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ISOLATION AND SYNTHESIS OF 1-METHYLISOGUANOSINE, A POTENT PHARMACOLOGICALLY ACTIVE CONSTITUENT FROM THE MARINE SPONGE TEDANIA DIGITATA

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ABSTRACT: A new pharmacologically active agent isolated from the marine sponge Tedania digitata has been identified as 1-methylisoguanosine (1) by spectral and degradative chemical methods and synthesis from a β-D-ribofuranosylimidazole (2).

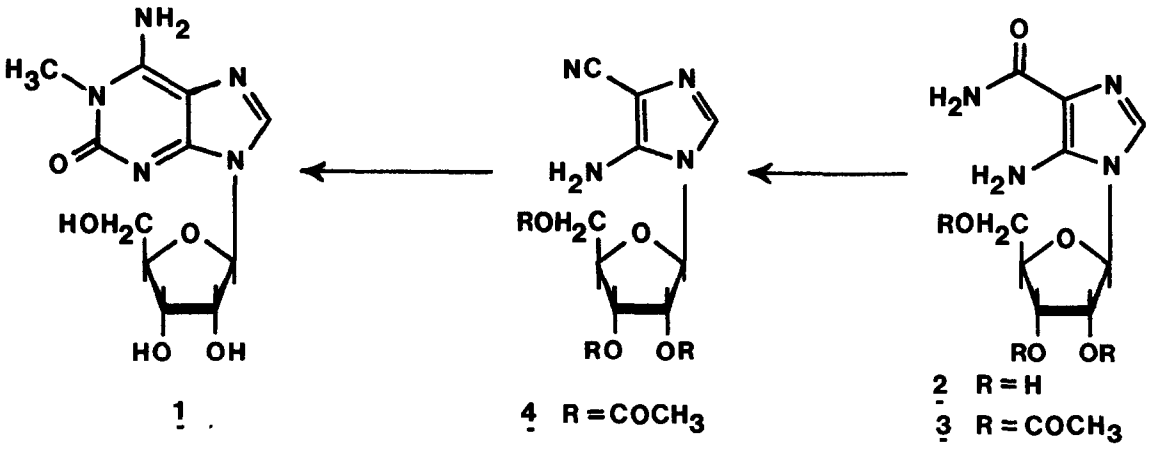
Much speculation has arisen over the occurrence in marine organisms of biologically active substances with therapeutic potential[1]. In order to further examine this potential we have undertaken a broadly-based screening procedure to test crude extracts of various marine phyla for pharmacological activity. We found that aqueous ethanolic extracts of Tedania digitata Schmidt (Demospongiae, Tedaniidae), collected off Newport Reef and Fairlight, Sydney, Australia, had muscle relaxant, anti-inflammatory and other pharmacological activities. We now report the isolation, structure determination and synthesis of the active constituent.

While the crude extract displayed a number of pharmacological effects, fractionation was guided by monitoring for hind limb paralysis and hypothermia in mice at doses equivalent to 1g crude extract/kg. The active constituent was found to adsorb to a sulfonic acid cation exchange resin from buffered crude extract at pH 3.5 and an active fraction was eluted with ammonium formate (pH 5.3, 0.1M formate). Re-chromatography under the same conditions, followed by recrystallisation from water gave the active constituent (1) in 0.71% yield from the crude extract. All the pharmacological effects of the crude extract were attributable to 1. It produced muscle relaxation and hypothermia in mice, hypotension associated with bradycardia, anti-inflammatory and anti-allergic activity in rats.

High resolution mass spectral analysis established the formula $C_{11}H_{15}N_5O_5$[2] which was supported by the ^{13}C NMR spectrum which showed signals for eleven carbon atoms. Both the mass

spectral fragmentation pattern and the ^{13}C NMR spectrum were consistent with a nucleoside structure. The presence of a C_5 sugar as a furanoside was apparent from doublets at 87.6, 86.0, 72.9, 70.7 and a triplet at 61.8 ppm[3,4] and this was confirmed by acid hydrolysis to give D-ribose. Singlets in the ^{13}C NMR spectrum at 153.8, 152.1, 151.5, 108.9 assignable to C_2, C_4, C_6, C_5 and a doublet at 138.0 ppm assignable to C_8 confirmed a purine nucleus while the other resonance at 30.0 ppm (quartet) was assigned to a N-methyl group. Reaction of (1) with concentrated hydrochloric acid under reflux[5] gave 1-methylxanthine establishing the position of methylation and confirming the purine nucleus. The UV maxima at 250 and 294nm[6] and a 6.5 ppm upfield shift of the C_5 resonance, relative to 1-methylguanosine, in the ^{13}C NMR spectra established the purine moiety of 1 as 1-methylisoguanine.

Acetylation of 5-amino-4-carbamoyl-1-β-D-ribofuranosylimidazole (2) with acetic anhydride in pyridine gave the triacetyl derivative (3), which was converted to the cyanide (4) using phosphorous oxychloride/triethylamine in chloroform. Reaction of (4) with methyl isocyanate in dimethylformamide at 100° for 5hr, removal of solvent and treatment with methanolic ammonium hydroxide gave a product (39% overall yield) identical (mixed MP, IR, UV, ^1H and ^{13}C NMR, MS, TLC, HPLC, rotation) to the natural product. Thus the structure was established as 9-β-D-ribofuranosyl-1-methylisoguanine (1).



1 . 4 R=COCH3 2 R=H 3 R=COCH3

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