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SHORT COMMUNICATIONS

Occurrence of *N*-urocanylhistamine in the soft tissues of the gastropod mollusc *Drupa concatenata* Lam

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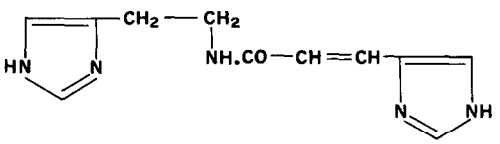
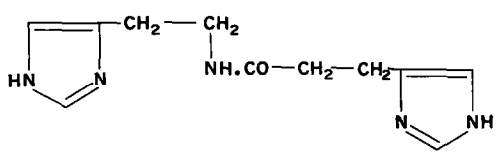
IT HAS been shown, in a preceding paper,[1] that methanol extracts of the total soft tissues of *Drupa concatenata* Lam., a gastropod of the Philippines, contained large amounts of *N*-imidazolepropionylhistamine, a *N*-acylated histamine derivative so far unknown in nature. In addition to *N*-imidazolepropionylhistamine the above extracts contained another new histamine derivative. This has now been identified as *N*-urocanylhistamine.



N - Urocanylhistamine



N - Imidazolepropionylhistamine

Several hundred of specimens of *Drupa concatenata* were collected near Dumaguete City (Negros Oriental). The whole soft tissues were removed from the living animals after cautious rupture of the shell and immediately extracted with 5 parts (w/v) of methanol.

After 10–30 days the supernatant liquid was decanted and the tissues re-extracted with 5 parts of 80% methanol.

The extracts were mixed and filtered. Part of them was studied as such but the greatest part was submitted to chromatography on an alcaline alumina column which was eluted with descending concentrations of ethanol.

Both the crude extracts and the eluates from the alumina column were submitted to paper chromatography, thin-layer chromatography, high voltage paper electrophoresis and bioassay, using the isolated guinea-pig ileum. Chromatograms and pherograms were developed with Pauly reagent, the NNCD reagent (2-chloro 4-nitrobenzenediazonium naphthalene-2-sulphonate), the dichloroquinone chlorimide reagent, and finally the Dragendorff reagent.

Synthetic urocanic acid, histamine dihydrochloride, *N*-acetylhistamine and *N*-imidazolpropionylhistamine were available for comparison.

Following chromatography on alumina column, the histamine derivative supposed to be *N*-urocanylhistamine (UCH) emerged in the 98–95% ethanol eluate, just prior to, and partly together with, *N*-imidazolepropionylhistamine.

A 98% ethanol eluate containing only UCH was submitted to hydrolysis with hydrochloric acid (6N HCl, 6 hr at 100°). Two imidazole derivatives were obtained which were indistinguishable by paper and thin-layer chromatography, paper electrophoresis and bioassay from urocanic acid and histamine, respectively.

For each mole of histamine, as estimated by bioassay, 1 mole of urocanic acid, as estimated by the Pauly reaction, was liberated. Synthetic *N*-urocanylhistamine was as yet not prepared, but there is no doubt that UCH may be identified with it, also because no other compound may exist which, upon acid hydrolysis, liberates histamine and urocanic acid in equimolar amounts.

A number of characteristics of UCH have been assessed: orange red colour with the Pauly reagent, and lilac colour with the Dragendorff reagent; mobility towards the cathode on high voltage electrophoresis, $E_{1\cdot2} = 0{\cdot}95$–$0{\cdot}98$ His, $E_{5\cdot8} = 0{\cdot}84$–$0{\cdot}9$ His.

R_f values in paper chromatography: 0·3–0·35 in *n*-butanol acetic acid water (4:1:5); 0·52–0·57 in *n*-butanol 35% methylamine (8:3); 0·52–0·55 in 1-pentanol pyridine water (40:40:10); 0·48–0·50 in methylethylketone pyridine water 35% methylamine (65:15:10:0·5); and finally 0·28–0·3 in 20% KCl.
—R_f values in thin-layer chromatography on silica gel: 0·15–0·2 in *n*-butanol acetic acid water (4:1:5); 0·63–0·67 in *n*-butanol 35% methylamine (8:3); and 0·29–0·31 in *n*-butanol ethanol 35% methylamine (22:7:1).

The content in *N*-urocanylhistamine of a crude extract of total soft tissues prepared from a large batch of snails was approximately 200 μg/g fresh tissue.

Dye-secreting gastropods belonging to the families *Muricidae*, *Thaididae* and *Drupae* contain in their soft tissues, more precisely in their hypobranchial gland, more or less conspicuous amounts of murexine and/or dihydromurexine, i.e. of the choline esters of urocanic acid and imidazolepropionic acid, respectively.

From present data on imidazolehistamines obtained in *Drupa concatenata* it appears evident that each of the above two imidazole acids may occur in the gastropod body either linked to choline, with a rather labile ester bond, or to histamine, with a much more tenacious peptide bond.

Research is in progress to investigate whether the new histamine derivatives are used by *Drupa concatenata*, a carnivorous gastropod, in the capture of the prey.

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The effect of sulfisoxazole (Gantrisin) and albumin on bilirubin conjugation in cultures of a clonal cell line with liver-like functions

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The elimination of bilirubin from the organism involves several processes: uptake of bilirubin by the liver cells, conjugation of bilirubin with glucuronic acid, and excretion of bilirubin-glucuronide. Variations in each of these processes may influence the total rate of bilirubin elimination.[1–3] In the blood, unconjugated bilirubin is transported bound to albumin.[4,5] The uptake of bilirubin into the liver cells seems to involve the dissociation of bilirubin from the albumin molecule before bilirubin enters the cells.[6]

Several drugs are bound to serum albumin under *in vivo* conditions, and it is well known that one drug can displace another from its binding to albumin. It has also been shown that various substances, endogenous as well as exogenous, influence the binding of bilirubin to albumin.[7–9] Sulfisoxazole (Gantrisin) is extensively bound to albumin (at a plasma concentration of 100 ng/ml approx. 84 per cent are protein