

Oxytocic Principle of Red Alga *Amphiroa fragilissima*

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Abstract

The crude aqueous methanolic extract of the marine red alga *Amphiroa fragilissima* has been reported as exhibiting oxytocic and spasmogenic activity at a dose of 50 µg/ml. The activity is located in the water soluble fraction and has been found to be due to the presence of a biogenic amine. Histamine was characterized on the basis of spectral data and the identity confirmed by comparison with an authentic sample.

Introduction

Naqvi *et al.* (1980) reported that the aqueous alcoholic extract of the marine alga, *Amphiroa fragilissima* (L.) Lamouroux had oxytocic and spasmogenic activities. In this communication we described the isolation and identification of an oxytocic principle present in this red alga.

Material and Methods

Fresh algal specimens (3 kg dry wt.) were hand picked from the intertidal zone of Anjuna beach (Goa coast), washed, air dried and extracted thrice by immersion in 90% methanol. After filtration the filtrate was concentrated to dryness on a rotary evaporator at 50 °C. This crude extract and the fractions obtained from it by sequential extraction with methylene chloride, ethyl acetate and water were tested for oxytocic activity on isolated guinea pig and rat uteri using the method of Mehrotra *et al.* (1985). The methylene chloride and ethyl acetate fractions being inactive, were discarded.

Solutions of different concentrations of the testing crude extract and fractions were prepared in either distilled water or 50% ethanol depending on the solubility. Standard contractions were produced with oxytocin. Test substances were added to both test

preparations in different doses and allowed to act for 5 minutes to produce contraction.

TLC analysis of the active fraction in NH₃-acetonitrile (10:90) using Pauly's reagent (diazo-benzo-sulfonic acid) as the detecting agent (Ames and Mitchell 1952) indicated the presence of compounds with a β-imidazole nucleus one of which had the same RF value as histamine. This active fraction was extracted thrice with dilute HCl. The acidic aqueous extract, after concentration under reduced pressure, was evaporated to dryness in a vacuum desiccator over KOH pellets and then chromatographed over florisil. The column was developed with increasing concentrations of NH₃ in ethanol. Column effluence was monitored by TLC. Material eluted with 4% NH₃ in ethanol contained, as a major constituent, the substance having the same RF value as histamine. It was tested again for the observed activity. Further purification of this histamine-like substance was carried out by repeated chromatography over florisil and then subjected to spectral analysis, yield 23 mg.

Results and Discussion

A methanolic extract of *Amphiroa fragilissima* produced contractions which were not concentration dependent, indicating that the oxytocic agent was prob-

ably an histamine-like substance (Mehrotra *et al.* 1985, Dews and Graham 1946). Sequential extraction of the dry extract with methylene chloride, ethyl acetate and water, and testing these extracts for oxytocic activity, showed that the activity was produced only by the aqueous extract.

Thin-layer chromatographic examination revealed a compound with identical characteristics to histamine. The active compound was isolated by column chromatography and shown to be histamine from m. p. (240 °C), mixed m. p., 1 HNMR spectroscopic and mass spectral data.

It has been observed that the occurrence of this biogenic amine in the alga is seasonal. *Amphiroa fragilissima* grows on *Gemmaria sp.*, a zooanthid, the water soluble fraction of which exhibited hypotensive activity (Kamat *et al.* 1981). The zooanthid contains this amine when it is absent in the alga and is devoid of it when present in the seaweed. This observation indicated that there might exist some symbiotic relationship between these two marine organisms but

it is not clear whether the histamine isolated is an algal metabolite or has been originated from the zooanthid. Even though TLC of the acid mixture indicated the presence of four more compounds possessing a β -imidazole nucleus, these metabolites could be transformation products of the histamine coming from the zooanthid. The presence of histamine in the red alga *Furcellaria lumbricallis* has also been reported recently by Barwell (1979).

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