[Chem. Pharm. Bull. 30(2) 502—508 (1982)]

Chemistry of Paragracine, A biologically Active Marine Base from *Parazoanthus gracilis* (LWOWSKY)[1)]

YASUO KOMODA,* MASATO SHIMIZU, SHIGERU KANEKO, MASAKO YAMAMOTO and MASAYUKI ISHIKAWA

Research Institute for Medical and Dental Engineering, Tokyo Medical and Dental University, 2-3-10 Surugadai, Kanda, Chiyoda-ku, Tokyo

(Received June 16, 1981)

A biologically active and strongly fluorescent marine base, paragracine (**1**), was isolated from an anthozoan, *Parazoanthus gracilis* (LWOWSKY), and the structure of **1** was determined to be 2-dimethylamino-6-methyl-8-methylamino-1*H*-1,3,7,9-tetrazacyclopent-[*e*]azulene. Chemical reactions were also carried out to investigate the reactivity of **1**.

Keywords——paragracine; *Parazoanthus gracilis*; *Dentitheca habereri*; 1,3,7,9-tetrazacyclopent[*e*]azulene; anthozoa; zoanthoxanthins; papaverine-like activity; anti-histamine activity; guinea-pig ileum; fluorescence

In the course of research on the isolation of biologically active substances from marine natural products,[2)] a methanolic extract of an anthozoan, *Parazoanthus gracilis* (LWOWSKY) (Parazoanthidae; Japanese name: Sennari-sunaginchaku) together with *Dentitheca habereri* (STECHOW) (Plumulariidae, Japanese name: Sudare-gaya), which is parasitized with the former organism, showed histamine-like activity on isolated guinea-pig ileum strips. During isolation of the active principle, however, we found that several substances having anti-histamine activity were also contained in the methanolic extract. A major one of them has been isolated and elucidated its structure as **1**. This compound, named paragracine, was a yellow-colored base exhibiting strong yellowish-green fluorescence in solution.

The polyps of *P. gracilis* usually cover the branches of *D. habereri* and the overall shapes brighten to a yellow color in the sea. When touched by hand for collection, some of the yellowish-green fluorescent material dissolved into sea water. One of these fluorescent materials seems to be paragracine; its isolation, structure, and chemical reactions are described in this report.

Isolation and Structure of Paragracine

Fractionation of the methanolic extract was carried out to isolate paragracine as shown in Chart 1, guided by the activity on isolated guinea-pig ileum strips.

The methanolic extract (fraction A) which showed histamine-like activity was partitioned between water and ethyl acetate to afford fractions B and C. A water solution of fraction B was passed through an Amberlite XAD-2 column, which was washed with water and then eluted with methanol. The combined water eluate gave non-adsorbed compounds (fraction D), and the methanol eluate afforded adsorbed and methanol-elutable compounds (fraction E). Histamine-like activity found in fraction A was transferred into fractions B and D. However, fraction D was 1.5 times more active than fraction B in terms of contracting activity. This result suggested that fraction B contained some compounds having anti-histamine activity as well as the histamine-like active compounds, and that the former were adsorbed on Amberlite XAD-2 and thus separated from the latter, which were not adsorbed. This was confirmed by the significant anti-histamine activity of fraction E.

Fraction E was chromatographed on neutral alumina to isolate the anti-histamine components, and the 10% methanol–chloroform eluate gave a yellow-crystalline substance, which was recrystallized from methanol to afford a major biologically active and strongly fluorescent

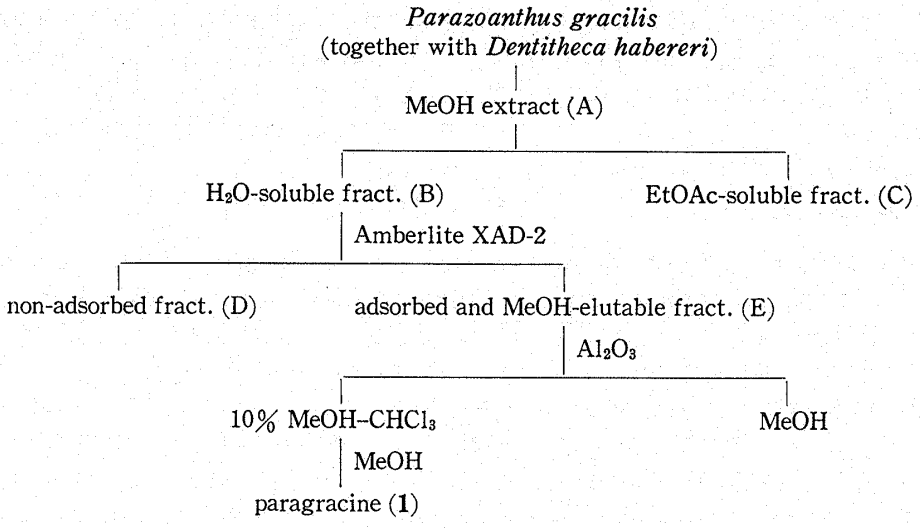


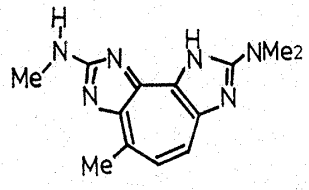
Chart 1

compound, paragracine (**1**, 0.1% of the undried animal materials), mp 258—262°C (dec.).

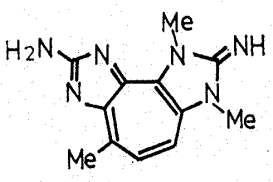
The structure of paragracine was determined to be 2-dimethylamino-6-methyl-8-methylamino-1*H*-1,3,7,9-tetrazacyclopent[*e*]azulene from its chemical and spectral properties, and by means of X-ray crystallographic analysis of both paragracine dihydrobromide trihydrate and paragracine monohydrate 1/2 ethanol.[1,3]

Paragracine has the same basic skeleton, with a 1,3,7,9-tetrazacyclopent[*e*]azulene system, as pseudozoanthoxanthin (**2**) and 3-norpseudozoanthoxanthin (**3**), which were isolated from *Epizoanthus arenaceus*[4] and whose structures were proposed on the basis of chemical and spectroscopic evidence.[5] Recently, a similar metabolite was isolated from a *Parazoanthus* sp. and its structure was determined to be **4** by X-ray crystallographic analysis.[6]

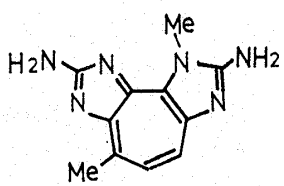
On the other hand, another structural series having a basic skeleton with a 1,3,5,7-tetrazacyclopent[*f*]azulene system was isolated from *E. arenaceus* and *P. axinellae*.[4,5,7] Parazoanthoxanthin A (**5**) is the least-substituted metabolite of this series, and the other five metabolites were shown to differ only in the number and position of *N*-methyl groups, in the same manner as with the series including paragracine (**1**). All metabolites included in both structural series are generically called zoanthoxanthins.[7c] The least-substituted zoanthoxanthins of both series have been synthesized.[8]



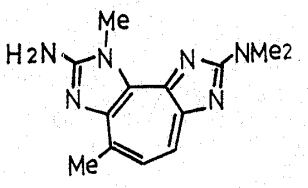
1



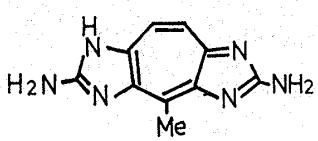
2



3



4



5

Chart 2

Investigations of the pharmacological actions of paragracine on various autonomic effector organs revealed that paragracine has papaverine-like activity, and the details have been published.[9] An additional interesting biological activity of paragracine, which selectively blocks sodium channels of squid axon membranes in a frequency-dependent manner, has recently been reported.[10]

Reactions of Paragracine[11]

Several reactions of paragracine were carried out as shown in Chart 3, in order to investigate the reactivity of paragracine having a unique structure and the relationship between its structure and biological activity.[12] Chemical shifts of paragracine and each product in proton nuclear magnetic resonance (^{1}H NMR) spectra are listed in Table I.

When refluxed with 6N hydrochloric acid for 46 h in a sealed tube, paragracine (**1**) gave the hydrolyzed product (**6**) along with a considerable amount of the starting material. Treatment of **6** with phosphorus oxychloride produced the chloride (**7**) in 85% yield. This product was further transformed into paragracine by reaction with monomethylamine. These reactions should be useful for introducing various substituents at C-8 of paragracine.

A methanolic solution of paragracine was reacted with ethereal diazomethane at room temperature and the products obtained were separated by column chromatography to afford two isomeric mono-*N*-methyl derivatives (**8**) and (**9**) in the ratio of 5 to 2. Compounds **8** and **9** were further treated with sodium hydride in dimethylformamide then with methyl iodide to yield the di-*N*-methyl derivatives (**10**) and (**11**), respectively. Product **10** was also obtained directly from paragracine by the latter methylation method along with a trace of **11**.

The ^{1}H NMR spectra in $CDCl_3$ indicated that the *N*-methyl groups newly introduced into the mono-*N*-methyl derivatives (**8**) and (**9**) are located on one of four cyclic nitrogens

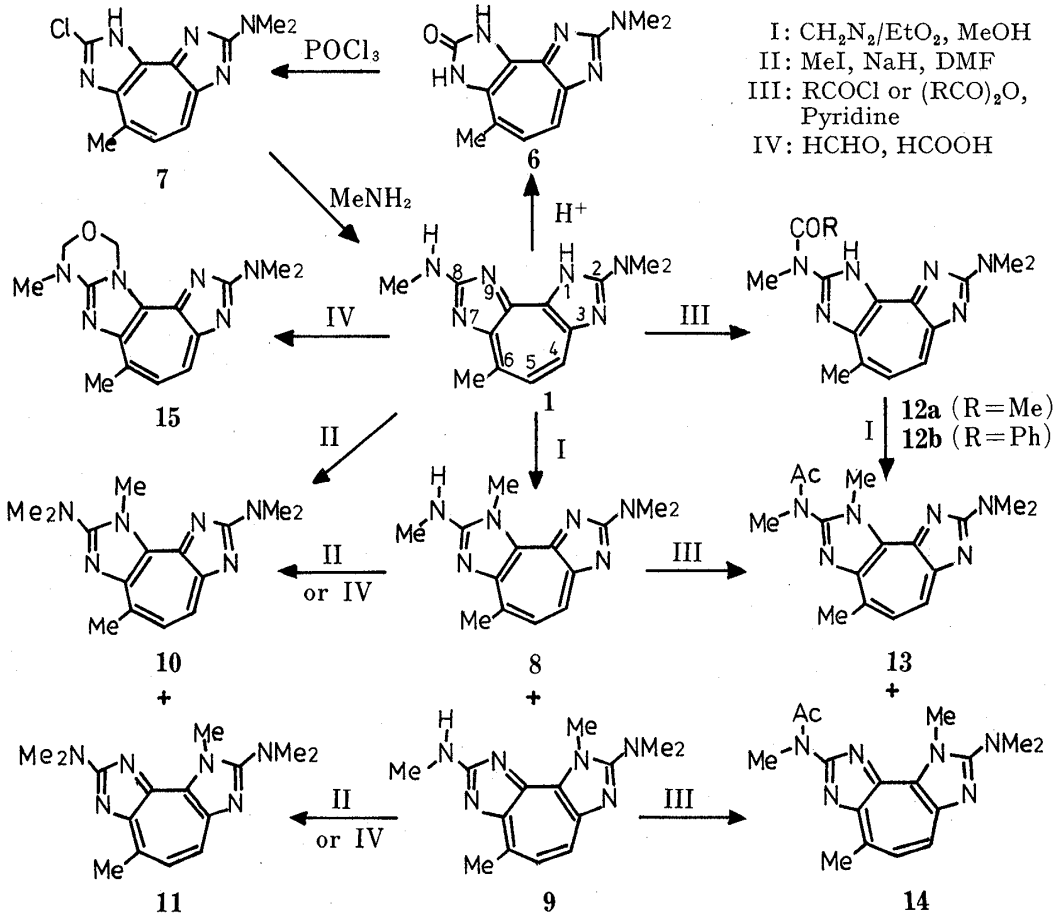


Chart 3

Table I. Chemical Shifts (ppm) and Signal Patterns in ^{1}H NMR Spectra[a]

Compd.	2N′–CH_3 (6H, s)	8N′–CH_3	1–CH_3 (3H, s)	9–CH_3 (3H, s)	6–CH_3 (3H, s)	4–H or 5–H (1H, d J=11)	4–H or 5–H (1H, d J=11)		
1	3.21	3.09 (3H, s)	—	—	2.84	7.52	7.82		
6[b]	3.30	—	—	—	2.56	7.45	7.72	4.30 (NH)	
7[c]	3.24	—	—	—	2.90	7.54	7.88		
8	3.34	3.23 (3H, s)	—	4.33	2.82	7.45	7.86	4.90 (NH)	
8[d]	3.57 (9H, s)		—	4.63	3.00	8.07	8.30		
9	3.10	3.28 (3H, s)	4.30	—	2.93	7.49	7.82	5.52 (NH)	
9[d]	3.53	3.37 (3H, s)	4.51	—	2.90	7.99 (2H, s)			
10	3.39	3.16 (6H, s)	—	4.30	2.85	7.49	7.91		
11	3.09	3.40 (6H, s)	4.29	—	2.91	7.44	7.76		
12a	3.34	3.64 (3H, s)	—	—	2.79	7.45	7.89	2.45 (Ac)	9.77 (NH)
12b[c]	3.27 (9H, s)		—	—	2.73	7.00—7.90 (7H, m)			
13	3.40 (9H, s)		—	4.37	2.88	7.54	8.00	2.04 (Ac)	
14	3.16	3.72 (3H, s)	4.33	—	3.05	7.76	8.14	2.78 (Ac)	
15	3.30	3.24 (3H, s)	—	—	2.77	7.38	7.77	4.87 (2H, s)	6.26 (2H, s)

a) Taken in $CDCl_3$; s: singlet, d: doublet, m: multiplet; coupling constants in Hz.
b) Taken in DMSO-d_6.
c) Taken in $CDCl_3$+CD_3OD.
d) Taken in CF_3COOH.

because of their relatively low chemical shifts (4.33 and 4.30 ppm, respectively) in comparison with those of the exocyclic *N*-methyl groups (3.10—3.34 ppm). This conclusion was also supported by the differences in the chemical shifts between the *N*-methyl groups (4.30 and 4.29 ppm, respectively) and the two *N*-dimethyl groups (3.09—3.40 ppm) of the di-*N*-methyl derivatives (**10**) and (**11**), whose newly introduced methyl groups are located at 8-N′. Prota *et al.* reported that the ^{1}H NMR spectra in CF_3COOH of pseudozoanthoxanthin (**1**) and 3-norpseudozoanthoxanthin (**3**) showed the signals of the 1-methyl groups at 4.61 and 4.56 ppm, respectively, whereas that for the 3-methyl group of **2** appeared at 4.05 ppm.[5] The chemical shifts in CF_3COOH of the cyclic *N*-methyl groups of the mono-*N*-methyl derivatives (**8**, 4.63 ppm and **9**, 4.51 ppm) were very close to those of the 1-methyl groups of **2** and **3**. This result indicated that the position of methylation of paragracine by diazomethane was the 1- or 9-position. The chemical shifts in $CDCl_3$ of the *N*-dimethyl groups of the mono-*N*-methyl derivatives (**8**, 3.34 ppm and **9**, 3.10 ppm) showed a relatively large difference, and the lower chemical shift of **8** was considered to be due to the influence of the ring current of the 1,3-diazazulene system. Such large differences of chemical shifts were also observed in the case of the *N*-dimethyl groups of **10** and **11**. Therefore, the cyclic *N*-methyl groups of **8** and **9** are located on the 9- and 1-positions, respectively. The di-*N*-methyl derivatives (**10**) and (**11**) were also obtained from **8** and **9** by methylation with formaldehyde and formic acid, respectively.

Acetylation of paragracine (**1**) with acetic anhydride and pyridine gave the monoacetate (**12a**), which was further treated with ethereal diazomethane in methanol to yield the mono-*N*-

methyl acetate (**13**) along with a trace of the isomer (**14**). Compounds **13** and **14** were also obtained from the mono-*N*-methyl derivatives (**8**) and (**9**), respectively, by acetylation. Therefore, the acetyl group of **12a** is located at 8-N′. Benzoylation of paragracine with benzoyl chloride and pyridine occurred at the same position, 8-N′, as in the case of acetylation, to afford **12b**.

When reacted with formaldehyde and formic acid, paragracine (**1**) was converted to **15**, which might be produced by successive cyclization, dehydration and reduction of the 8-*N*′, 9-di-*N*-formyl intermediate.

The nature of the minor bases related to paragracine isolated from *P. gracilis* will be reported in the near future.

Experimental

All melting points were taken with a Yanagimoto microscope hot stage and are uncorrected. NMR spectra were obtained using a JEOL JNM-C-60H spectrometer at 60 MHz for ^{1}H NMR and a JEOL JNM-FX100 spectrometer at 25 MHz for ^{13}C NMR. Chemical shifts (δ) are reported in ppm downfield from internal tetramethylsilane and the following abbreviations are used: s=singlet, d=doublet, t=triplet, q=quartet and m=multiplet. Infrared (IR) spectra were measured on a Hitachi 285 spectrometer, mass spectra (MS) were obtained with a Hitachi RMU-7M instrument, and ultraviolet (UV) spectra were taken on a Hitachi 323 spectrometer. Column chromatography was carried out using Al_2O_3 (neutral, Merck) and silica gel (Wakogel C-200, Wako Pure Chemical). Thin-layer chromatography (TLC) was performed on silicagel 60 F_{254} and Al_2O_3 150 F_{254} plates (Merck).

Bioassay——A strip of ileum (about 20 mm in length) was prepared from isolated ileum of a male or female guinea-pig (250—500 g), and a load (4 g) was applied at 37°C in a Magnus bath (10 ml) containing Mg^{2+}-free Tyrode's solution through which air was blown. The isometric contractions obtained upon addition of samples dissolved in H_2O to the Magnus bath were recorded with a Nihon-Koden SB-1T force-displacement transducer connected to a Nihon-Koden RJG-3024 recticorder.

Extraction of *Parazoanthus gracilis*——Samples (1.91 kg) of *P. gracilis* (Japanese name: Sennari-sunaginchaku) together with *Dentitheca habereri* (Stechow) (Japanese name: Sudaregaya) (the former is parasitic on the latter) were collected in Nov. 1974 at Shimoda, Sagami Bay. They were crushed in a mortar and extracted with MeOH (2.0 l) for 24 h at room temperature. The mixture was filtered and the filtrate was concentrated *in vacuo* at 35—40°C to give a methanolic extract (fraction A), which showed histamine-like activity in the bioassay.

Isolation of Paragracine (Chart 1)——The MeOH extract (fraction A) was partitioned between EtOAc (4×100 ml). The combined EtOAc layer was washed with H_2O (50 ml), dried over anhydrous Na_2SO_4 and concentrated *in vacuo* to afford EtOAc-soluble materials (fraction C, 4.31 g). The combined H_2O layer (fraction B) was passed through an Amberlite XAD-2 column (500 ml). The column was washed with H_2O (500 ml) and then eluted with MeOH (3.0 l). The combined H_2O solution and the MeOH solution were each concentrated *in vacuo* to give the non-adsorbed compounds (fraction D), and the adsorbed and MeOH-elutable compounds (fraction E, 3.42 g), respectively. Histamine-like activity found in fraction A was transferred to fractions B and D, and fraction D (from which histamine was purified as an active compound) was 1.5 times more active than fraction B. On the other hand, fraction E showed anti-histamine activity.

Fraction E was suspended in 10% MeOH–$CHCl_3$ (300 ml) and put on top of the column (Al_2O_3, 34 g), which was eluted with 10% MeOH–$CHCl_3$ and then MeOH. The first fraction eluted with 10% MeOH–$CHCl_3$ (350 ml) gave a crystalline substance (2.217 g), which was recrystallized from MeOH to yield a major biologically active and strongly fluorescent compound, paragracine (**1**, 1.120 g). The materials obtained by combining the mother liquor, the second eluate with 10% MeOH–$CHCl_3$ (200 ml) and the MeOH eluate (500 ml) were further chromatographed on Al_2O_3 to afford paragracine (803 mg) after recrystallization as well as minor components related to paragracine which will be described elsewhere. The total yield of paragracine was 1.923 g (0.1% of the undried animal materials).

Paragracine (**1**)——Yellow needles, mp 258—262°C (dec., MeOH). UV λ_{max}^{MeOH} nm (ε): 229 (shoulder (sh), 16500), 252 (11900), 309 (sh, 49000), 314 (52000), 372 (12600), 409 (13100), 422 (sh, 6550); $\lambda_{max}^{MeOH+H^+}$ nm (ε): 245 (13500), 301 (54800), 363 (6900), 410 (21300), 423 (sh, 13100). ^{1}H NMR (DMSO-d_6) δ: 2.71 (3H, s, 6-CH_3), 3.05 (3H, s, 8-N′CH_3), 3.25 (6H, s, 2-N′$(CH_3)_2$), 7.12 (2H, br s, D_2O-exchangeable, 2×NH), 7.36 (1H, J=11 Hz, 4- or 5-H), 7.60 (1H, d, J=11 Hz, 5- or 4-H). ^{13}C NMR ($CDCl_3$+CD_3OD) δ: 23.3 (q, 6-$\underline{C}H_3$), 29.5 (q, 8-N′$\underline{C}H_3$), 38.4 (q, 2-N′$(\underline{C}H_3)_2$), 120.5 (d, 5), 131.5 (d, 4), 132.8 (s, 6), 138.4 (s), 140.3 (s), 153.6 (s), 154.3 (s), 163.8 (s), 165.0 (s). MS *m/e*: 256.1449 (M^+, $C_{13}H_{16}N_6$=256.1435), 241, 227. *Anal.* Calcd for $C_{13}H_{16}N_6$: C, 60.92; H, 6.29; N, 32.79. Found: C, 60.84; H, 6.33; N, 32.66.

Paragracine was recrystallized from 50% EtOH to obtain crystals of paragracine monohydrate 1/2 ethanol suitable for X-ray crystallographic analysis.

Paragracine Dihydrobromide Trihydrate——When paragracine (**1**) (51 mg) was added to a solution of conc. hydrobromic acid (0.5 ml) and H_2O (0.5 ml) at room temperature, the crystals dissolved and then newly formed crystals appeared. The crystals were filtered off, washed with acetone and recrystallized from H_2O to afford yellow needles, mp 280—282°C (dec.), suitable for X-ray crystallographic analysis of paragracine dihydrobromide trihydrate.

Hydrolysis of Paragracine (**1**)——A solution of **1** (226 mg) in 6 N HCl (0.5 ml) was refluxed in a sealed tube for 46 h. The mixture was basified with 10% NaOH solution and passed through an Amberlite XAD-2 column (100 ml) which was washed with H_2O and eluted with 50% MeOH–$CHCl_3$. The residue (232 mg) obtained by evaporation of the solvent *in vacuo* was chromatographed on Al_2O_3 to give unchanged **1** (137 mg) and the hydrolyzed product (**6**, 43 mg), yellow needles, mp >300°C (dec., CH_2Cl_2+MeOH). UV λ_{max}^{MeOH} nm (ε): 249 (17400), 294 (36200), 297 (sh, 35700), 314 (sh, 14500), 374 (13500), 411 (12300). IR ν_{max}^{KBr} cm^{-1}: 1735 (C=O). MS *m/e*: 243 (M^+), 228, 214. *Anal.* Calcd for $C_{12}H_{13}N_5O$: C, 59.25; H, 5.39; N, 28.79. Found: C, 59.13; H, 5.48; N, 28.57.

Chlorination of 6——A solution of **6** (13 mg) in $POCl_3$ (2.0 ml) was refluxed for 25 h. After decomposition of excess $POCl_3$ with H_2O, the mixture was basified with 10% NaOH solution and applied to an Amberlite XAD-2 column. The crude product obtained was purified by column chromatography on Al_2O_3 to afford the chloride (**7**, 11 mg), yellow needles, mp 195—198°C (dec., MeOH). UV λ_{max}^{MeOH} nm (ε): 237 (sh, 14700), 266 (sh, 20900), 283 (34400), 351 (5900), 385 (sh, 5800), 406 (sh, 13400), 422 (22600). MS *m/e*: 263 (M^++2), 261 (M^+), 246, 232. *Anal.* Calcd for $C_{12}H_{12}N_5Cl$: C, 55.06; H, 4.62; N, 26.76. Found: C, 55.27; H, 4.79; N, 26.51.

Conversion of 7 to Paragracine (**1**)——A solution of **7** (5.5 mg) and monomethylamine hydrochloride (290 mg) in MeOH (2.0 ml) and acetic acid (0.3 ml) in the presence of NaOAc (310 mg) was heated in a sealed tube for 18.5 h at 110°C. After removal of insoluble materials by filtration, the solvent was evaporated off *in vacuo* and the residue was chromatographed on Al_2O_3 to give yellow needles (2.0 mg), which were identical with **1** by comparisons of UV, mass and IR spectra.

Methylation of Paragracine (**1**) **with Ethereal CH_2N_2**——A solution of **1** (240 mg) in MeOH (5.0 ml) was treated with ethereal CH_2N_2 and the mixture was allowed to stand for 15 min at room temperature. The solvent was evaporated off *in vacuo* and the residue (249 mg) was chromatographed on silica gel with a mixture of MeOH and $CHCl_3$. Fractions eluted with 1—2% MeOH–$CHCl_3$ gave 1-methylparagracine (**9**, 46 mg), yellow prisms, mp 208—209°C (EtOAc). UV λ_{max}^{MeOH} nm (ε): 259 (14400), 306 (sh, 40400), 315 (46100), 374 (11500), 412 (12700). MS *m/e*: 270 (M^+), 255, 241. *Anal.* Calcd for $C_{14}H_{18}N_6$: C, 62.20; H, 6.71; N, 31.09. Found: C, 62.31; H, 6.67; N, 31.13.

The fractions eluted with 10% MeOH–$CHCl_3$ yielded 9-methylparagracine (**8**, 115 mg), yellow prisms, mp 270—272°C (dec., MeOH). UV λ_{max}^{MeOH} nm (ε): 259 (12700), 310 (56200), 317 (sh, 49600), 376 (12400), 417 (20200). ^{13}C NMR ($CDCl_3$+CD_3OD) δ: 23.4 (q, 6-$\underline{C}H_3$), 29.6 (q, 8-N′$\underline{C}H_3$), 31.9 (q, 9-$\underline{C}H_3$), 38.2 (q, 2-N′-($\underline{C}H_3$)$_2$), 119.7 (d, 5), 129.9 (d, 4), 131.6 (s), 133.7 (s, 6), 148.3 (s), 149.5 (s), 157.9 (s), 159.4 (s), 171.8 (s). MS *m/e*: 270 (M^+), 255, 241. *Anal.* Calcd for $C_{14}H_{18}N_6$: C, 62.20; H, 6.71; N, 31.09. Found: C, 62.25; H, 6.55; N, 30.94.

8-N′,9-Dimethylparagracine (**10**)——(a) By Methylation of 9-Methylparagracine (**8**) with CH_3I and NaH: A solution of **8** (20 mg) in dimethylformamide (DMF) (0.5 ml) was stirred with NaH (50% in mineral oil, 14 mg) for 10 min at room temperature and then a solution of CH_3I (100 mg) in DMF (0.5 ml) was added. The mixture was stirred for an additional 2 h at room temperature, then H_2O was added and the whole was extracted with $CHCl_3$. The $CHCl_3$ layer was washed with H_2O, dried over anhydrous Na_2SO_4 and concentrated *in vacuo*. The residue was purified by preparative TLC (silica gel) to give **10** (16 mg), yellow prisms, mp 156—158°C (EtOAc+*n*-hexane). UV λ_{max}^{MeOH} nm (ε): 262 (16200), 314 (sh, 42000), 320 (45400), 378 (13700), 420 (21800). MS *m/e*: 284 (M^+), 269, 255. *Anal.* Calcd for $C_{15}H_{20}N_6$: C, 63.35; H, 7.09; N, 29.56. Found: C, 63.23; H, 7.31; N, 29.47. **10** was also obtained in 61% yield from paragracine (**1**) along with a trace of 1,8-*N′*-dimethylparagracine (**11**) on direct treatment with CH_3I and NaH, and work-up as described above.

(b) By Methylation of **8** with HCHO and HCOOH: A solution of **8** (90 mg) in 37% HCHO (1.5 ml) and HCOOH (3.0 ml) was refluxed for 20 h, basified with 10% NaOH solution and extracted with $CHCl_3$. The $CHCl_3$ layer was washed with H_2O, dried over anhydrous Na_2SO_4 and concentrated *in vacuo*. The residue (85 mg) was purified by preparative TLC (silica gel) to afford **10** (6 mg) together with unchanged **8** (70 mg).

1,8-*N′*-Dimethylparagracine (**11**)——1-Methylparagracine (**9**, 20 mg) was treated with NaH (50% in mineral oil, 11 mg) and CH_3I (100 mg) in DMF and worked up as described above for **10** to yield **11** (10 mg), yellow needles, mp 146—148°C (EtOAc). UV λ_{max}^{MeOH} nm (ε): 261 (15600), 312 (sh, 35400), 320 (38300), 381 (12500), 418 (16200). MS *m/e*: 284 (M^+), 269, 255. *Anal.* Calcd for $C_{15}H_{20}N_6$: C, 63.35; H, 7.09; N, 29.56. Found: C, 63.28; H, 7.19; N, 29.78. **11** was also obtained in 33% yield from **9** by treatment with HCHO and HCOOH as described above for **10**.

8-*N′*-Acetylparagracine (**12a**)——A solution of paragracine (**1**, 128 mg) in Ac_2O (1.0 ml) and pyridine (1.0 ml) was allowed to stand for 17 h at room temperature. The residue obtained by evaporation *in vacuo* was purified by column chromatography on Al_2O_3 to give **12a** (124 mg), red needles, mp 230—233°C (dec., MeOH). UV λ_{max}^{MeOH} nm (ε): 260 (17000), 287 (sh, 23400), 269 (26300), 305 (27100), 313 (sh, 25300), 361 (8200),

379 (7600), 406 (sh, 13300), 421 (18100). IR ν_{max}^{KBr} cm^{-1}: 3380 (NH), 1680 (C=O). MS *m/e*: 298 (M$^+$), 283, 269, 255, 241, 227. *Anal.* Calcd for $C_{15}H_{18}N_6O$: C, 60.38; H, 6.08; N, 28.17. Found: C, 60.35; H, 6.11; N, 28.13.

8-*N'*-Acetyl-9-methylparagracine (13)——A solution of **12a** (55 mg) in MeOH (5.0 ml) was treated with ethereal CH_2N_2 and the mixture was allowed to stand for 1 h at room temperature. The residue (65 mg) obtained by removal of the solvent was chromatographed on Al_2O_3 to give **13** (53 mg), yellow needles, mp 220—223°C (dec., C_6H_6). UV λ_{max}^{MeOH} nm (ε): 253 (sh, 26000), 258 (28900), 275 (13500), 287 (14300), 307 (20700), 315 (sh, 19400), 364 (sh, 7300), 380 (8600), 414 (sh, 14700), 430 (19500). IR ν_{max}^{KBr} cm^{-1}: 1685 (C=O). MS *m/e*: 312 (M$^+$), 297, 283, 269, 255, 241, 227. *Anal.* Calcd for $C_{16}H_{20}N_6O$: C, 61.52; H, 6.45; N, 26.91. Found: C, 61.65; H, 6.58; N, 26.78. A trace of **14**, which was a minor product of this reaction, was found in the mother liquor of **13** on TLC. **13** was also obtained from 9-methylparagracine (**8**) by acetylation with Ac_2O and pyridine.

8-*N'*-Acetyl-1-methylparagracine (14)——A solution of 1-methylparagracine (**9**, 10 mg) in Ac_2O (0.5 ml) and pyridine (0.5 ml) was allowed to stand for 16 h at room temperature and then concentrated *in vacuo*. The residue was purified by column chromatography on silica gel to yield **14** (9 mg), yellow needles, mp 165—167°C (dec., EtOAc). UV λ_{max}^{MeOH} nm (ε): 260 (18500), 303 (46800), 364 (11700), 415 (11200). IR ν_{max}^{KBr} cm^{-1}: 1680 (C=O). MS *m/e*: 312 (M$^+$), 297, 283, 269, 255, 241, 227. *Anal.* Calcd for $C_{16}H_{20}N_6O$: C, 61.52; H, 6.45; N, 26.91. Found: C, 61.78; H, 6.40; N, 27.11.

8-*N'*-Benzoylparagracine (12b)——A suspension of paragracine (**1**, 128 mg) in pyridine (3.0 ml) was treated with benzoyl chloride (2.0 ml) and the whole was stirred for 6 h under ice-cooling then for 2 h at room temperature. The solvent was evaporated off *in vacuo*, then cold water was added to the residue and the whole was extracted with $CHCl_3$. The $CHCl_3$ layer was washed with H_2O, dried over anhydrous Na_2SO_4 and concentrated *in vacuo*. The residue was purified by column chromatography on silica gel to afford **12b** (61 mg), yellowish needles, mp 225—229°C (dec., C_6H_6). UV λ_{max}^{MeOH} nm (ε): 262 (21100), 290 (sh, 28400), 306 (29900), 315 (sh, 28000), 363 (8100), 406 (sh, 13800), 426 (22700). IR ν_{max}^{KBr} cm^{-1}: 3390 (NH), 1660 (C=O). MS *m/e*: 360 (M$^+$), 345, 331, 317, 303, 255, 239. *Anal.* Calcd for $C_{20}H_{20}N_6O$: C, 66.65; H, 5.59; N, 23.32. Found: C, 66.48; H, 5.69; N, 23.27.

Reaction of Paragracine (1) with HCHO and HCOOH——A solution of **1** (200 mg) in 37% HCHO (3.0 ml) and HCOOH (12.0 ml) was refluxed for 9 h, basified with 10% NaOH solution and extracted with $CHCl_3$. The $CHCl_3$ layer was washed with H_2O, dried over anhydrous Na_2SO_4 and concentrated *in vacuo*. The residue (177 mg) was chromatographed on silica gel to give unchanged starting material (60 mg) and the cyclic ether (**15**, 111 mg), yellow needles, mp 246—249°C (dec., MeOH). UV λ_{max}^{MeOH} nm (ε): 257 (12200), 312 (50700), 321 (51800), 358 (sh, 7900), 379 (14900), 419 (20300). MS *m/e*: 298 (M$^+$), 268, 253, 239, 226. *Anal.* Calcd for $C_{15}H_{18}N_6O$: C, 60.38; H, 6.08; N, 28.17. Found: C, 60.53; H, 6.29; N, 27.98.

Acknowledgement We wish to thank Dr. Y. Hirosaki for the collection and identification of the animal materials.

References and Notes

1) A part of this work was presented at the 1st International Symposium on Marine Natural Products, Aberdeen, Scotland, September 1975, and at the 19th Symposium on the Chemistry of Natural Products, Hiroshima, October 1975, Abstracts, p. 255, and published in a preliminary communication, Y. Komoda, S. Kaneko, M. Yamamoto, M. Ishikawa, A. Itai, and Y. Iitaka, *Chem. Pharm. Bull.*, **23**, 2464 (1975).
2) Y. Komoda, T. Kanayasu, and M. Ishikawa, *Chem. Pharm. Bull.*, **27**, 2491 (1979).
3) A full account of the X-ray crystallographic analysis of paragracine will be published elsewhere.
4) The species formerly considered as *E. arenaceus* is probably *P. axinellae* and *vice versa*; a private communication from Professor G. Prota.
5) L. Cariello, S. Crescenzi, G. Prota, and L. Zanetti, *Tetrahedron*, **30**, 4191 (1974).
6) R.E. Schwartz, M.B. Yunker, P.J. Scheuer, and T. Ottersen, *Tetrahedron Lett.*, **1978**, 2235.
7) *a*) L. Cariello, S. Crescenzi, G. Prota, F. Giordano, and L. Mazzarella, *Chem. Commun.*, **1973**, 99. *b*) L. Cariello, S. Crescenzi, G. Prota, S. Capasso, F. Giordano, and L. Mazzarella, *Tetrahedron*, **30**, 3281 (1974); *c*) L. Cariello, S. Crescenzi, G. Prota, and L. Zanetti, *Experientia*, **30**, 849 (1974); *d*) *Idem. Tetrahedron*, **30**, 3611 (1974).
8) M. Braun, G. Büchi, and D.F. Bushey, *J. Am. Chem. Soc.*, **100**, 4208 (1978); M. Braun and G. Büchi, *ibid.*, **98**, 3049 (1976).
9) S. Kaneko, *Ochanomizu Med. J.*, **24**, 63 (1976).
10) I. Seyama, C.H. Wu, and T. Narahashi, *Biophys. J.*, **29**, 531 (1980); C.H. Wu, I. Seyama, and T. Narahashi, *ibid.*, **25**, 135a (1979). (Abstr.).
11) The preliminarily elucidated structures[1,9] of the derivatives (**8**, **9**, **10**, **11**, **13** and **14**) obtained from paragracine should be corrected as described in this report.
12) The preliminary results of studies on the relationship between the structure and biological activity of paragracine and its derivatives have been published,[9] and a full account will be published elsewhere.