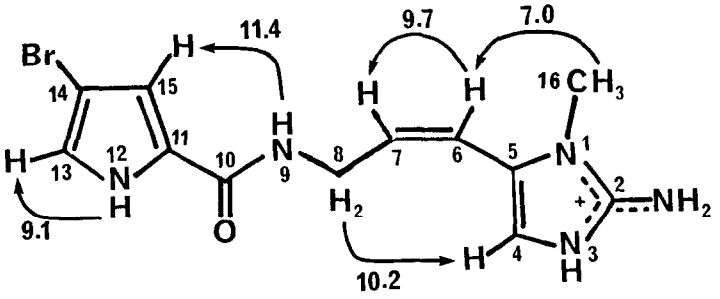

Fig. 1. Chemical structure of keramadine 1 and n.O.e.s (%), H(irradiated)⟶ H(enhanced).

Table 1. ^{13}C n.m.r. (22.5 MHz) and ^{1}H n.m.r. (90MHz) spectral data for keramadine 1 in DMSO-d_6.

Position	δH^a	H, m, J (Hz)	δC^b	m^c	J_{H-C} (Hz)
2			146.9	s	
3	11.96	brs			
4	7.02	s	112.0		
5			123.7	d	200
6	6.20	AB, d, 11	133.3	d	160
7	5.81	ABX_2, dt, 11 and 5.6	113.8	d	158
8	4.01	t, 5.6	38.6	t	
9	8.22	brt, 5.6			
10			159.6	s	
11			126.7	s	
12	11.58	brs			
13	6.92	dd, 2.9, 1.5	121.3	d	185
14			95.0	s	
15	6.80	dd, 2.9, 1.5	111.7	d	177
16	3.38	s	29.2	q	142
N(2)	7.59	brs			

a; δ in ppm, 70°C. b; δ in ppm and assignments are based on single-frequency decoupling experiments. c; Multiplicity in the off-resonance decoupled spectrum.

-CO-NH-CH_2-CH=CH-(cis) [δ 8.22 (9-H,exchangeable, brt, J=5.6 Hz), 4.01 (8-H, t, J=5.6 Hz), 5.81 (7-H, ABX, J=11 and 5.6 Hz) and 6.20 (6-H, d, J=11 Hz)]. The large H-C coupling constants (Table 1) indicated the presence of nitrogen containing heteroaromatic rings[4,5], in agreement with the u.v. absorption, λmax(MeOH) 269 nm (ε 21400). The ^{1}H n.m.r. spectrum of 1 contained an exchangeable signal at δ11.58 (12-H) and signals for two aromatic protons at δ6.80 (15-H, dd, J_{13-15}=1.5 Hz, J_{12-15}=2.9 Hz) and 6.92 (13-H, dd, J_{15-13}=1.5 Hz, J_{12-13}=2.9 Hz), indicating the existence of a 2,4-disubstituted pyrrole ring. The pyrrole ring was further suggested by a positive color test with Ehrlich reagent. The substitution pattern of the pyrrole ring was determined on the basis of H-C coupling constants of C-13 (185 Hz) and C-15 (177 Hz)[4] and chemical sifts of C-11 (δ126.7) and C-14 (δ95.0)[4,6]. An N-methyl-2-aminoimidazole unit was suggested by the signals at δ146.9 (C-2), 112.0 (C-4), 123.7 (C-5) and 29.2 (C-16) and a large H-C coupling constant of C-4 (200 Hz)[5,6,9]. The substitution position of the methyl group and the relation among these partial structures were elucidated by n.O.e. experiments (Fig. 1).

Keramadine 1 appears to be closely related biogenetically to bromine-containing alkaloids oroidin[7,8] and sceptrin[9] which have been isolated from sea sponges of the same genus Agelas as antimicrobial substances. However, the configuration of double bond at 6 position in 1 is the reverse of those in these compounds. In addition, in the isolated rabbit aorta the contractile response to serotonine (10^{-6}M) was abolished by 1 (1.5×10^{-5}M), whereas the responses to potassium chloride (4×10^{-2}M) and norepinephrine (10^{-7}M) were not affected by 1.

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