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FISH ATTRACTANTS FROM MARINE INVERTEBRATES

ARCAMINE FROM *ARCA ZEBRA* AND STROMBINE FROM *STROMBUS GIGAS*

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Abstract—The isolation and characterisation of two fish attractants, a new dipeptide (Arcamine, hypotauryl-2-carboxyglycine), **1** and Strombine (C-methyl-imino diacetic acid), **2** are described.

Previous attempts to identify fish attracting principles from natural sources have implicated mixtures of amino acids as being mainly responsible for the activity,[1-3] characterised as Exploratory Feeding Behaviour.[4] Two specific compounds which have elicited this response have been isolated and characterised.

Arcamine. Arcamine was isolated by freeze drying as a colourless syrup after ion exchange chromatography of an *Arca zebra* extract on Dowex 50. Thin layer chromatography of the sample immediately on silica gel showed one spot only in three different solvent systems. After a few hours a second chromatogram showed traces of a second spot which was identified as hypotaurine by comparison with an authentic sample. This sample, which contained hypotaurine, was used for a two-dimensional chromatography on MN 300 cellulose powder and for degradative studies as well as NMR and field desorption mass spectrometry. The freeze dried product was also purified by cellulose powder thin layer chromatography. The pure material from a small preparative plate was converted to the trimethylsilyl derivative which was used for mass spectroscopy. Further oxidative degradation on standing or mild base hydrolysis yielded three additional spots, one identified as taurine, the second as glycine through its DNP derivative, and the third tentatively as 2-amino malonic acid.

An NMR spectrum of the syrup known to contain hypotaurine, in D_2O with TMS as external standard, had peaks at δ 3·1 (triplet), δ 3·7 (triplet) assigned to adjacent methylene groups and a peak at δ 4·8 (singlet) assigned to a single hydrogen. The IR spectrum showed the presence of amide (1639 cm^{-1}) and carboxylate ion (1580 cm^{-1}), as well as free amino (3300 cm^{-1}). A field desorption mass spectrum[5] (Fig. 1) showing prominent peaks at m/e 166 (M^+-CO_2), 149 (M^+-H_2O, COOH), 145 (M^+-SOOH), 127 (M^+-H_2O, COOH) supports the structure **1**, though the molecular ion (M^+ 210) is absent, confirming the instability of the molecule.

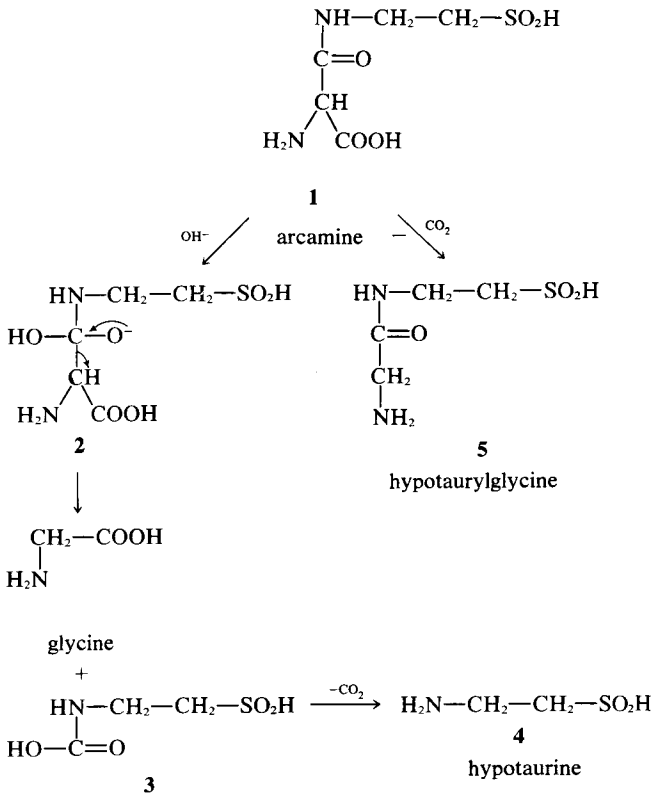
Arcamine purified by cellulose powder thin layer chromatography was also shown by electrophoresis to be different from hypotaurine. The gum dissolved in water, however, produced hypotaurine and therefore taurine as well as glycine within minutes. The hydrolysis of the molecule is explained by Scheme A. The decarboxylation of the β-keto acid is also feasible, but the postulated hypotaurylglycine **5**, which would be formed, was not detected by thin layer chromatography or NMR spectroscopy.

The base peak at m/e 61 is postulated as arising either by hydrogen transfer and loss of sulphur monoxide with retention of hydroxyl in arcamine (Scheme B) or alternatively from hypotaurine without evoking H transfer (Scheme C).

The mass spectrum of the silylated material is also compatible with the structure proposed for arcamine. The peak at m/e 356, which is the main high mass peak, represents the bis-trimethylsilylated fragment of the tetra-trimethylsilylated derivative (498 − 142). Peaks at 341 (loss of CH_3, 356 − 15); 284 (loss of $Si(CH_3)_3$–H, 356 − 72); and 239 (loss of CO_2–$Si(CH_3)_3$, 356 − 117) are evident in the high mass region as well as several other peaks of low intensity in the high mass region. This is in accordance with results obtained by Baker, Shaw and Williams[6] from TMS-amino acids and peptides. The unusual base peak at m/e 57 can be explained as due to the ion $CH_2{=}Si^+{-}CH_3$ produced from $\overset{+}{Si}(CH_3)_3$.

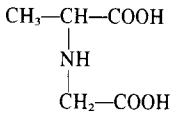
Strombine. Strombine was isolated as its magnesium-salt from an aqueous/ethanolic extract of *Strombus gigas.*

The magnesium salt, melting above 300°C, was converted to the hydrochloride by dissolving in dilute hydrochloric acid and then precipitating the magnesium as phosphate with ammonium phosphate and ammonia. Strombine was recovered as the hydrochloride salt by cation exchange on Dowex-50 followed by anion exchange on Dowex-1. Strombine hydrochloride m.p. 131°C, λ_{max} (nujol) 3080 cm^{-1}), has the molecular formula $C_5H_{10}NO_4Cl$ (microanalysis and potentiometric titration equivalent weight average 184). The IR spectrum is characteristic of an amino acid hydrochloride. The NMR spectrum (in D_2O with an external TMS standard) is fully in agreement with the structure **2** and shows a doublet at δ



Scheme A

2·0 (3H) J = 7·0 Hz, a singlet at δ 4·18 (2H) and a quartet at δ 4·17 (1H) J = 7·0 Hz.



2

Strombine hydrochloride was synthesised by condensing alanine and monochloroacetic acid at a controlled pH of approximately 7. The product was isolated by ion exchange chromatography as described above.

EXPERIMENTAL

M.ps were determined on a Kofler block, IR spectra on a Perkin Elmer 137 Infracord. NMR spectra, Varian A 60, (TMS as external standard); Thin layer chromatography on silica gel or cellulose powder in solvent systems (1) acetone/water 3:1, (2) phenol/water 3:1, (3) butanol/acetic acid/water 3:1.

Isolation of Arcamine. In a typical experiment three hundred mussels (2 kg.) were homogenised and allowed to stand overnight at low temperature (4°C) in 75% aqueous ethanol (10 l.). The extract was filtered on a hyflo pad and evaporated *in vacuo* to yield a clear gum (25 g.) The gum (12 g.) was dissolved in water and chromatographed on Dowex-50 resin (H^+) form. The active fraction was weakly absorbed and was eluted with dilute (0·1 N) ammonium hydroxide solution. The fraction was evaporated and again put onto a column of Dowex-50 (H^+) form. On prolonged washing with water arcamine was eluted and freeze dried to colourless sticky droplets which decomposed to hypotaurine, taurine and glycine. Hypotaurine was identified on isolation by TLC and comparison with an authentic sample.

Hydrolysis of Arcamine—Preparative thin layer chromatography. 40 mg. Arcamine was dissolved in 2 ml 0·05 N sodium hydroxide. At room temperature after two hours, two dimensional thin layer chromatography showed five spots. Preparative thin layer chromatography was used to isolate glycine, confirmed as its 2,4-dinitrophenyl derivative, and taurine, confirmed by IR spectral comparison with an authentic sample.

Isolation of Strombine (Mg salt). In a typical experiment the flesh of ten conchs (593 g) was homogenised with distilled water

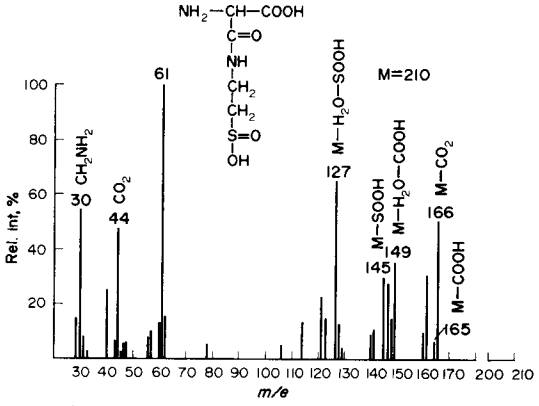
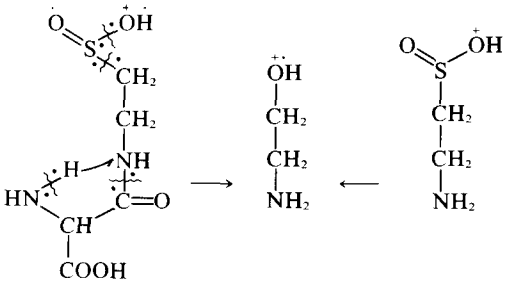


Fig. 1. Field desorption mass spectrum of arcamine.



m/e 61

Scheme B Scheme C

and then mixed with alcohol (total volume 10 l) and allowed to stand overnight at low temperature (4°C). The extract was filtered and evaporated *in vacuo* to 500 ml. On standing overnight in the freezer Strombine Mg salt crystallised out (8·2 g). Further standing gave small quantities of mytilitol (20 mg). The filtrate was evaporated to yield a brown gum (22 g).

Synthesis of Strombine hydrochloride. To a concentrated solution of alanine (1·84 g) 1 M sodium carbonate and 1 M monochloroacetic acid solution were added slowly, each from a burette. The temperature was maintained at 60°C and the pH at 7·0 by monitoring with a pH meter. The reaction was followed by TLC sampling at half-hour intervals, and the reaction was stopped when alanine had disappeared from the reaction mixture.

The synthetic product was isolated by column chromatography and elution from Dowex 1 with 1 M hydrochloric acid. The product was recrystallized from ethanol and ether, m.p. 131°C; yield 1·98 g (52%) (Found: C, 32·70; H, 5·50; N, 7·65; O, 34·85; Cl. 19·30. Calc. for $C_5H_{10}NO_4Cl$: C, 32·70; H, 5·60; N, 7·60; O, 35·05; Cl, 19·60%).

Fish attraction. Dilutions of 10^{-8} g/l of **1** and **2** were active in inducing small test fish to display feeding behaviour in an experimental aquarium.[3,4]

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