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# 의충동물 개불의 화학적 성분 연구

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# A Study on the Chemical Constituents from the Echiura *Urechis unicintus*

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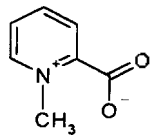
Marine natural product research programs have provided a spectacular array of novel compounds and now marine organisms have been recognized as sources of novel drugs due at least in part to the unique environmental conditions. Several thousand marine natural products[1] have been chemically defined; many of these are biologically active compounds possessing novel functional groups and molecular structures. Thus, hundreds of unique, biologically-active natural products isolated from marine organisms provide an untapped resource for future biomedical applications.

*Urechis unicintus* belongs to the phylum Echiura and lives in the intertidal mudflats in U-shaped burrows. Although much effort has been made to obtain bioactive compounds from marine animals, little is known about the secondary metabolites of these animals. In the previous report,[2,3] four compounds of anthraquinone derivatives and a couple of sterols were isolated from dichloromethan extracts of body wall of *Urechis unicintus*.

We were interested in bioactive compounds of this animal because methanol extracts showed the hemolytic activity against human erythrocytes (red blood cells) and rat erythrocytes.[4] We report here the results of our investigation of methanol extracts of *U. unicintus*. Chemical investigation of this animal resulted in the isolation and characterization of a variety of nucleosides, amino acids and known compounds.

*Urechis unicintus* was collected at seashore of west side sea of choongchungnam-do in January, 1997. Three individuals of *U. unicintus* were dissected into the mantle, viscera(all internal organs) and coelomic fluid. The freeze-dryed mantle of *U. unicintus* (740 g) were extracted sequentially in hexane, chloroform, methanol and 30% acqueous methanol. The *U. unicintus* methanol extract (1.5 g) was fractionated by gel filteration (Sephadex LH-20) with methanol as eluent. The second LH-20 fraction (75 mg) was purified by $C_{18}$ reverse-phase HPLC using $H_2O$-MeOH (9:1) to yield a fraction whose $^1H$ NMR spectrum revealed structural features of homarine[5] (1) (10 mg).



Homarine 1

The $^1$H NMR spectrum of homarine (1) in MeOH-$d_4$ revealed four aromatic signals and one methyl signal. The doublets at 8.76 ppm ($J$=5.8 Hz) and 8.09 ppm ($J$=8.0 Hz), and the triplets at 8.52 ppm ($J$=8.0 Hz) and 7.94 ppm ($J$=8.0 Hz) in its $^1$H NMR spectum were suggestive of four aromatic protons existing at 1, 2, 3, 4 conjugated positions. The $^{13}$C and DEPT NMR spectra showed the presence of seven carbons, including one carbonyl signal at 164.47 ppm, five aromatic signals from 146.28 to 126.76 ppm, and one methyl signal at 46.72 ppm. The high-resolution mass spectrum of homarine (1) supported a molecular formular of $C_7H_7NO_2$ which appeared as a pyridine derivative. Correlations of the methyl protons at 4.43 ppm to the 2, 6 pyridine carbons via HMBC spectroscopy allowed assignment of a homarine as *N*-methyl picolinic acid. We prepared homarine from picolinic acid (Aldrich) for use as a standard. Picolinic acid (1.23 g, 1 mmol) was refluxed for 10hr in ethanol with methyl iodide (2.82 g, 20 mmol). Homarine was recrystallized from methanol to yield 1.28 g (93%). NMR spectrum data of homarine were correct to those of the synthetic one.

Homarine has been found in a wide diversity of marine invertebrates and has been suggested to serve as an osmolyte.[6] Additionally homarine has been demonstrated to have chemical bioactivity, significantly inhibiting the growth of the potentially fouling benthic marine pennate diatom *Navicula salinicola* at naturally occurring concentrations.[7]

The third LH-20 fraction (950 mg) was subjected to Silica gel normal-phase HPLC with $CHCl_3$-MeOH-$H_2O$ (4:5:1). Further purification was achieved by CLC-$NH_2$ HPLC with $H_2O$-AcCN (7:3) to yield three compounds, taurine (95 mg), alanine (93 mg), glycine (112 mg).

Alanine and glycine showed typical amino acid $^1$H NMR peaks which were exchangable protons of amine and carboxylic acid in DMSO-$d_6$. Chemical shifts of these compounds were the same as those of commercial samples.

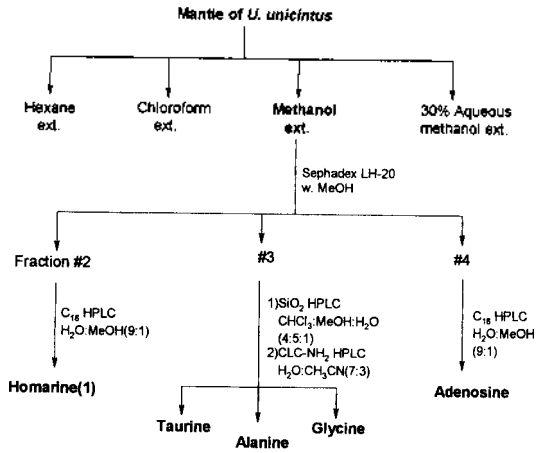
The $^1$H NMR spectrum of taurine in DMSO-$d_6$ revealed two ethylene signals and broad exchangable amine and hydroxy proton signals. The mass spectrum of taurine showed the molecular weight of 125 amu ($C_2H_7NO_3S$).

Taurine(2-aminoethane sulfonate), a nonprotein amino acid, is a small organic solute that can be accumulated to high intracellular concentrations without perturbing macromolecules.[8] Although the functional roles of taurine are numerous and still being defined,[9] its role in osmoregulation is well established. Marine invertebrates, most notably molluscs and arthropods, have high concentrations of taurine that regulate tissue osmolarity.[10]

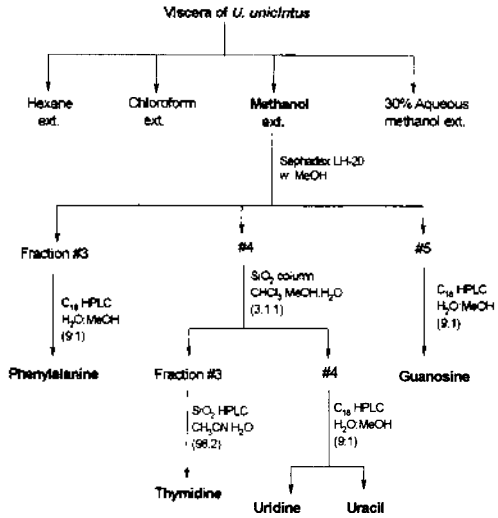
The fourth LH-20 fraction (132 mg) was purified by $C_{18}$ reverse-phase HPLC using $H_2O$-MeOH (9:1) to give adenosine (12 mg) (*Scheme* 1).

Adenosine showed also the typical $^1$H NMR nucleoside peaks which represented the sugar and purine base. Retention time and NMR data of adenosine were correct to those of the standard sample.

The freeze-dryed viscera of *U. unicintus* were extracted as the same method as the mantle part. The majority of LH-20 fractions displayed $^1$H




*Scheme* 1.




*Scheme* 2.

NMR signals indicative of amino acids and nucleosides. Separation and purification of these fractions was achieved by reversed phase and Silica gel normal phase chromatography, resulting in the isolation of phenylalanine, thymidine, uridine, uracil and guanosine (*Scheme* 2).

These nucleosides also showed the same value of chemical shifts of NMR data of standard samples.

In summary, the polar constituents including homarine, taurine, alanine, glycine and adenosine were first isolated and characterized from methanol extracts of mantle of *U. unicintus*. Also, phenylalanine, thymidine, uridine, uracile and guanosine were first purified and characterized from methanol extracts of viscera of *U. unicintus*. Those compounds have been unreported from this animal. We are investigating for the hemolytic activity and anti-platelet aggregation test using these isolated compounds.

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