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Mucronatine, a new *N*-methyl purine from the French mediterranean marine sponge *Stryphnus mucronatus*

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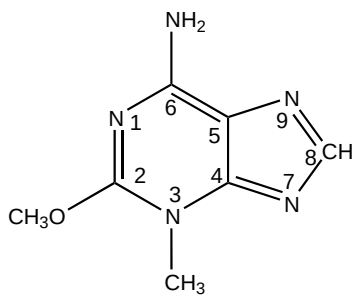
Abstract—A new *N*-methyl purine, mucronatine **1**, was isolated from the French marine sponge *Stryphnus mucronatus*. Its structure was determined by detailed 2D spectroscopic analysis, including ^{15}N spectral data assignments.

The discovery in 1951 by Bergmann and co-workers of three new nucleosides from the marine sponge *Cryptothethya crypta*[1–3] and subsequent developments of the two synthetic analogues: the antiviral Ara-A and the antileukemic Ara-C, have proven the medicinal potential of this class of compounds. Since, unusual nucleosides such as the hypotensive doridosine,[4,5] the antitumoral tubercidin[6] and the cytotoxic mycalisines A and B[7] continue to stimulate intense biomedical studies. Modified purines have also displayed potent biological activities with the 1,3-dimethylisoguanine[8,9] and the caissarone,[10,11] which both stimulated mammalian gut motility and the antifungal phidolopin.[12]

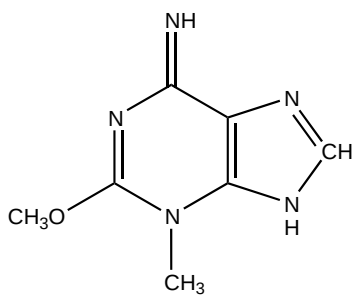
As a continuation of our studies on bioactive compounds from Porifera, we have examined the CH_2Cl_2 extract of the marine sponge *Stryphnus mucronatus* (class Demospongiae, order Astrophorida, family Stelletidae), collected in the Mediterranean Sea off La Ciotat (France). We have isolated a new *N*-methyl isoguanine **1**, we named mucronatine.

To the best of our knowledge, reports from the marine sponge *Stryphnus mucronatus* are only limited to its sterols derivatives:[13] fucosterol, the predominant sterol and 24-methylenecholesterol.

The CH_2Cl_2 extract (2.5 g) obtained from the freeze-dried marine sponge *Stryphnus mucronatus* was fractionated on a silica gel column using a gradient MeOH/CH_2Cl_2. The fraction eluted with 20% MeOH was further purified on another silica gel column to afford the major compound of the crude extract (0.7% dry weight) as a white solid: mucronatine **1** mp 200–202°C.



mucronatine **1**



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Keywords: purine; isoguanine; sponge metabolite; *Stryphnus mucronatus*.
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The molecular formula of **1** was deduced as $C_7H_9N_5O$, [M^+, 179.0801, Δ–0.6 mmu] by HREIMS, indicating the presence of six unsaturations in the molecule. The ^1H NMR spectrum of **1** (DMSO-d_6, 400 MHz), indicating the presence of two deshielded methyl groups at δ 3.66 and 4.03 ppm, a methine proton at δ 7.68 ppm and two broad NH protons at δ 8.76 and 8.98 ppm, suggested the purine nature of **1**. This was further substantiated by ^{13}C NMR data, which showed signals for four quaternary carbons at δ 153.8, 152.3, 151.7 and 115.1 ppm, one methine carbon at δ 150.3 ppm, one *O*-methyl carbon at δ 56.2 ppm and one *N*-methyl carbon at δ 31.2 ppm (Table 1).

HMBC correlations observed from the *N*-Me at δ 3.66 ppm with carbons at δ 152.3 and 151.7 ppm and from the *O*-methyl group at δ 4.03 ppm with carbon at δ 152.3 ppm placed them on two adjacent atoms. The proton at δ 7.68 ppm gave long-range correlations with carbons at δ 151.7, 115.1 and 153.8 ppm. This information added to the coupling constant values observed ^3J (C_4–H_8) 9.9 Hz and ^3J (C_5–H_8) 9.3 Hz, suggested the presence of an isoguanine base[14] substituted with one *N*-methyl and one *O*-methyl groups. This was confirmed by the fragment ion at m/z 121, observed in the EIMS, corresponding to the loss of the CH_3NCO moiety.[8,9] At this stage two tautomeric forms **1** and **2**, as it was reported for the 1,3-dimethylisoguanine[15] isolated from the marine sponge *Amphimedon viridis*,[8,9] could be proposed for mucronatine **1**.

The ^1H–^{15}N HMQC spectrum, which was demonstrated to be a valuable experiment for structure elucidation of alkaloids,[16] furnished decisive information for the new purine. In addition to the ^{15}N signal at δ 128.6 ppm, which correlated with protons at δ 3.66 ppm and was assigned to the *N*-methyl group, the two signals at δ 8.98 and 8.76 ppm showed one bond ^{15}N correlation to the same N at δ 105.2 ppm, clearly indicating the presence of an amine function. Furthermore, at 323 K the proton signal at δ 7.68 ppm correlated with two distinguished ^{15}N signals at δ 231.6 and 229.3 ppm, which could be assigned to *N*-7 and *N*-9 in mucronatine. Hence, structure **1** can be unambiguously assigned for mucronatine. This result is in accordance with a X-ray diffraction analysis of 1,3-dimethylisoguanine,[15] which has demonstrated that this molecule possesses a NH_2 group rather than the imine one initially proposed.

Table 1. $^1H^a$, $^{13}C^b$ and $^{15}N^c$ NMR data recorded in DMSO-d_6 for mucronatine **1**

	^1H δ, m	^{13}C δ	^{15}N δ
Position			
N-1			
2		152.3	
N-3			
4		151.7	
5		115.1	
6		153.8	
N-7			231.6[d]
8	7.68 s	150.3	
N-9			229.3[d]
CH_3-O	4.03 s	56.2	
CH_3-N	3.66 s	31.2	128.6
NH_2	8.76 brs/8.98 brs		105.2

[a] Recorded at 400.13 MHz relative to the solvent at δ 2.49 ppm.
[b] Recorded at 100.13 MHz relative to the solvent at δ 39.5 ppm.
[c] Recorded at 40.54 MHz relative to NH_4, in natural abundance.
[d] These values may be interchanged.

To the best of our knowledge, this is the first report of **1** as either a natural or synthetic product.

Mucronatine **1** showed no cytotoxic activity on KB cells until 0.055 mM and displayed weak toxicity in the brine shrimp assay[17] with 50% inhibition at 2.8 mM. However, mucronatine **1** exhibited 37% inhibition in the phenoloxydase bioassay,[18] suggesting potential antifouling properties.

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