CORALLISTINE, A NEW POLYNITROGEN COMPOUND FROM THE SPONGE *Corallistes fulvodesmus*

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Abstract: Two polynitrogen compounds 1-methyl-pteridine-2.4-dione 1b and corallistine 2 were isolated from the new-caledonian sponge *Corallistes fulvodesmus*. The structure of corallistine was determined by X-ray single crystal analysis of its 6'-isobutyloxycarbonyl derivative 3.

In the course of our search for new biologically active substances in deep water marine invertebrates, we undertook an investigation of the extracts of the new-caledonian sponge *Corallistes fulvodesmus*. This lead us to the isolation of two polynitrogen compounds 1-methyl-pteridine-2.4-dione 1b and a new compound with an original structure corallistin 2.

The sponge was ground, freeze-dried and extracted with 80% ethanol. The alcohol was evaporated under reduced pressure and the aqueous residue extracted with methylene chloride. Silica gel chromatography of the extract using increasing concentrations of methanol in methylene chloride provided crude compounds 1b and 2, which were purified respectively using repeated recrystallisation and rechromatography.

1-Methyl-pteridine-2.4-dione 1b was straightly identified by comparing its physical and spectral data, as well as those of its N-methyl derivative 1c, with the data reported in the literature. Pteridines are widely distributed in animal kingdom and have also been found in marine organisms, but 1-methyl-pteridine-2.4-dione 1b, in the opposite of its N-demethyl-derivative, lumazine, 1a, has never been isolated previously from natural sources.

Corallistine 2 crystallized from methanol to afford light yellow crystals m.p. 192° (dec). It had the molecular formula C10H13N5OS on the basis of a molecular ion at m/z 251,08797 (calc. 251,09463) and elemental analysis.

The 1H NMR spectrum in CDCl3-MeOH showed the presence of only three tertiary methyl groups (3s, each 3H) at 2.62, 3.17 and 3.65 ppm and two olefinic protons (2s, each 1H) at 6.50 and 7.46 ppm. On the 13C NMR spectrum a signal at 163 ppm could be assigned to a SMe group resoning at 2.62 ppm on the 1H NMR spectrum. The other two methyl groups located at 24.9 and 33.2 ppm (3.17 and 3.65 ppm on the 1H NMR spectrum) were obviously N-methyl groups.
On the other hand, the basic properties of the molecule, as well as a band at 3360 cm⁻¹ on the IR spectrum and a broadened singlet of two exchangeable protons at 7.36 ppm on the ¹H NMR spectrum (DMSO-d₆), suggested that a primary amine group was present. This was confirmed through acylation (CICOOiBu, pyridine, rt, 1 h) giving rise to a monoacyl derivative 3 (SM: Mᵢ 351) showing one exchangeable proton as a singlet at 11.70 ppm on the ¹H NMR spectrum (DMSO-d₆).

All other spectral data of 2 and 3⁶,⁷ fitted an heterocyclic compound possibly a pteridine derivative close to the pteridine dione 1b previously isolated, but did not prove the connectivity of the different C and N atoms. An X-ray crystal structure determination was therefore undertaken.

As suitable crystals of corallistaine could not be obtained we turned to the acyl derivatives. After some experiments, we chose the 6'-isobutyloxycarbonyl derivative 3 which crystallized from methanol, giving yellow crystals m.p. 204°. A crystal (0.9 x 0.4 x 0.05 mm) was mounted on an automatic 4-circle diffractometer with graphite monochromatized CuKα radiation (λ = 1.5418 Å). The crystal space group was triclinic P₁, with a = 18.110 (5), b = 8.238 (2), c = 6.303 (1) Å, α = 108.53 (1), β = 92.38 (1), γ = 96.84 (1)°, V = 882.10 Å³, Z = 2. From 3187 measured independent reflexions, 1092 only with I > 1.5 σ (I) were included in the computations. The reflexions were corrected for Lorentz and polarisation effects, but not for absorption.

The structure was solved by direct methods. Atomic coordinates and anisotropic thermal parameters were refined by least squares refinements to a discrepancy factor of R = 5.12% and Rw = 5.17%. The minimized function in the refinement was Σw [(Fo) - (Fc)]² with a final weighting scheme, w = 1/[Fo]² + 0.0009 Fo². All hydrogen atoms were located on difference-Fourier maps. However, to take into account the deficient number of data with regard of the number of refinement parameters, H atoms were refined using rigid groups (methyl) or in theoretical position (C-H, N-H) and assigned the equivalent isotropic thermal parameter of C or N bounded atoms. The highest residue on the final electronic density map was 0.4 e/Å³.

The final X-ray model of the 6'-isobutyloxy carbonyl derivative 3 is illustrated in Fig.1. The peculiar planar molecular conformation is due to an intramolecular bifurcated hydrogen bond:

\[
\text{N-1} - \text{H-2} \quad \quad \text{O-8}^{\prime}
\]
Except for the C-13' methyl group, all non H-atoms are located in the mean plane of the molecule, the maximum deviation from planarity being 0.3 Å.

![Computer generated drawing of the final X-ray model of the 6'-isobulyoxycarbonyl derivative 3](image)

**Fig.1.** Computer generated drawing of the final X-ray model of the 6'-isobulyoxycarbonyl derivative 3

On the basis of the above X-Ray results, corallistine was assigned structure 2, which is fairly different from the pteridine 1b, except that two rings both with two heterocyclic nitrogen are also present. The imidazolidone type portion shown as the preferred endocyclic imino tautomer has been found previously in marine organisms attached to a tryptophane derivative in the same way as to the 2-thiolhistidine derivative in 2. The geometry of the central double bond was shown or presumed as E in most of the tryptophane compounds. The geometry of the Δ6,1' double bond of corallistin must be Z as for its 6'-isobutylxycarbonyl derivative 3. Although stabilisation of the Z configuration by hydrogen bonding as in 3 was ruled out, isomerisation during acylation was very unlikely, as migration of the double bond to the Δ1' position involved in the isomerisation process was electronically unfavorable. No Z-E isomerisation was observed for 2 itself which appeared as a pure compound and H-6 was not exchangeable for deuterium in neutral (MeOD, DMSO-d6) or acidic medium (CF3COOD). The 2-thiolhistidine type portion is found in ergothioneine (2-thiolhistidine trimethylbetaine) a constituent of numerous alive tissues also extracted from *Linulus polyphemus* L. (Crustacea) and part of the molecule of clithioneine recently isolated from the Japanese fungus *Clitocybe acromegalga*. However a 2-methylthioimidazole moiety as present in 2 has never been found before in a natural product, corallistine representing thus an original compound.

The chloromethylenic extract of *Corallistes fulvodesmus* was cytotoxic against KB and P388 cells (DI50 < 10 μg), but corallistine itself showed no toxicity against those cells.
References and Notes

1. Animal Material: The sponge was collected in course of the dragging campaigns of the ORSTOM-CNRS Programme "Substances Marines d'Intérêt Biologiques" (SMIB) by the N/O Vauban at the point 22°55'S and 167°15.9'E, at a depth of 500 m. A zoologic sample is kept at the Orstom Centre in Noumea under the reference R 1385. The sponge has been identified by Prof. C. Levi, whom we wish to acknowledge.


6. Spectral Data of corallisthen 2: UV (MeOH): 206 nm (ε 18000), 222 nm (sh. ε 11000), 363 nm (ε 22600); IR (CHCl₃): 3360, 1730, 1670 cm⁻¹; ¹³C NMR (CDCl₃): 163 (SCH₃), 24.9 and 33.2 (NCH₃), 99.0 and 123.3 (CH), 128.5, 137.9, 144.9, 153.2, 165.0 (C).

7. Spectral Data of 3: SM: UV (MeOH): 203 nm (ε 15900), 233 nm (ε 11000), 478 nm (ε 17500); IR (CHCl₃): 3310, 1730, 1680, 1650, 1610 cm⁻¹; ¹H NMR (CDCl₃): 1.00 (6H, d, J=6, CH₃ i-Bu), 2.08 (1H, m, CH i-Bu), 2.90 (3H, s, SCH₃), 3.27 (3H, s, NCH₂H₂), 3.61 (3H, s, NCH₃); 4.00 (ZH, d, J=6, CH₂O), 6.68 (1H, d, CH), 7.25 (1H, s, CH).


9. Tables of structural data are available from the Cambridge Crystallographic Data Centre, University Chemical Laboratory, Lensfield Road, Cambridge CB2 1EW, U.K.

10. O-8' ---- H-2' 2.1 Å, N-1 ---- H-2' 2.1 Å; < O-8' ---- H-2' ---- N-1 127°, < N-1 ---- H-2' ---- N-1 119°, < O-8' ---- H-2' ---- N-1 114°.


