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 determinated by X-ray single crystal analysis of its 6'-isobutyloxycarbonyl derivative **3**.

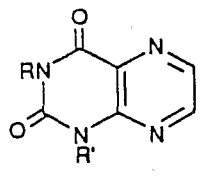
In the course of our search for new biologically active substances in deep water marine invertebrates, we undertook an investigation of the extracts of the new-caledonian sponge *Corallistes fulvodesmus*.[1] This lead us to the isolation of two polynitrogen compounds 1-methyl-pteridine-2-4-dione **1b** and a new compound with an original structure corallistin **2**.

The sponge was ground, freeze-dried and extracted with 80% ethanol. The alcool was evaporated under reduced pressure and the aqueous residue extracted with methylene choride. 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 recristallisation and rechromatography.

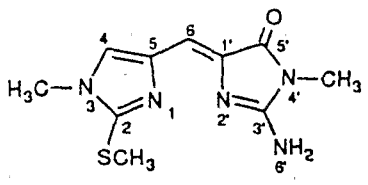
1-Methyl-pteridine-2-4-dione **1b** was straightly identified by comparing its physical and spectral data, as well as those of its N-methyl derivative **1c**, with the data reported in the literature.[2,3] Pteridines are widely distribuated in animal kingdom[4] and have also be found in marine organisms[5], 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** cristallized from methanol to afford light yellow crystals m.p. 192° (dec). It had the molecular formula $C_{10}H_{13}N_5OS$ on the basis of a molecular ion at m/z 251,08797 (calc. 251,09463) and elementar analysis.

The ^1H NMR spectrum in $CDCl_3$-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 ^{13}C NMR spectrum a signal at 16,3 ppm coud be assigned to a SMe group resoning at 2,62 ppm on the ^1H NMR spectrum. The other two methyl goups located at 24,9 and 33,2 ppm (3,17 and 3,65 ppm on the 1H NMR spectrum) were obviously N-methyl groups.



1 a R = R' = H
1 b R = H, R' = CH_3
1 c R = R' = CH_3



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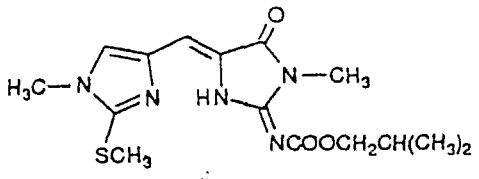


3

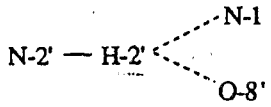
On the other hand, the basic properties of the molecule, as well as a band at 3360 cm-1 on the IR spectrum and a broadened singlet of two exchangeable protons at 7,36 ppm on the ^{1}H NMR spectrum (DMSO-d_6), suggested that a primary amine group was present. This was confirmed through acylation (ClCOOiBu, pyridine, rt, 1 h) giving rise to a monoacyl derivative 3 (SM : M^+ 351) showing one exchangeable proton as a singlet at 11,70 ppm on the ^{1}H NMR spectrum (DMSO-d_6).

All others spectral data of 2 and 3[6,7] 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 corallistine could not be obtained we turned to the acyl derivatives. After some experimentations, we chose the 6'-isobutyloxycarbonyl derivative 3 which cristallized 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 $P\bar{1}$, 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 Å^3, 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.[8] 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 $\Sigma w (|Fo| - |Fc|)^2$ with a final weighting scheme $w = 1/[\sigma 2 (Fo) + 0{,}0009\ Fo^2]$. All hydrogen atoms were located on difference-Fourier maps. Howewer, to take in 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/Å^3.[9]

The final X-ray model of the 6'-isobutyloxycarbonyl derivative 3 is illustrated in Fig.1. The peculiar planar molecular conformation is due to an intramolecular bifurcated hydrogen bond[10]



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.

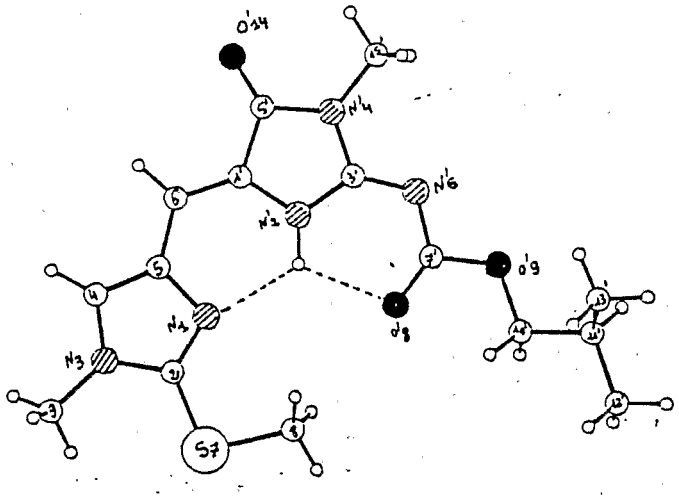


Fig.1. Computer generated drawing of the final X-ray model of the 6'-isobulyoxycarbonyl derivative **3**

On the basis of the above X-Ray results, corallistine was assigned structure **2**, wich 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[11] has been found previously in marine organisms[12] 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 $\Delta^{6,1'}$ double bond of corallistin must be Z as for its 6'-isobutyloxycarbonyl 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 $\Delta^{1'}$ 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-d_6) or acidic medium (CF_3COOD). The 2-thiolhistidine type portion is found in ergothioneine (2-thiolhistidine trimethylbetaine) a constituent of numerous alive tissues[13] also extracted from *Linulus polyphemus* L. (Crustacea)[14] and part of the molecule of clithioneine recently isolated from the japanese fungus *Clitocybe acromegalga*.[15] Howewer a 2-methylthioimidazole moiety as present in **2** has never be found before in a natural product, corallistine representing thus an original compound.

The chloromethylenic extract of *Corallistes fulvodesmus* was cytotoxic againt KB and P388 cells ($DI_{50} < 10$ µg), but corallistine itself showed no toxicity against that cells.

References and Notes

1. Animal Material : The sponge was collected in course of the dragging campaigns of the ORSTOM-CNRS Progamme "Substances Marines d'Intérêt Biologiques" (SMIB) by the N/O Vauban at the point 22°55,5' 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 whish to acknowledge.
2. W. Pfleiderer, Chem. Ber., 1957, **90**, 2582-2587.
3. K. Tsuzuki and M. Tada, J. Heterocyclic Chem., 1986, **23**, 1299-1301.
4. W. Pfleiderer, in "Comprehensive Heterocyclic Chemistry", A.R. Katritzky and CH.W. Rees Eds., Vol. 3, Part B, Pergamon Press, Oxford, 1984.
5. G. Prota, in "Marine Natural Products : Chemical and Biological Perspectives", P.J. Scheuer Ed., Vol. 3, Academic Press, 1980, Chapter 3.
6. Spectral Data of corallistine **2** : UV (MeOH) : 206 nm (ε 18000), 222 nm (sh. ε 11000), 363 nm (ε 22600); IR ($CHCl_3$) : 3360, 1730 (w), 1670 cm^{-1}; ^{13}C NMR ($CDCl_3$) : 16.3 (SCH_3), 24.9 and 33.2 (NCH_3), 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 ($CHCl_3$) : 3310, 1730, 1680, 1650, 1610 cm^{-1}; ^1H NMR ($CDCl_3$) : 1.00 (6H, d, J=6, CH_3 i-Bu), 2.08 (1H, m, CH i-Bu), 2.90 (3H, s, SCH_3), 3.27 (3H, s, NCH_3), 3.61 (3H, s, NCH_3), 4.00 (2H, d, J=6, CH_2O), 6.68 (1H, d, CH), 7.25 (1H, s, CH).
8. G.M. Sheldrick, SHELXS86, Program for Crystal Structure Solution, University of Göttingen, FRG, 1986.
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°.
11. G.L. Kenion and G.L. Rowley, J. Am. Soc. Chem., 1971, **93**, 5552-5560.
12. R. Kazlaukas, P.T. Murphy, R.J. Quinn et R.J. Wells, Tetrahedron Lett., 1977, 61-64; K.H. Hollenbeak and F.J. Schmitz, LLoydia, 1977, **40**, 479. R.J. Wells, Pure and Appl. Chem., 1979, 51, 1832; P. Djura and D.J. Faulkner, J. Org. Chem.,1980, **45**, 735; P. Djura; D.B. Stierle, B. Sullivan and D.J. Faulkner, ibid., 1435; A.A. Tymiak, K.L. Rinehart Jr and G.J. Bakus, Tetrahedron, 1985, **41**, 1039; E. Fattorusso, V. Lanzotti, S. Magno et E. Novellino, J. Nat. Prod., 1985, **48**, 924.
13. D.B. Melville, Vitamines and Hormones, Academic Press, 1959, **17**, 155.
14. D. Ackerman and P.H. List, Z. Physiol. Chem., 1958, **313**, 30.
15. K. Konno, H.Shirahama and T. Matsumoto, Phytochemistry, 1984, **23**, 1003.