

3-METHYLADENINE FROM THE MARINE SPONGE *TOPSENTIA GENITRIX*

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Recently we reported the isolation and structure determination of three ichthyotoxic, bisindole alkaloids, topsentins A, B1, and B2, from the Mediterranean sponge *Topsentia genitrix* Schmidt (Epipolasiidae) (1). A further examination of the most polar fraction of the MeOH extract of this sponge led to the isolation of 3-methyladenine identified on the basis of its spectral properties. This identification was further proved by direct comparison with an authentic sample prepared by methylation of adenine with MeI in DMF (2).

A few free purine derivatives (3–5) and some glycosylpurines (6–9) already have been isolated from sponges. Moreover, several diterpenic purine derivatives have been reported from *Agelas* species (10–15).

As a free base, 3-methyladenine has never been reported from natural sources, but 3-methyladenine-DNA and 3-methyladenine-RNA are formed when cells or cell extracts are treated with methylating agents. These apurinic sites may be selectively incised by an endonuclease with subsequent excision repair by the action of specific enzymes (16).

## EXPERIMENTAL

**BIOLOGICAL MATERIAL.**—A large specimen of *T. genitrix* (339 g dry wt) was collected by scuba diving near Banyuls, France. A voucher specimen has been deposited in the laboratory of Bio-organic Chemistry of the University of Brussels (no. XXIII.64).

**ISOLATION AND IDENTIFICATION.**—The sponge was cut into small pieces and exhaustively extracted with MeOH. Evaporation of the organic solvent under reduced pressure led to an aqueous solution which was extracted successively with CH<sub>2</sub>Cl<sub>2</sub> and CH<sub>2</sub>Cl<sub>2</sub>-EtOH (3:2). The residual aqueous solution was evaporated to dryness, and the resulting solid was treated with EtOH at room temperature for 1 h. The topsentins were isolated from this extract (1). A second treatment of the residual solid with EtOH (24 h) led to a new extract from which, after two successive chromatographies on Si gel (eluent CHCl<sub>3</sub>-MeOH-NH<sub>3</sub>, 9:1:2) and two recrystallizations from EtOH, 3-methyladenine could be isolated (0.45% of the dry sponge). Mp 302–305°; ms *m/z* 149 [M]<sup>+</sup>, 121, 94, 69; uv (MeOH) λ max 273 (14,010); <sup>1</sup>H nmr (DMSO-*d*<sub>6</sub>, 250 MHz, δ, TMS) 3.9 (3H, s), 7.76 (1H, s), 7.82 (2H, bs), 8.29 (1H, s); <sup>13</sup>C nmr (DMSO-*d*<sub>6</sub>, 62.5 MHz, δ, TMS, BBD, and DEPT) 37.7 (q, CH<sub>3</sub>), 120.3 (s, C-5), 143.6 (d, C-2), 150.3 (s, C-4), 152.4 (d, C-8), 154.9 (s, C-6). These spectral properties are entirely compatible with those reported for 3-methyladenine (17, 18) and 3-butyladenine (17). An authentic sample (11 mg) was prepared by methylation of adenine using the procedure described by Abshire and Berlinguet (2) (adenine 20 mg, MeI 15 μl, DMF 0.3 ml, reflux 2.5 h). Comparison of the properties of the natural derivative with those of the reference compound shows that they are identical (*R*<sub>f</sub>, mmp, <sup>1</sup>H nmr, uv, ms).

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