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Structure of Paragracine, A Biological Active Marine Base from *Parazoanthus gracilis* (Lwowsky)

A papaverine-like active compound named paragracine was isolated from *Parazoanthus gracilis* (Lwowsky).

The structure of paragracine was determined to be **1** from its chemical and spectral properties and by X-ray crystallographic analysis of paragracine dihydrobromide trihydrate.

In the course of a research for isolation of biological active substances from marine natural products, a methanol extract of *Parazoanthus gracilis* (Lwowsky) collected at Sagami Bay in Japan showed histamine-like activity. On the way to isolate the active principle, however, we found that papaverine-like active substances were also contained. We herein report the isolation and structural elucidation of paragracine (**1**), a main component of the marine bases with papaverine-like activity in *Parazoanthus gracilis*.

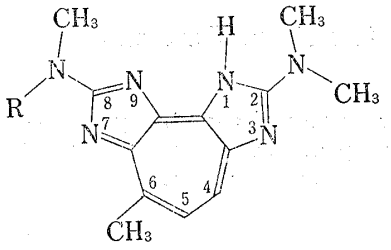
A water solution of the methanol extract of *Parazoanthus gracilis* (Japanese name: Sennari-sunaginchaku) together with *Dentitheca habereri* (Stechow) (Japanese name: Sudaregaya) (the former is parasitic on the latter) was past through an Amberlite XAD-2 column, which was then eluted with methanol. The methanol eluate was further purified by column chromatography on alumina to give a strong fluorescent substance, paragracine (**1**), yellow needles, mp 258—262° (decom.), $C_{13}H_{16}N_6$, *m/e* 256 (M^+), UV $\lambda_{max}^{ethanol}$ nm (ε): 230 (shoulder, 15000), 252 (13000), 305 (shoulder, 47900), 316 (62200), 373 (15000), 409 (14400), and 428 (shoulder, 3800), NMR (DMSO-d_6) τ: 7.29 (3H, s, 6-CH_3), 6.95 (3H, s, 8-NCH_3), 6.75 (6H, s, 2-$N(CH_3)_2$), 2.88 (2H, broad s, D_2O-exchangeable, 2×NH), 2.64 (1H, d, J=11 Hz, 4- or 5-H), and 2.40 (1H, d, J=11 Hz, 5- or 4-H). When treated with acetic anhydride and pyridine, paragracine afforded monoacetyl paragracine (**2**), mp 233—235° (decomp.), $C_{15}H_{18}N_6O$, *m/e* 298 (M^+), NMR ($CDCl_3$) τ: 7.55 (3H, s, 8-$NCOCH_3$), 7.22 (3H, s, 6-CH_3), 6.65 (6H, s, 2-$N(CH_3)_2$), 6.35 (3H, s, 8-NCH_3), 2.57 (1H, d, J=11 Hz, 4- or 5-H), 2.14 (1H, d, J=11 Hz, 5- or 4-H), and 0.23 (1H, broad s, D_2O-exchangeable, NH).

With hydrobromic acid paragracine formed dihydrobromide, which was submitted to X-ray crystallographic analysis to determine the structure.

The crystals of paragracine dihydrobromide trihydrate are monoclinic, space group $P2_1/n$ with a=9.828, b=32.231, c=5.990 Å, β=95.9°, Z=4, D_x=1.665 g/cm^3. Intensity data were collected on a Philips automated four-circle diffractometer, using graphite monochromated Cu Kα radiation. A total of 2858 independent reflections, which were above 2σ(I) level, were used for structure determination. The structure was solved by the Patterson and heavy atom method. All the hydrogens were found from a difference electron-density map calculated after refinement by several cycles of block-diagonal least-squares with anisotropic temperature factors for the non-hydrogen atoms. The final refinement which includes the hydrogen atoms with isotropic temperature factors gave the R-factor of 0.039.

As shown in Fig. 1, the molecule has a good planarity as a whole with the mean deviation of atoms from the least-square plane, 0.009Å. Bond lengths also indicate that all the bonds excepting the bonds related to the terminal methyl groups bear a considerable amount of double-bond character. Especially, the bond lengths of the two exocyclic C-N bonds are significantly short (1.317 and 1.313Å). One of the bromide ions forms hydrogen bond to the secondary amine, and the other indirectly binds to the ring nitrogens mediated by water molecules. All the four ring nitrogen atoms form hydrogen bonds to the water molecules,

1) L. Cariello, S. Crescenzi, G. Prota, and L. Zanetti, *Tetrahedron*, **30**, 4191 (1974).



1: R=H
2: R=$COCH_3$

Chart 1

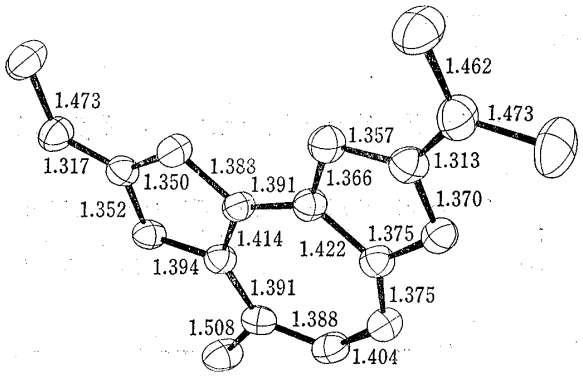


Fig. 1. An ORTEP Drawing of the Paragracine Molecule viewed down along a-Axis with the Bond Lengths

The mean standard deviation of the bond lengths is 0.006Å.

in which the three nitrogen atoms, N(3), N(7) and N(9), donate their hydrogen atoms but the one, N(1), accepts the hydrogen atom from the water molecule.

The structure of paragracine has the same basic skelton with a 1,3,7,9-tetrazacyclopent-[*e*]azulene system as that of pseudozoanthoxanthins[1] which were isolated from *Epizoanthus arenaceus* and whose structures were proposed by chemical and spectroscopic evidences.

Paragracine showed anti-$BaCl_2$, anti-histamine, anti-bradykinin and anti-acetylcholine activities on the contraction of the various isolated organs. These results suggest that paragracine has the similar activity to that of papaverine. The details of the biological activity of paragracine will be reported elsewhere.

The isolation and structural elucidation of the other minor components related to paragracine in *Parazoanthus gracilis* is now in progress.

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