

## THE OCCURRENCE OF N-METHYLTAURINE IN THE SEA ANEMONE *BUNODOSOMA CAISSARUM*

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**ABSTRACT:** Significant amounts of N-Methyltaurine together with the recently described novel purine Caissarone, were isolated from the acetonic and methanolic extracts of the whole body wall of the sea anemone *Bunodosoma caissarum*. The structure of N-Methyltaurine was deduced by spectroscopic methods.

**KEY WORDS:** Sea anemones; Cnidaria; *Bunodosoma caissarum* Corrêa; N-methyltaurine; caissarone.

### INTRODUCTION

Sea anemones belong to the *phylum* Cnidaria, one of the morphological features of which is the nematocyst or cnidocyst apparatus, also known as stinging cells, useful for prey capture. Fishes and small invertebrates are paralyzed<sup>3</sup> on the discharge of the nematocysts which may also inflict injurious effects on man, ranging from mild dermatitis to severe local necrosis<sup>10</sup>. Since toxicity is indicative of potent physiological activity, these marine organisms have attracted considerable attention because of their potential biological value. Chemical investigations have gained growing interest since the finding of tetramine, a curare-like toxin, in the tentacles of *Actinia equina*<sup>1</sup>. The various papers appearing over the last decades include descriptions of neuro- and cardio-active polypeptides<sup>2</sup>, hemolytic factors<sup>4,18</sup>, antitumoral bioassays<sup>8,20</sup> and organo-sulphuric<sup>21</sup>, organo-phosphoric<sup>14</sup>, indolic<sup>17</sup> and guanidinic<sup>6</sup> compounds.

*Bunodosoma caissarum* Corrêa (Anthozoa, Actiniaria) is a sea anemo-

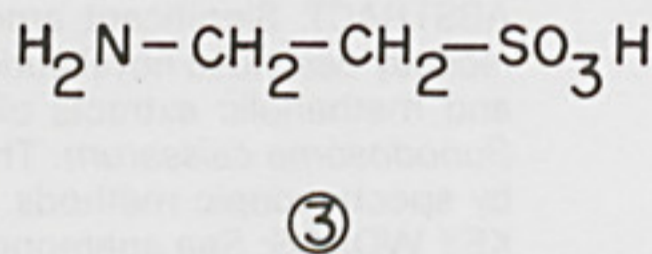
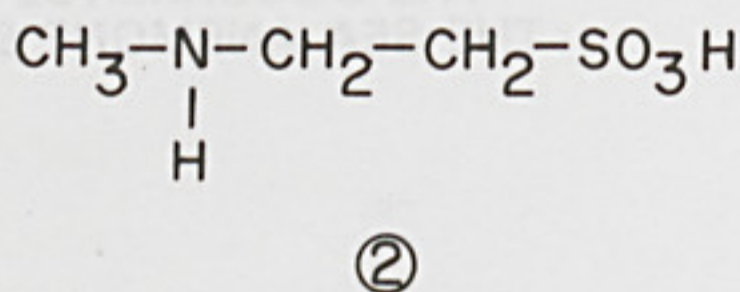
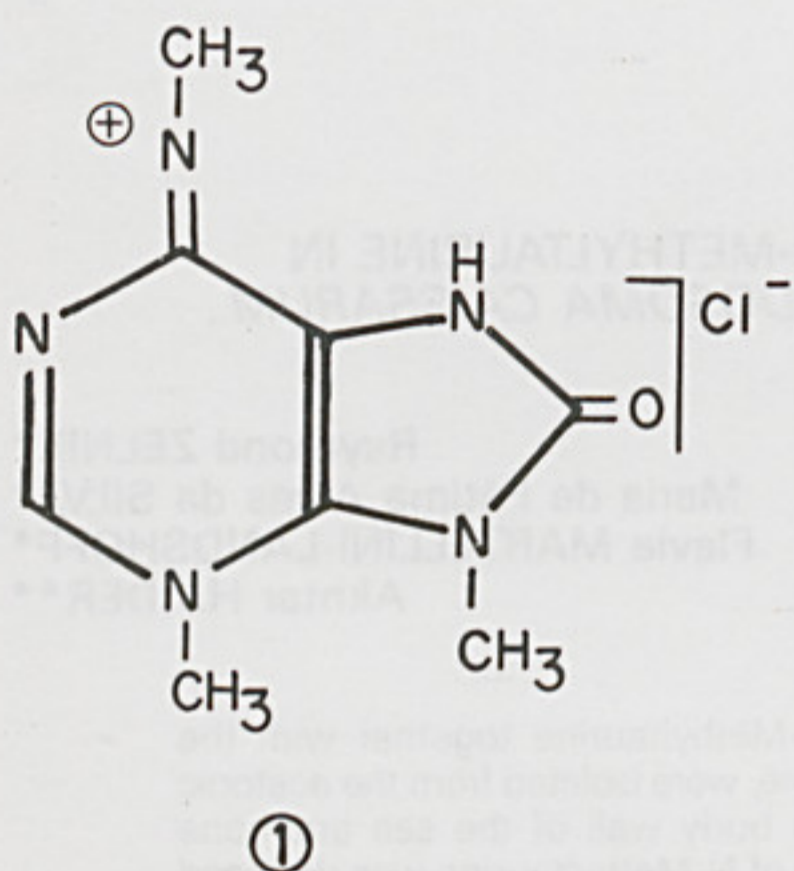
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ne abundant on reefs along the Brazilian coast<sup>7</sup>. Material released by nematocyst discharge has been shown to be active on cholinceptive preparations<sup>19</sup> and a cardiotoxic polypeptide has been recovered from a methanolic extract of the anemone<sup>13</sup>. We have recently isolated a novel purine derivative, caissarone 1, from the whole body walls of this cnidarian<sup>24</sup>. The presence of further components as revealed by thin-layer chromatography of a methanolic extract, led us to re-examine this marine organism and from which we now report the isolation of N-methyltaurine 2.

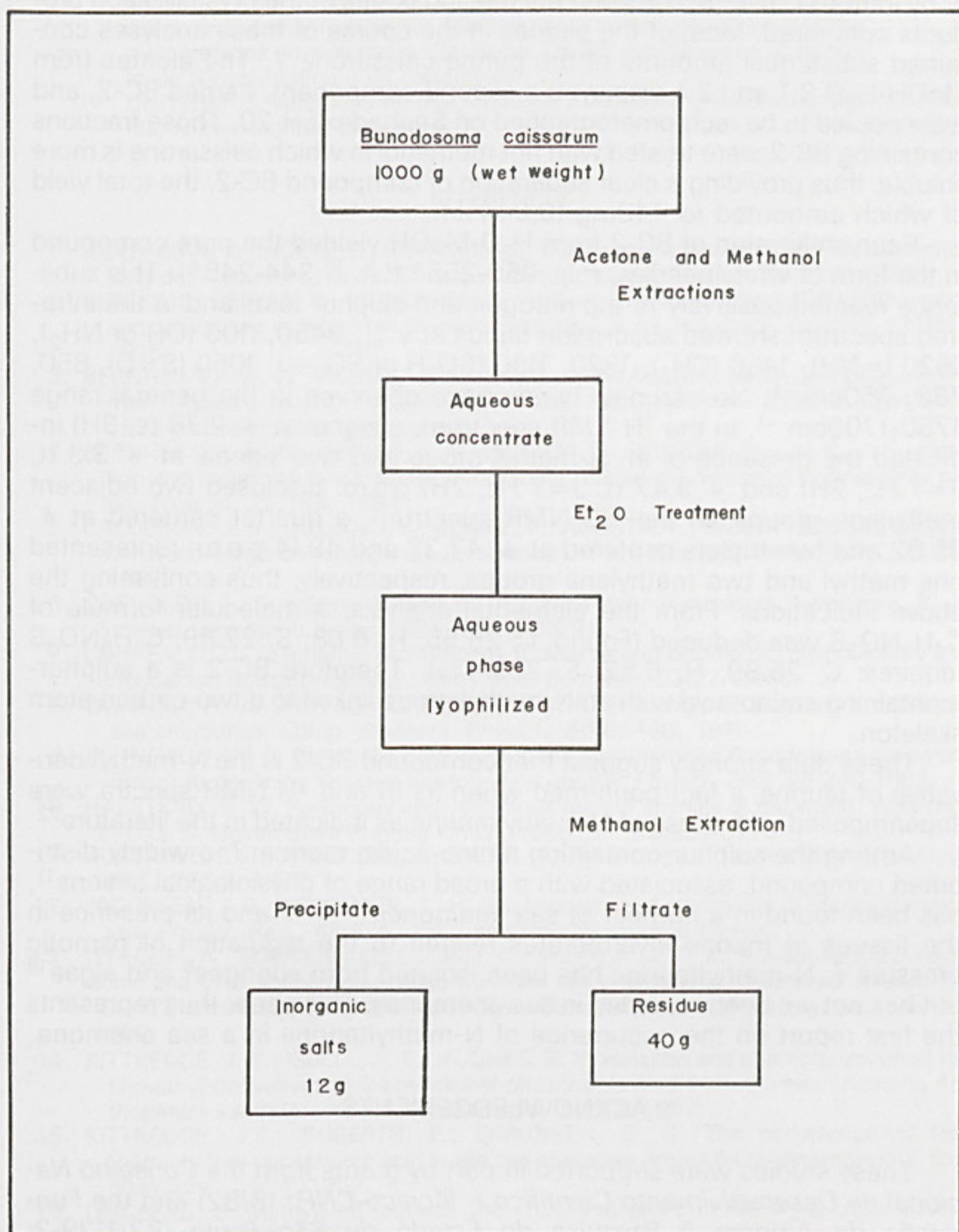


## MATERIAL AND METHODS

Specimens were collected on the São Sebastião coast, State of São Paulo, and frozen for transport. The homogenized anemones (1.000g, wet weight) were extracted with acetone (3L) and methanol (4L) at room temperature, the extracts filtered and the combined organic phases evaporated under reduced pressure. The aqueous concentrate was then treated with diethyl ether to remove pigments, lipids and sterols and lyophilized to a viscous paste which was triturated in methanol to give a whitish precipitate (12g), containing mainly inorganic salts. The methanolic filtrate was evaporated to dryness and the residue (40g) analyzed by t.l.c. (Silica H, Merck; methanol-water 2:1; iodine vapours), revealing the presence of at least three components, one of which was readily identified as caissarone 1 by comparison with an authentic sample. Table 1 summarizes the sequence of steps used in the extraction procedure.

Melting points were determined on a Reichert-Kofler hot-stage apparatus and are uncorrected. Infra-red spectra were performed in KBr pellets, using a Perkin-Elmer 737 instrument. <sup>1</sup>H and <sup>13</sup>C NMR spectra were recorded on a Bruker WH-360 spectrometer, operating at 360 and 90.5MHz, respectively, in D<sub>2</sub>O solution.

TABLE 1



## RESULTS

The material obtained from the last methanolic filtrate (see Table 1) was chromatographed on Sephadex LH-20 (Sigma Chemical C<sup>a</sup>, St. Louis, MO, USA) in batches of 3g each and the elutions were performed with graded proportions of methanol-water. The fractions were then analyzed by t.l.c.

or by infra-red spectroscopy and the melting points of the crystallization products compared. Most of the eluates in the course of these analyses contained substantial amounts of the purine caissarone 1. The eluates from MeOH-H<sub>2</sub>O 3:1 and 2:1 displayed a second component, named BC-2, and were pooled to be rechromatographed on Sephadex LH-20. Those fractions containing BC-2 were treated with hot methanol in which caissarone is more soluble, thus providing a clear separation of compound BC-2, the total yield of which amounted to 440mg (0.044%).

Recrystallization of BC-2 from H<sub>2</sub>O-MeOH yielded the pure compound in the form of white needles, m.p. 250-255° (Lit. <sup>16</sup>:244-245°). This substance reacted positively to the nitrogen and sulphur tests and in the infra-red spectrum showed absorption bands at  $\nu_{\max}$  3450, 3100 (OH or NH<sub>2</sub>), 1620 (=NH), 1480 (CH<sub>3</sub>), 1220, 1190 (SO<sub>3</sub>H or SO<sub>3</sub>-), 1050 (S=O), 850, 780, 750cm<sup>-1</sup>. No carbonyl bands were observed in the general range 1750-1700cm<sup>-1</sup>. In the <sup>1</sup>H NMR spectrum, a signal at  $\delta$  2.78 (s, 3H) indicated the presence of an N-methyl group and two signals at  $\delta$  3.3 (t, J=7 Hz, 2H) and  $\delta$  3.47 (t, J=7 Hz, 2H) p.p.m. disclosed two adjacent methylene groups. In the <sup>13</sup>C NMR spectrum, a quartet centered at  $\delta$  35.82 and two triplets centered at  $\delta$  47.32 and 49.14 p.p.m. represented one methyl and two methylene groups, respectively, thus confirming the above indications. From the elemental analysis, a molecular formula of C<sub>3</sub>H<sub>9</sub>NO<sub>3</sub>S was deduced (Found: C, 25.55; H, 6.68; S, 22.89; C<sub>3</sub>H<sub>9</sub>NO<sub>3</sub>S requires: C, 25.89; H, 6.52; S, 23.04%). Therefore BC-2 is a sulphur-containing amino-acid with an N-methyl group linked to a two-carbon atom skeleton.

These data strongly suggest that compound BC-2 is the N-methyl derivative of taurine, a fact confirmed when its IR and <sup>1</sup>H NMR spectra were superimposed with those of N-methyltaurine as indicated in the literature<sup>22</sup>.

Among the sulphur-containing amino-acids, taurine 3, a widely distributed compound, associated with a broad range of physiological actions<sup>11</sup>, has been found in a number of sea anemones<sup>21,9,23,15</sup> and its presence in the tissues of marine invertebrates related to the regulation of osmotic pressure<sup>12</sup>. N-methyltaurine has been isolated from sponges<sup>5</sup> and algae<sup>16</sup> but has not yet been recorded in sea anemones. This paper thus represents the first report on the occurrence of N-methyltaurine in a sea anemone.

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RESUMO: A anêmona-do-mar *Bunodosoma caissarum* Corrêa foi submetida a um tratamento extrativo com acetona e metanol e os extratos cromatografados em colunas de Sephadex LH-20, resultando no isolamento de N-metiltaurina e de caissarone.

UNITERMOS: Anêmona-do-mar; cnidaria; *Bunodosoma caissarum* Corrêa; N-metiltaurina; caissarone.

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