161. Corallistin A, a Second Example of a Free Porphyrin from a Living Organism. Isolation from the Demosponge *Corallistes* sp. of the Coral See and Inhibition of Abnormal Cells

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It is shown that the demosponge *Corallistes* sp. (Tetractinomorpha, Lithistida, Corallistidae) collected in the Coral Sea, contains corallistin A (**1**), the second example, of a free porphyrin from a living organism. The compound proved to be active against the Kb cell line. In contrast with the geoporphyrins which do not bear any O-atom corallistin A (**1**) carries two carboxylic groups.

1. Introduction. – Although a large number of geoporphyrins have been isolated from both oil shale [1] and coal [2], at our knowledge there is no record of free porphyrins isolated from a living organism, except for chlorophyll C. Except for the chlorophylls, the compounds most closely related to porphyrins which have been isolated from living organisms are chlorins. Examples are bonellin, isolated from the Enteropneusta marine worm *Bonellia viridis* [3], tunichlorin, isolated from the ascidian *Tridemnum solidum* [4], and 13^2,17^3-cyclopheophorbide enol, isolated from the marine demosponge *Darwinella oxeata* (Dendroceratida) [5].

We report on a free porphyrin isolated from a demosponge of the Coral Sea, *Corallistes* sp.

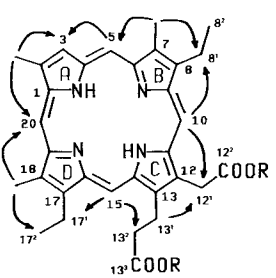
2. Results and Discussion. – Key observations about the nature of the novel pigment **1** isolated as methyl ester **2** from the sponge *Corallistes* sp., which belongs to the family Corallistidae of the order Lithistida, are: *i)* a strong *Soret* absorption at 400 nm and weaker absorptions from 498 to 618 nm *(Exper. Part)*; *ii)* resonance at *ca.* 10 ppm for protons which, unusually, do not exchange with D_2O *(Table)*; *iii)* the presence of Me groups which, in spite of their ^{1}H-NMR signals at > 3 ppm, are not bound to heteroatoms; in fact, their ^{13}C-NMR signal is at such a high field (*ca.* 10 ppm; *Table*) to suggest CH_3–C bonding; *iv)* a broad ^{1}H-NMR *s* at -3.9 ppm for two protons at N-atoms.

The above NMR data, in particular the low-field CH and the high-field NH resonances, indicate strong ring currents such as in porphyrins [6]. In accordance with this hypothesis [7], the ^{13}C-NMR spectrum *(Table)* does not show resonances for the sixteen C-atoms of pyrrol rings. The lacking ^{13}C-NMR signals, which are accounted for in the mass spectrum (M^+ 566), can be detected for the Zn(II) complex **3**, prepared from **2** and $Zn(OAc)_2$. In complex **3**, all signals can be assigned from long-range ^{13}C,^{1}H correlations [8] *(Table)*.

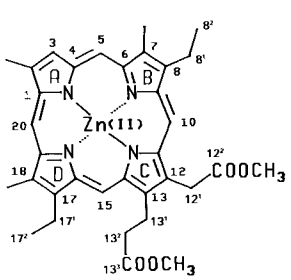
Table. *^{1}H-NMR Data for Corallistin A Methyl Ester* (**2**) *and ^{13}C-NMR Data for Both* **2** *and its Zn(II) Complex* (**3**)[a])

	^{13}C-NMR (δ)		^{1}H-NMR (δ)
	2	**3**	**2**
C(1)		147.17 (*s*)	
C(2)		139.62 (*s*)	
CH(3)		128.44 (*d*)	8.99 (*d*, *J*(3, Me–C(2)) = 1.2)
C(4)		146.74 (*s*)	
CH(5)	99.71 (*d*)	99.32 (*d*)	9.98 (*s*)
C(6)		147.80 (*s*)	
C(7)		134.94 (*s*)	
C(8)		142.31 (*s*)	
C(9)		146.28 (*s*)	
CH(10)	96.89 (*d*)[b])	96.37 (*d*)	10.18 (*s*)
C(11)		145.01 (*s*)	
C(12)		139.58 (*s*)	
C(13)		131.39 (*s*)	
C(14)		144.47 (*s*)	
CH(15)	96.72 (*d*)[b])	96.24 (*d*)	10.12 (*s*)
C(16)		145.84 (*s*)	
C(17)		141.95 (*s*)	
C(18)		134.73 (*s*)	
C(19)		146.87 (*s*)	
CH(20)	96.98 (*d*)[b])	96.37 (*d*)	10.09 (*s*)
Me–C(2)	13.79 (*q*)	13.17 (*q*)	3.70 (*d*, *J*(Me–C(2), 3) = 1.2)
Me–C(7)	11.36 (*q*)[c])	11.14 (*q*)	3.64 (*s*)
Me–C(18)	11.42 (*q*)[c])	11.01 (*q*)	3.66 (*s*)
*Me*OOC(12^2)	52.39 (*q*)	52.20 (*q*)	3.76 (*s*)[f])
*Me*OOC(13^3)	51.83 (*q*)	51.76 (*q*)	3.73 (*s*)[f])
CH_2(8^1)	19.80 (*t*)	19.49 (*t*)[e])	4.14 (*q*, *J*(8(1), 8(2)) = 7.5)
CH_3(8^2)	17.62 (*q*)[d])	17.62 (*q*)	1.89 (*t*, *J*(8(2), 8(1)) = 7.5)
CH_2(12^1)	33.03 (*t*)	32.41 (*t*)	5.10 (*s*)
C(12^2)	172.31 (*s*)	172.24 (*s*)	
CH_2(13^1)	22.09 (*t*)	21.68 (*t*)	4.45 (*t*, *J*(13(1), 13(2)) = 8.0)
CH_2(13^2)	37.41 (*t*)	37.24 (*t*)	3.35 (*t*, *J*(13(2), 13(1)) = 8.0)
C(13^3)	173.79 (*s*)	173.78 (*s*)	
CH_2(17^1)	19.80 (*t*)	19.43 (*t*)[e])	4.15 (*q*, *J*(17(1), 17(2)) = 7.5)
CH_3(17^2)	17.65 (*q*)[d])	17.62 (*q*)	1.89 (*t*, *J*(17(2), 17(1)) = 7.5)
NH_2			–3.9 (br. *s*)

[a]) $CDCl_3$ solutions, chemical shifts δ in ppm rel. to TMS (= 0 ppm), coupling constants *J* in Hz.
[b])[c])[d])[e])[f]) Resonances labeled with the same letter can be interchanged.



1 R = H
2 R = CH_3, ↷ = NOE



3

Fine details in the ^{13}C-NMR are decisive in proving the porphyrin hypothesis, revealing 4 *d* for the *meso* C-atoms C(5), C(10), C(15), and C(20). The other ^{13}C-NMR resonances *(Table)* are compatible with 3 Me, 2 Et, 1 CH_2COOMe, and 1 CH_2CH_2COOMe peripheral substituents. This is supported by ^{1}H-NMR data *(Table)* which also show that the unsubstituted pyrrole position must be occupied by the proton resonating at 8.99 ppm.

The location of the peripheral substituents on the porphyrin ring rests on positive differential NOE's both with the *meso* H-atoms and among the substituents themselves; such NOE effects are listed in the *Exper. Part* (see also *Formula* **2**).

It is to be remarked that the porphyrins so far isolated from oil shale [1] and coal [2] lack O-atoms, whereas corallistin A (**1**) is oxygenated. Corallistin A is structurally closer to heme than to chlorophyll C, which has a carbocycle. Therefore, derivation of corallistin A from protoporphyrin *via* heme rather than *via* Mg protoporphyrin can be envisaged.

Whereas ester **2** was inactive in cellular screening, corallistin A (**1**) proved active against the Kb cell line. However, *in vivo* assays were negative.

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Experimental Part

1. *General.* All evaporations were carried out at reduced pressure. HPLC: *Merck-LiChrosorb CN* (7 μm); reverse-phase HPLC, *Merck-LiChrosorb RP18* (7 μm); 25 × 1 cm columns, solvent flux 5 ml/min, UV monitoring at 320 nm. UV (λ_{max} in nm, ε in mol^{-1} l cm^{-1}): *Perkin-Elmer-Lambda-3.* NMR: *Varian-XL-300; δ*'s (ppm) relative to internal Me_4Si (= 0 ppm); probe temperature 21°; ^{1}H-NMR at 300 MHz, *J* in Hz, couplings obtained from double irradiations and COSY experiments; NOE experiments with 8 s of preirradiation; ^{13}C-NMR at 75.43 MHz, multiplicities from DEPT [8], chemical shift assignments from ^{13}C,^{1}H correlations [9]. EI-MS (*m/z* (%)): home-built spectrometer based on the *ELFS-4-162-8-Extranuclear* quadrupole [10].

2. *Collection and Isolation.* The sponge (1 kg wet) was collected by beam trawl South-East of Noumea, New Caledonia, Fresh sponge was lyophilized and a portion (2/3) of the lyophilizate extracted with 80% EtOH. Excess solvent was evaporated and the aqueous residue extracted with CH_2Cl_2. The org. phase was evaporated to leave a dark resinous residue which proved untractable by chromatographic methods. However, following the observation of a COOH band in IR spectra, a portion (0.5 g) of this material was dissolved into abs. EtOH and treated with CH_2N_2; the solvent was evaporated and the residue subjected to HPLC with hexane/AcOEt/$(CH_3)_2CHNH_2$ 93:7:0.2 isolating material at t_R 18 min. This material was subjected to reverse-phase HPLC with MeOH/H_2O 96:4 to give pure **2** at t_R 14 min.

3. *Corallistin A Methyl Ester (= Methyl 8,17-Diethyl-12-[(methoxycarbonyl)methyl]-2,7,18-trimethylporphyrin-13-propanoate;* **2**). UV ($CHCl_3$): 618 (2000), 565 (6500), 538 (10000), 498 (12000), 400 (190000). IR ($CHCl_3$): 3330, 1730. Differential NOE effects ($CDCl_3$; irradiated proton → % NOE effect on the observed proton(s)): 8.99 → 10.5% on 9.98; 9.98 → 17% on 8.99; 10.18 → 2.6% on 4.14, 4% on 5.10; 10.12 → 3.8% on 4.45, 2.1% on 4.15; 3.70 → 12% on 10.09, 9.4% on 8.99; 3.64 → 14% on 9.98, 1.3% on 4.14; 3.66 → 13% on 10.09, 1.8% on 4.15; 4.14 → 1.7% on both 10.18 and 10.12 (in this experiment, the protons at 4.15 were irradiated, too); 5.10 → 13% on 10.18; 4.45 → 2% on 10.12, 1.5% on 5.10. MS: 566 (100, $M^{+\cdot}$), 551 (8, $M^{+\cdot} - 15$), 539 (9), 493 (32), 429 (20), 355 (27), 281 (21), 221 (25).

4. *Corallistin A Methyl Ester Zn(II) Complex (= Diacetato[methyl 8,17-diethyl-12-[(methoxycarbonyl)methyl]-2,7,18-trimethylporphyrin-13-propanoato]zinc(II);* **3**). A mixture of **2** (0.02 g) and $Zn(OAc)_2$ (excess) in 0.5 ml of MeOH was refluxed for 80 min. The mixture was then filtered over *Amberlite XAD-2* in a glass filter. After washing with H_2O, **3** was eluted with $CHCl_3$. The impure product was subjected to reverse-phase HPLC with MeOH/H_2O 92:8: pure **3** at t_R 10.5 min.

5. *Corallistin A (= 12-(Carboxymethyl)-8,17-diethyl-2,7,18-trimethylporphyrin-13-propanoic Acid;* **1**) *from* **2**. Compound **2** (0.01 g) was dissolved in $CHCl_3$/MeOH/10% KOH 3:3:1 and allowed to stand overnight. The soln. was acidified and filtered over *Amberlite XAD-2* as above with **3**: pure **1**.

6. *Biological Assays.* The raw CH_2Cl_2 extract from the sponge proved active against the Kb cell line in experiments carried out at *ORSTOM*, Noumea. In experiments carried out at the Institut de Chimie des Substances Naturelles, Gif-Sur-Yvette, methyl ester **2** proved inactive both against the Kb cell line and in the tubulin assay; however, corallistin A (**1**), while negative in the tubulin assay, proved active against the Kb cell line (% inhibition at x μg/ml): 65 at 100, 48 at 10, 10 at 5, 4 at 1, and 0 at 0.1. In experiments carried out at *Rhône-Poulenc*, within the agreement *CNRS-ORSTOM-Rhône-Poulenc*, **1** proved inefficacious against both leukemic and solid tumor cell cultures.

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