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STUDIES ON THE EXTRACTIVE COMPONENTS OF ASCIDIANS II.[1] HALOCYNINE, A NOVEL BETAINE ISOLATED FROM THE MUSCLE OF ASCIDIAN *HALOCYNTHIA RORETZI*

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Abstract: A new betaine, halocynine, was isolated from the muscle of ascidian *Halocynthia roretzi* and its structure was elucidated to be (R)-2-hydroxy-6-trimethylammoniohexanoate.

A variety of betaines has been found in marine invertebrates.[2-4] In the course of our continuing studies on the nitrogenous compounds in the muscle extract of ascidian *H. roretzi*,[1] we have encountered a Dragendorff-positive compound, whose TLC properties implied its novelty. The isolation and structural elucidation of the metabolite named halocynine (1) are the subject of this paper. Halocynine is a 2-hydroxylated analogue of laminine (2)[5] which is occasionally found in the algal extracts or in some proteins.[6-9]

1 R=OH 2 R=NH_3^+

The aqueous ethanol extract of the ascidian muscle (200 g) was partitioned between water and diethyl ether. The aqueous phase was chromatographed on Amberlite IRC 50 (H^+ form), Amberlite CG 400 (OH^- form), Dowex 50-X4 (H^+ form) and Dowex 50-X4 (pyridinium form) columns, in order, to obtain 84 mg of halocynine as colorless gum.

Halocynine was positive to Dragendorff reagent but negative to ninhydrin. Mobilities in ion exchange chromatographies suggested it to be a mono-basic and mono-acidic molecule. Its hygroscopic nature did not allow measurements of the IR and melting point. Attempts to prepare an oxalate or a hydrochloride were unsuccessful. However, as in the case with other betaines,[10] the FDMS and the FABMS gave the $(M + H)^+$ ion at m/z 190; combined with the presence of 9 carbons in the ^{13}C NMR, the molecular formula of $C_9H_{19}NO_3$ was obtained. The ^1H NMR spectrum (D_2O, acetone=2.22 ppm) revealed the presence of a trimethylammonio group (δ3.08, 9H, s), a carbinol methine adjacent to a methylene and a carboxylic acid (δ4.02, 1H, t), a methylene placed between a methylene and a nitrogen (δ3.03, 2H, t), and three contiguous methylenes (δ1.76, 4H, m and 1.40, 2H, m). The ^{13}C NMR [D_2O, dioxane=67.4 ppm, 180.8s(C1), 72.3d(C2), 67.4t(C6), 53.6q(3 x N-CH_3), 34.0t(C3), 22.9t(C5), 22.0t(C4)] supported the presence of above structural units. These data readily assign the structure of halocynine as 2-hydroxy-6-trimethylammoniohexanoate. In order to confirm the position and the kind of substituents,

chemical shift values in the ^{13}C NMR of halocynine were compared with those of laminine.[11] Carbons C1 to C3 of halocynine were suffering considerable downfield shifts due to the presence of a hydroxyl group instead of an amino group, whereas C4 to C6 as well as the three N-methyl groups gave the same chemical shifts. This is also true of the ^{1}H NMR; the protons on C2 and C3 showed different values, while other protons exhibited essentially the same shifts.[12] On the other hand, CD spectrum of halocynine showed a negative Cotton effect at 208 nm (1 N HCl, $[\theta]=-97 \times 10^3$) to assign the D(2R)-configuration.[13] Thus halocynine is (R)-2-hydroxy-6-trimethylammoniohexanoate.

In order to verify the assigned structure, L-lysine was thoroughly methylated as Cu complex[14] followed by treatment with $NaNO_2$ in 0.1 M pyridine-acetic acid buffer (pH 3.75).[15] The synthetic product gave identical TLC, NMR and FABMS features to the natural one, though the CD spectrum exhibited an opposite Cotton effect.[16]

Among lysine-derived betaines, halocynine is the first example of a 2-hydroxylated variant, though 2-oxo derivative is known as biosynthetic intermediate.[17] As halocynine possesses D-configuration, it is obviously biosynthesized from L-lysine. Considerable amount of halocynine is present in the tissue of the ascidian; its accumulation mechanism and physiological role in the animal may be an interesting problem.

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