168. Imidazolone and Imidazolidinone Artifacts of a Pivotal Imidazolthione, Zyzzin, from the Poecilosclerid Sponge *Zyzza massalis* from the Coral Sea. The First Thermochromic Systems of Marine Origin

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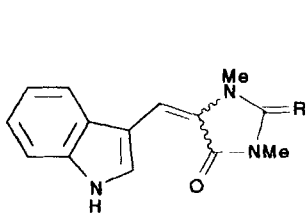
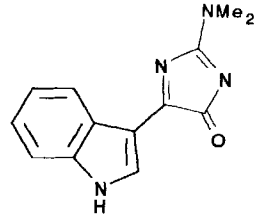
Dry acetone extracts of the freeze-dried, deep-red powder of the poecilosclerid sponge *Zyzza massalis* (DENDY) from the Coral Sea gave the orange alkaloid zyzzin (= 4-(1*H*-indol-3-yl)-1,5-dihydro-5-thioxo-1*H*-imidazol-2-one; **14**), which underwent exchange of the 5-thioxo for the 5-oxo group during aqueous chromatographic workup, giving yellow **13**. Both **13** and **14** added hydroxylic solvents at C(4)=N giving colourless, racemic products, **3** and **8**, respectively, by incorporation of MeOH and **10** and **11**, respectively, by incorporation of H_2O. On warming **3** or **10** in DMSO, **13** was recovered, thus furnishing a novel thermochromic system. Optically active (+)-**12**, which may be viewed to derive from enzymatic reduction of **14** at C(4)=N followed by S → O exchange, was also isolated from this sponge, along with the linear amides **16** and **19**. Compound **3** proved to have antibacterial and antifungal activities.

1. Introduction. – A group of marine alkaloids are known that entail a 4*H*-imidazol-4-one ring, such as aplysinopsin (**1a**) and 2-deimino-2-oxoaplysinopsin (**1b**) ((*E*/*Z*)-mixtures), isolated from sponges and dendrophylliid corals [1], compound **2**, obtained from the colonial ascidian *Dendrodoa grossularia* [2], and polyandrocarpamide D (**3**; *Scheme 1*), isolated from another colonial ascidian, *Polyandrocarpa* sp. [3].



1a R=NH
b R=O



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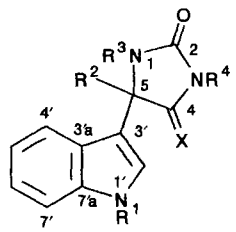
We suggest here that thioxo metabolites may be chemical precursor of the oxo compounds of type **2** and **3**. This has emerged on examination of the sponge *Zyzza massalis* (DENDY) (= *Plocamia massalis* DENDY, 1921 = *Zyzza massalis* DE LAUBENFELS, 1936 = *Damirina verticillata* BURTON, 1959), Poecilosclerida, Cornulidae [4] from south-eastern New Caledonia in the Coral Sea. *Zyzza* is a genus that has no precedent in the natural-product literature[1]).

[1]) According to a personal communication from Prof. *P.R. Bergquist*, University of Auckland, the reported *Zyzzya cf. marsailis* [5] was misspelled and, more important, it cannot belong to the genus *Zyzza*. Anyway, the products reported for that sponge [5] are quite different from those in this work, though all probably derive biogenetically from tryptophan.

2. Results and Discussion. – 2.1. *The Colourless Products.* Ethanol extraction of the freeze-dried red powder of *Z. massalis*, followed by workup with MeOH, led to the new thioamide **8** (see *Scheme 1*). Both EI- and FAB-MS failed to reveal the molecular ion for **8**, which could, however, be detected for the corresponding N^1-acetyl derivative **9**, yielding the molecular formula $C_{14}H_{13}N_3O_3S$, in agreement with the ^{13}C-NMR spectrum. Substitution at C(3′) in compound **8** was suggested by ^1H- and ^{13}C-NMR spectra (*Tables 1* and *2*), selective irradiations, $^1H,^1H$ correlations, and $^1H,^{13}C$ one-bond and long-range

Scheme 1[a])



3 R^2 = MeO, R^1 = R^3 = R^4 = H, X = O
a) → **4** R^2 = MeO, R^1 = R^4 = H, R^3 = Ac, X = O
b) → **5** R^2 = MeO, R^1 = R^3 = R^4 = Me, X = O
→ **6** R^2 = OH, R^1 = R^3 = R^4 = Me, X = O
→ **7** R^2 = MeO, R^1 = H, R^3 = R^4 = Me, X = O

a) → **8** R^2 = MeO, R^1 = R^3 = R^4 = H, X = S
→ **9** R^2 = MeO, R^1 = R^4 = H, R^3 = Ac, X = S
c) → **10** R^2 = OH, R^1 = R^3 = R^4 = H, X = O
→ **11** R^2 = OH, R^1 = R^3 = R^4 = H, X = S
(+)-**12** R^2 = H, R^1 = R^3 = R^4 = H, X = O

a) Ac_2O, pyridine, r.t., overnight. *b*) MeI, K_2CO_3, acetone, r.t., overnight. *c*) During *RP18*-HPLC (MeCN/H_2O 1:3).

[a]) Arbitrary numbering is used for spectral-data presentation and discussion; for systematic names for retrieval purposes, see *Exper. Part.*

Table 1. *^{13}C-NMR Data for the Alkaloids Isolated from the Sponge* Zyzza massalis (in$(CD_3)_2SO$, unless otherwise specified). For numbering, see *Schemes 1* and *2*.

	3	**8**	**10**	**11**[a])	**(+)-12**	**13**	**16**	**19**[b])
C(2′)	124.60 (*d*)	124.58 (*d*)	124.12 (*d*)	125.52 (*d*)	125.35 (*d*)	138.29 (*d*)	130.71 (*d*)	129.02 (*d*)
C(3′)	110.61 (*d*)	112.96 (*s*)	113.15 (*s*)	115.80 (*s*)	110.86 (*s*)	105.58 (*s*)	110.55 (*s*)	111.89 (*s*)
C(3′a)	124.57 (*s*)	124.38 (*s*)	124.67 (*s*)	126.14 (*s*)	126.48 (*s*)	125.50 (*s*)	126.32 (*s*)	127.31 (*s*)
C(4′)	120.27 (*d*)	120.0 (*d*)	119.79 (*d*)	120.95 (*d*)	119.68 (*d*)	122.62 (*d*)	121.91 (*d*)[c])	121.43 (*d*)[c])
C(5′)	119.10 (*d*)	119.08 (*d*)	118.81 (*d*)	120.60 (*d*)	120.15 (*d*)	121.96 (*d*)	121.24 (*d*)[c])	122.10 (*d*)[c])
C(6′)	121.46 (*d*)	121.30 (*d*)	121.29 (*d*)	122.90 (*d*)	122.67 (*d*)	123.84 (*d*)	122.50 (*d*)[c])	122.91 (*d*)
C(7′)	111.63 (*d*)	111.75 (*d*)	111.67 (*d*)	112.78 (*d*)	112.54 (*d*)	112.61 (*d*)	112.07 (*d*)	112.51 (*d*)
C(7′a)	136.68 (*s*)	136.61 (*s*)	136.74 (*s*)	138.83 (*s*)	137.99 (*s*)	136.91 (*s*)	136.27 (*s*)[d])	137.56 (*s*)[e])
C(2)	156.17 (*s*)	156.25 (*s*)	156.01 (*s*)	158.80 (*s*)	157.59 (*s*)	166.64 (*s*)[e])[f])	–	–
C(4)	172.52 (*s*)	206.09 (*s*)	172.98 (*s*)	211.45 (*s*)	174.69 (*s*)	167.87 (*s*)[e])[f])	–	–
C(5)	88.55 (*s*)[g])	93.85 (*s*)[h])	83.91 (*s*)	91.48 (*s*)	57.16 (*d*)	167.87 (*s*)[e])[f])	–	–

[a]) In CD_3OD. [b]) In $(CD_3)_2CO$. [c]) Interchangeable data within the same column. [d]) NCO–C(3′) at 165.31 (*s*) and $CONH_2$ at 154.79 (*s*). [e]) $CONH_2$ at 167.90 (*s*). [f]) δ 168.84, 168.18, 167.40 interchangeable in $(CD_3)_2CO$. [g]) *Me*O–C(5) at 50,16 (*q*). [h]) *Me*O–C(5) at 49.32 (*q*).

Table 2. *^{1}H-NMR Data for the Alkaloids Isolated from the Sponge* Zyzza massalis (in $(CD_3)_2SO$, unless otherwise stated)

	3	**8**	**10**	**11**	**(+)-12**	**13**	**14**	**16**[a])	**19**
H–C(2′)	7.35 (*d*, *J* = 2.7)	7.35 (*d*, *J* = 2.6)	7.31 (*d*, *J* = 2.4)	7.31 (*d*, *J* = 2.7)	7.44 (*d*, *J* = 2.4)	8.81 (*d*, *J* = 2.7) 8.93[a])	8.83 (*d*, *J* = 3.0) 9.22[a])	8.53 (*d*, *J* = 3.0)[b])	8.03 (*d*, *J* = 2.4)
H–C(4′)	7.67 (*dd*, *J* = 8.1, 0.6)	7.53 (br. *d*, *J* = 7.8)	7.54 (br. *d*, *J* = 7.6)	7.53 (br. *d*, *J* = 7.8)	7.57 (br. *d*, *J* = 8.1)	8.24 (*m*) 8.42[a])	8.26 (*m*) 8.54[a])	8.30 (*m*)	8.28 (br. *d*, *J* = 7.8)
H–C(5′)	7.01 (*ddd*, *J* = 7.8, 7.2, 0.9)	7.01 (*ddd*, *J* = 7.8, 7.1, 1.1)	6.98 (br. *dd*, *J* = 7.8, 7.2)	6.98 (*ddd*, *J* = 7.8, 7.2, 0.9)	7.01 (*ddd*, *J* = 8.1, 7.0, 1.2)	7.34 (*m*)	7.34 (*m*)	7.24 (*m*)	7.13 (*ddd*, *J* = 8.1, 7.2, 1.2)
H–C(6′)	7.11 (*ddd*, *J* = 7.8, 7.2, 0.9)	7.09 (*ddd*, *J* = 7.8, 7.2, 1.2)	7.08 (br. *dd*, *J* = 8.1, 7.2)	7.08 (br. *dd*, *J* = 8.1, 7.2)	7.15 (*ddd*, *J* = 8.1, 7.0, 1.2)	7.34 (*m*)	7.34 (*m*)	7.24 (*m*)	7.46 (br. *d*)
H–C(7′)	7.38 (*dd*, *J* = 8.1, 0.9)	7.36 (*dd*, *J* = 7.9, 0.9)	7.36 (br. *d*, *J* = 8.1)	7.36 (br. *d*, *J* = 8.1)	7.43 (*dd*, *J* = 8.1, 1.2)	7.60 (*m*)	7.61 (*m*)	7.54 (*m*)	7.54 (*m*)
R^1	11.27 (br. *s*)	11.24 (br. *d*, *J* = 2.6)	11.15 (br. *s*)	11.14 (br. *s*)	10.35 (br. *s*)	11.57 (br. *s*)	11.60 (br. *s*)	11.50 (br. *s*)	10.71 (br. *s*)
R^2	3.23 (*s*)[c])	3.23 (*s*)[c])	–	–	5.46 (*dd*, *J* = 1.2, 0.6)	–	–	9.50 (br. *s*)[d])	–
R^3	9.05 (*s*)	9.35 (br. *s*)	8.79 (br. *s*)	8.79 (br. *s*)	7.25 (br. *s*)	–	–	–	–
R^4	11.01 (br. *s*)	12.71 (br. *s*)	–	–	7.25 (br. *s*)	–	10.70 (br. *s*)	–	–

[a]) In $(CD_3)_2CO$. [b]) δ 8.50 in $(CD_3)_2SO$ or 8.17 in CD_3OD. [c]) δ 3.38 in CD_3OD. [d]) δ 8.50 and 6.40 (2 br. *s*, $CONH_2$).

heterocorrelations (^{13}C-NMR: deshielded *s* at δ 206.09 (C=S)[2]), shielded *s* at δ 156.25 ppm (imide C=O); low-field NMR signals for *Me*O–C(5) (*Tables 1* and *2*)). Lack of optical rotation for **8** in the whole 365–589-nm range indicated a racemic C(5) centre, in agreement with incorporation of MeOH during workup. Thus, **8** is an analogue of polyandrocarpamide D [3] (**3**), which was also found as a component of the sponge extracts.

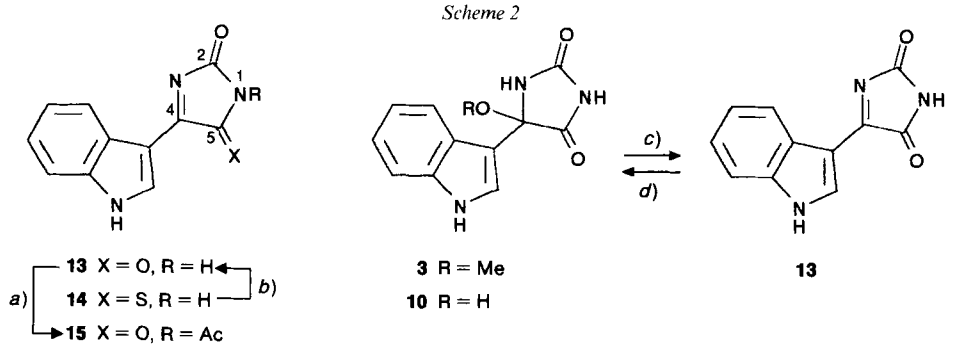
Acetylation of **3** occurred mainly at N(1)[3]) giving **4** that showed the molecular ion (EI-MS), whereas methylation of **3** with MeI/K_2CO_3 in acetone gave the permethylated **5** [3], besides the partially methylated **7** and the hydroxy compound **6** (*Scheme 1*). Product **6** probably arose from MeOH elimination under the basic reaction conditions, followed by hydration during workup.

The artifact nature of both **3** and **8** was confirmed by extracting the sponge with acetone, followed by reversed-phase HPLC purification with MeCN/H_2O, which led to the racemic 5-hydroxy analogues **10** and **11**.

Interestingly, slow partial transformation of the thioxo group into the oxo group was observed during reversed-phase HPLC elution of **11** with MeCN/H_2O (*Scheme 1* and *Exper. Part*), and similar observations were also made for both **8** and **14** (the latter isolated as indicated below). These observations recall the decomposition of a marine-derived trithiane yielding the corresponding ketone *via* an elusive thioketone [8]. However, the isolation of thio analogues **8**, **11**, and **14** reflects a higher stability of thioamides than of thioketones [9a].

2.2. *The Coloured Products.* MeOH or acetone extraction of freeze-dried *Z. massalis* led to mainly **13** or **14**[4]) (*Scheme 2*), according to the experimental procedure. While **14**

Scheme 2



a) AcCl, pyridine, r.t., or Ac_2O, pyridine, 4-(dimethylamino)pyridine, r.t. *b*) In wet $(CD_3)_2SO$, **14** gave mainly **13**, accompanied by minor amounts of **10** and **11**. *c*) Δ (–ROH). *d*) R.t. (+ROH).

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[2]) Thiolactams are known to exist preferentially in the thioketo rather than thioenol form [6]. In a thioamide, the ^{13}C-NMR C=S signal is normally shifted downfield by *ca.* 30 ppm with respect to an amide C=O [7]; this was indeed observed on comparison of **8** with **3** or of **11** with **10**.

[3]) The diacetylated form, previously reported as the sole product of acetylation of **3** [3], was observed here only as a trace product.

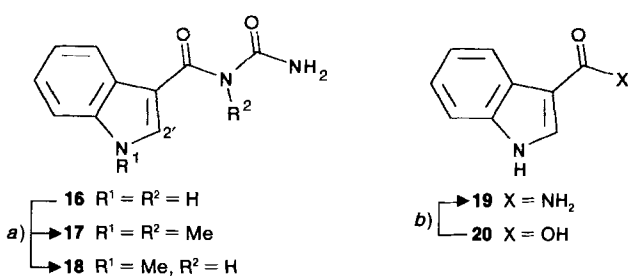
[4]) The imino group C(4)=N(3) in **13** and **14** was suggested by the absence of the ^{13}C-NMR signal of the quaternary C(4) present for **3**, **8**, **10**, and **11** (C(5) in *Table 1*) which was replaced by downfield signals at *ca.* 167 ppm. This was confirmed by acetylation of **13** → **15**. The tautomeric form with a C(2)=N(1) moiety for **13** can be ruled out by the lack of resonances in the δ(C) 180 area [2]. Tautomeric forms for **14** are more difficult to distinguish [9b].

was observed to change smoothly into **13**, reversed-phase HPLC of a MeOH solution of **13**, on elution with $MeCN/H_2O$, led to both **10** and **3** by addition of either H_2O or MeOH to C(4)=N (*Scheme 2*). Notably, the colourless solutions of either **3** or **10**, as obtained from reversed-phase HPLC, turned to yellow on partial evaporation at reduced pressure while warming at 40°; formation of **13** accounts for the colour change. A similar colour change was observed also for **8** and **11**, resulting from their partial transformation into **14**.

These phenomena could be directly observed by ^{1}H-NMR spectroscopy. A $(CD_3)_2SO$ solution of **3** at room temperature showed only the ^{1}H-NMR signals expected for this structure. On warming the sample, formation of **13** was observed at the expense of **3**, **13** prevailing at 60° and being the exclusive form at 100°[5]); on cooling to room temperature, form **3** or **10** were obtained back, suggesting an equilibrium process of solvent loss and addition (*Scheme 2*). Thus, the couples of compounds **3**/**13** and **10**/**13** constitute novel thermochromic systems [10]. When a colourless DMSO solution of **3** was heated at 80° for 1 h, a VIS absorption band was developed at 445 nm, due to the conjugated system of **13**. This situation changed on either addition of H_2O in traces, by which the intensity of the absorption at 445 nm decreased (**13** → **10**), or addition of 0.1M aqueous HCl, by which this VIS band was shifted to 410 nm and increased dramatically in intensity, very likely due to the formation of protonated **13**. In fact, on neutralization with NaOH, the original VIS band at 445 nm reappeared; thus the process proved to be reversible, in analogy to the behaviour of aniline (E-2 band, λ_{max} 230 nm) which exhibited a reversible *ca.* 30-nm shift on protonation to the anilinium cation (E-2 band, λ_{max} 203 nm) [11]. With **14**, as expected for a thiocarbonyl *vs.* a carbonyl system, the VIS absorption band was displaced towards longer wavelengths and more intense than for **13**.

2.3. *The Optically Active Form and Simpler Indol-3-yl Derivatives.* The only optically active compound isolated from *Z. massalis* was (+)-**12**. The structure of (+)-**12** was assigned mainly from heterocorrelation for H–C(5) (δ(H) 5.46 ppm (*s*), δ(C) 57.16 ppm (*d*)), HR-EI-MS for the molecular ion (*Exper. Part*), and spectral analogies with compounds discussed above. In view of the facile S → O exchange observed for **9**, **11**, and **14**, it can be assumed that (+)-**12** is a workup artifact originating from the corresponding thioamide.

Scheme 3



a) MeI, K_2CO_3, acetone, r.t., overnight. *b*) 1. 1,1'-Carbonylbis(imidazole), DMF, r.t., 0.5 h; 2. $NH_{3(g)}$, 2 h.

[5]) Similarly, heating a solution of **8** in $(CD_3)_2SO$ gave rise to **14**; however, on continued heating at 100°, while recording ^{13}C-NMR spectra, a complex mixture of products was formed.

Besides **14** and its artifacts, more simple indol-3-yl derivatives were isolated from *Z. massalis*, such as **16** (*Scheme 3*). The composition $C_{10}H_9N_3O_2$ was derived from HR-EI-MS, in agreement with NMR spectra that showed the signal for *H*–C(2′) (8.50 ppm (*d*, $J = 2.4$ Hz)) coupled to that of C(2′) (130.71 ppm (*d*)) and 4 br. *s* for the 4 protons bound to N-atoms (*Tables 1* and *2*). On treatment with excess MeI/K_2CO_3 in acetone, **16** gave the expected mono- and dimethyl derivatives **18** and **17**. The latter structure was confirmed by HR-EI-MS on the fragment *m/z* 188 formed by a *McLafferty* rearrangement (*Exper. Part*).

The 1*H*-indole-3-carboxamide (**19**, *Scheme 3*) was also isolated from *Z. massalis*, and the spectral data proved to match those of synthetic **19** obtained by treatment of the acylimidazole [12] from 1*H*-indole-3-carboxylic acid (**20**) with ammonia.

3. Conclusions. – That extraction procedures may alter the structure of compounds of nature is expected for products that are either fragile, or loosely bound to the organism's components, or protected from oxidation by cell compartmentalization. This study has provided illustrative examples. Apparently, of all compounds isolated from the sponge *Z. massalis*, only zyzzin (**14**) may represent a form occurring in nature. It undergoes C(5)=S to C(5)=O exchange giving **13**, which adds solvent at C(4)=N. None of the other tricyclic compounds described here can safely be considered as natural products as it is unknown if the S → O exchange or the H_2O addition phenomena described above occur in nature.

Because of these facile chemical transformations, it proved difficult to relate the marked antibacterial and antifungal activities of the sponge extracts (*Exper. Part*) to chemically defined compounds, though **3** could be easily assayed, proving its moderate antibacterial and antifungal action. Rewardingly, however, compounds **13** and **14** offer the first thermochromic systems of marine origin of which we are aware.

We thank Prof. *C. Lévi*, Musée National d'Histoire Naturelle, Paris, for the sponge identification, Prof. *P. R. Bergquist*, University of Auckland, for precious comments, Mrs *M. Rossi* and *A. Sterni* for technical contribution with product isolation and mass spectra, respectively, and *MURST* (Progetti 40%) and *CNR*, Roma, for financial support. This work was carried out within the collaborative program *ORSTOM-CNRS* on 'Marine Substances of Biological Interest'.

Experimental Part

1. *General.* All evaporations were carried out at reduced pressure. Yields are given on reacted compounds. DMF and pyridine were distilled from BaO and stored on flamed 4 Å molecular sieves. M.p.: *Kofler* hot-stage microscope. Flash chromatography (FC): *Merck Si-60*, 15–25 μm. TLC: *Merck* silica gel *60 PF_{254}* plates. HPLC: *Merck LiChrosorb RP18*, 7 μm, 25 × 1 cm columns; MeCN/H_2O 1:3, unless otherwise stated, flow 5 ml min^{-1}; UV monitoring λ 254 nm. Polarimetric data: *JASCO-DIP-181* polarimeter. UV/VIS: *Perkin-Elmer-Lambda-3* spectrophotometer; λ_{max} (ε) in nm. IR: *Perkin-Elmer-337* spectrometer; $\tilde{\nu}_{max}$ in cm^{-1}. NMR: δ values in ppm, in $CDCl_3$ rel. to internal $SiMe_4$ (= 0 ppm) and $CDCl_3$ (δ(C) 77.00 ppm), in $(CD_3)_2SO$ rel. to the solvent (δ(H) 2.49, δ(C) 39.50), in $(CD_3)_2CO$ rel. to the solvent (δ(H) 2.05, δ(C) 29.80), and in CD_3OD rel. to the solvent (δ(H) 3.30, δ(C) 49.30); *J* in Hz; *Varian-XL-300* spectrometer (^1H at 299.94 MHz; ^{13}C at 75.43 MHz, multiplicities from DEPT experiments [13]); $^1H,^1H$ [14] and $^1H,^{13}C$ assignments from one-bond [15a] and long-range $^1H,^{13}C$ COSY experiments [15b]; HMBC *via* the heteronuclear multiple-quantum coherence pulse sequence [16a], using a dedicated probe [16b]. EI-MS (*m/z*; %): *Kratos-MS80* mass spectrometer with home-built data system and equipped with a *Vacumetrics DIP* gun for FAB spectra.

2. *Collection, Isolation, and Biological Assays. Z. massalis* was first collected in March 1989 by dredging at a mean depth 235 m on the Norfolk Ridge Sea-Mounts, New Caledonia, in an area (23°40,5′ S, 168°00,26′ E) unusually rich in biodiversity. It appeared as a small (3–5 cm) glutinous, shapeless mass. The colour, orange-ycl-

low, proved to be stable upon exposure to the air. Immediately after collection, the sponge was deep frozen, then freeze-dried to give a red powder which was extracted with EtOH. After evaporated of the EtOH extract the residue was extracted with CH_2Cl_2 and the extract evaporated: 2.45 g of deep-red residue (R1452/616M). A second collection of this sponge in 1990 gave 0.64 g of the corresponding residue (R1452/617M). These residues proved to be antibacterial (on ten strains of marine *Vibrio* spp., which are pathogenic for mollusc, fish, and crustacean larvae in aquaculture, on two strains of pathogenic marine *Pseudomonas* spp., and on the human pathogenic *Staphylococcus aureus*), antifungal (on *Fusarium oxysporum* and *Candida albicans*), and toxic to the crustacean *Artemia salina*. Neither cytotoxicity on tumoral cell lines nor antiviral activity were detected. A portion (1.5 g) of these combined residues was subjected to FC (hexane/Et_2O, then hexane/AcOEt, gradient elution, and finally MeOH), collecting 24 fractions of 50 ml each. *Fr. 10–12* were evaporated to give **8** (reversed-phase HPLC, MeCN/H_2O 3:7, t_R 10.0 min; 35.2 mg). *Fr. 13–15* contained **13** and some **14**. *Fr. 16–18* were evaporated to give **3** (HPLC t_R 8.6 min; 39.0 mg). HPLC Purification of *Fr. 19–22* gave a colourless, optically inactive product (8.5 mg; t_R 7.0 min) whose NMR data are compatible with the general structure of the compounds in *Scheme 1*, although the nature of the R^2 substituent could not be ascertained[6]).

A third collection of this sponge in 1993 gave 110 g of freeze-dried red powder (R1452/667M) that was extracted with acetone. Evaporation gave 0.3 g of residue that was subjected to FC (hexane/AcOEt gradient elution, then acetone (300 ml), by which practically all red pigment was eluted), collecting 52 fractions of 50 ml each. *Fr. 26–46* were subjected to HPLC to give **10** (t_R 5.1 min; 11.0 mg) and **11** (t_R 7.8 min; 7.0 mg). The eluate with t_R 7.3 min was subjected to further reversed-phase HPLC purification (MeCN/H_2O 1:9) to give (+)-**12** (t_R 21.6 min; 2.0 mg) and **19** (t_R 23.4 min; 2.2 mg). The eluate with t_R 12.5 min, on evaporation, afforded **16** (3.5 mg), while the yellow eluate with t_R 15.8 min gave **13**. The red soln. that was afterwards eluted with neat MeCN, on evaporation, gave **14** that was further purified by reversed-phase HPLC (MeCN/H_2O 3:7; t_R 19.2 min).

5-(1H-Indol-3-yl)-5-methoxyimidazolidine-2,4-dione (**3**): $[\alpha]_D^{25} = 0.0$ ($c = 0.3$, MeOH). EI-MS: 213 (7), 144 (2), 116 (1), 115 (4). FAB-MS (Ar, glycerol): 246 (4, $[M + H]^+$). Moderately antibacterial (on *Staphylococcus aureus*) and weakly antifungal (on *Candida albicans*).

4-(1H-Indol-3-yl)-4-methoxy-5-thioxoimidazolidin-2-one (**8**): $[\alpha]_D^{25} = 0.0$ ($c = 0.1$, MeOH). EI-MS: 229 (1), 160 (2), 144 (12), 116 (5).

5-Hydroxy-5-(1H-indol-3-yl)imidazolidine-2,4-dione (**10**). Solid (AcOEt/hexane). M.p. 140° (dec.). $[\alpha]_D^{25} = 0.0$ ($c = 0.13$, MeOH). UV (MeOH): 340 (1900), 276 (3200), 269 (3300), 215 (13000). IR (neat): 3270, 1630, 1580. EI-MS: 231 (5, $M^{+\cdot}$), 215 (18), 116 (1), 213 (16), 144 (100), 142 (23), 116 (21).

4-Hydroxy-4-(1H-indol-3-yl)-5-thioxoimidazolidin-2-one (**11**): UV (MeOH): 340 (3000), 276 (14800), 217 (49000). EI-MS: 229 (2), 215 (35), 160 (15), 144 (68), 143 (34), 116 (21).

5-(1H-Indol-3-yl)imidazolidine-2,4-dione ((+)-**12**): White powder. $[\alpha]_D^{25} = +10.0$, $[\alpha]_{435}^{25} = +13.8$, $[\alpha]_{365}^{25} = +75.0$ ($c = 0.13$, MeOH). UV (MeOH): 277 (5200), 271 (5300), 217 (30700). EI-MS: 215 (36, $M^{+\cdot}$), 187 (5), 144 (47), 143 (35), 116 (12). HR-EI-MS: 215.0696 ± 0.0030 ($[C_{11}H_9N_3O_2]^+$, calc. 215.0695), 144.0674 ± 0.0030 ($[C_9H_8N_2]^+$, calc. 144.0687).

4-(1H-Indol-3-yl)-1H-imidazole-2,5-dione (**13**): Yellow, amorphous solid. UV/VIS (MeOH): 445 (300), 275 (11200), 263 (11700), 215 (36400). EI-MS: 213 (75, $M^{+\cdot}$), 142 (100), 116 (14), 89 (12), 71 (29), 43 (25). HR-EI-MS: 213.0538 ± 0.0020 ($[C_{11}H_7N_3O_2]^+$, calc. 213.0538).

4-(1H-Indol-3-yl)-1,5-dihydro-5-thioxo-1H-imidazol-2-one (**14**): Deep-orange amorphous solid. UV/VIS (MeOH): 475 (1200), 420 (1800), 345 (2200), 270 (5200), 212 (15800). EI-MS: 229 (45, $M^{+\cdot}$), 142 (82), 116 (12), 89 (11). HR-EI-MS: 229.0303 ± 0.0020 ($[C_{11}H_7N_3OS]^+$, calc. 229.0310).

N-(1H-Indole-3-carbonyl)urea (**16**): UV (MeOH): 289 (8100), 235 (8500), 212 (21700). EI-MS: 204 (12, $[M + H]^+$), 203 (23, $M^{+\cdot}$), 186 (16), 161 (13), 160 (24), 145 (27), 144 (100), 116 (25), 89 (24), 43 (18). HR-EI-MS: 203.0692 ± 0.0030 ($[C_{10}H_9N_3O_2]^+$, calc. 203.0694). FAB-MS (Ar, glycerol): 204.1 (11, $[M + H]^+$).

1H-Indole-3-carboxamide (**19**): White solid. M.p. (AcOEt/hexane) 201–203° ([17]: 201°). IR (nujol): 1660, 1605. UV (MeOH): 280 (9400), 213 (35000). EI-MS: 161 (23, $[M + H]^+$), 160 (59), 144 (100), 116 (25). HR-EI-MS: 160.0633 ± 0.0020 ($[C_9H_8N_2O]^+$, calc. 160.0636). FAB-MS (Ar, glycerol): 161 (100, $[M + H]^+$).

3. **11** → **10** *Change During Workup.* Compound **11** was subjected to anal. reversed-phase HPLC (MeCN/H_2O 1:3): t_R 5.8 min. The eluate was evaporated and immediately subjected to the same chromatographic procedure: peaks for both the starting **11** and the resulting **10** (t_R 3.9 min) in a 20.7:1 ratio (from peak-area integration).

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[6]) The most notable spectral differences for this compound with respect to **3** and **10** were observed for the ^{13}C-NMR *s* of C(5) which appeared downfield ($\delta(CD_3OD)$ 64.52). Only uninformative fragments were obtained in the MS under EI or FAB conditions for this compound and for its derivatives obtained on MeI/K_2CO_3 treatment. Elemental analyses gave erratic results.

4. *Acetamide Derivatives.* 4.1. *Acetylation of* **8**. A mixture of **8** (18 mg, 0.07 mmol), dry pyridine (1 ml), and excess Ac_2O was stirred for 3 h at r.t. After evaporation, the residue was subjected to FC ($CHCl_3$/MeOH 9:1): pure **9** (18 mg, 86%). ^{1}H-NMR ($(CD_3)_2SO$): 7.46 (*d*, *J* = 2.6, H–C(2′)); 7.26 (br. *d*, *J* = 8.1, 0.9, H–C(4′)); 6.99 (*ddd*, *J* = 8.1, 6.9, 0.9, H–C(5′)); 7.08 (*ddd*, *J* = 8.1, 7.0, 0.9, H–C(6′)); 7.36 (*dt*, *J* = 8.1, 0.9, H–C(7′)); 11.29 (br. *s*, H–N(1′)); 3.28 (*s*, MeO); 13.64 (br. *s*, H–N(3)); 2.42 (*s*, MeCO). ^{13}C-NMR ($(CD_3)_2SO$): 125.04 (*d*, C(2′)); 111.66 (*s*, C(3′)); 123.43 (*s*, C(3′a)); 118.10, 119.47, 121.18 (3*d*, C(4′), C(5′), C(6′)); 112.02 (*d*, C(7′)); 136.29 (*s*, C(7′a)); 153.16 (*s*, C(2)); 96.97 (*s*, C(5)); 202.64 (*s*, C(4)); 50.02 (*q*, MeO); 167.37 (*s*, Me*C*O); 25.27 (*q*, *Me*CO). EI-MS: 303 (48, M^+), 261 (6), 260 (4), 230 (24), 201 (100), 186 (12), 144 (41), 116 (15). HR-EI-MS: 303.0679 ± 0.0020 ($[C_{14}H_{13}N_3O_3S]^+$, calc. 303.0678); 230.0396 ± 0.0020 ($[C_{11}H_8N_3OS]^+$, calc. 230.0388); 186.0464 ± 0.040 ($[C_{10}H_6N_2O_2]^+$, calc. 186.0429); 144.0468 ± 0.0030 ($[C_9H_6NO]^+$, calc. 144.0449).

Under similar conditions, **3** (13 mg, 0.05 mmol) gave **4** (12 mg, 79%). ^{1}H-NMR ($CDCl_3$): 8.41 (br. *s*, HN(1′)); 7.49 (*d*, *J* = 2.7, H–C(2′)); 7.36 (*dd*, *J* = 8.0, 1.2, H–C(4′)); 7.11 (*ddd*, *J* = 8.0, 7.0, 1.2, H–C(5′)); 7.19 (*ddd*, *J* = 8.4, 7.0, 1.2, H–C(6′)); 7.35 (*dd*, *J* = 8.4, 1.2, H–C(7′)); 3.49 (*s*, MeO); 2.51 (*s*, MeCO). ^{13}C-NMR ($CDCl_3$): 124.82 (*d*, C(2′)); 113.40 (*s*, C(3′)); 123.27 (*d*, C(3′a)); 117.89, 120.91, 122.69 (3*d*, C(4′), C(5′), C(6′)); 111.98 (*d*, C(7′)); 136.40 (*s*, C(7′a)); 152.07 (*s*, C(2)); 168.20 or 167.37 (*s*, C(4)); 92.39 (*s*, C(5)); 51.62 (*q*, MeO); 25.89 (*q*, *Me*CO); 167.37 or 168.20 (*s*, Me*C*O). EI-MS: 287 (56, M^+), 256 (2), 244 (2), 214 (100), 201 (26), 186 (40), 144 (33), 143 (34), 116 (14).

4.2. *Acetylation of* **13**. To a soln. of **13** (3 mg) in dry pyridine (0.5 ml) were added Ac_2O in excess and 4-(dimethylamino)pyridine in catalytic amount. The mixture was stirred at r.t. overnight, then evaporated at r.t. and subjected to prep. TLC ($CHCl_3$/*i*-PrOH 9:1): pure **15** (2.1 mg, 59%). Similar results with AcCl in pyridine at 0°. ^{1}H-NMR ($(CD_3)_2CO$): 8.93 (br. *s*, H–C(2′)); 8.45 (*m*, H–C(4′)); 7.42 (*m*, H–C(5′), H–C(6′)); 7.68 (*m*, H–C(7′)); 2.67 (*s*, MeCO). FAB-MS (Ar, 3-nitrobenzyl alcohol): 256 (4, $[M + H]^+$), 212 (2).

5. N-*Methyl Derivatives.* 5.1. *Methylation of* **3**. To a soln. of **3** (6 mg, 0.024 mmol) in acetone (1 ml) were added K_2CO_3 and an excess of MeI (0.15 ml). The mixture was stirred overnight at r.t., filtered, and evaporated. The residue was subjected to prep. TLC (Et_2O), collecting the band with R_f 0.65 that afforded, after reversed-phase HPLC purification (MeCN/H_2O 1:1), **5** (t_R 7.8 min; 2.4 mg, 36%) and **7** (t_R 5.4 min; 1 mg, 10%). The band with R_f 0.1 gave, after reversed-phase HPLC purification, **6** (t_R 5.5 min; 3.8 mg, 53%).

*5-Methoxy-1,3-dimethyl-5-(1-methyl-1*H*-indol-3-yl)imidazolidine-2,4-dione* (**5**): ^{1}H-NMR ($(CD_3)_2SO$): 7.50 (*s*, H–C(2′)); 7.45 (br. *d*, *J* = 8.1, H–C(4′)); 7.05 (*ddd*, *J* = 8.1, 7.2, 0.9, H–C(5′)); 7.18 (*ddd*, *J* = 8.1, 7.0, 0.9, H–C(6′)); 7.24 (br. *d*, *J* = 7.8, H–C(7′)); 3.21 (*s*, MeO); 3.78 (*s*, Me–N(1′)); 2.99, 2.68 (2*s*, Me–N(1′), Me–N(3′)). ^{1}H-NMR ($CDCl_3$): 7.32 (*s*, H–C(2′)); 7.28–7.22 (*m*, H–C(4′), H–C(7′)); 7.10 (*ddd*, *J* = 8.1, 6.9, 1.2, H–C(5′), H–C(6′)); 3.78 (*s*, Me–N(1′)); 3.32 (*s*, MeO); 3.14 (*s*, Me–N(1)); 2.81 (*s*, Me–N(3)). ^{13}C-NMR ($(CD_3)_2SO$): 129.69 (*d*, C(2′)); 106.14 (*s*, C(3′)); 124.02 (*s*, C(3′a)); 118.36, 119.85, 121.58 (3*d*, C(4′), C(5′), C(6′)); 110.33 (*d*, C(7′)); 136.84 (*s*, C(7′a)); 155.08 (*s*, C(2′)); 172.84 (*s*, C(4)); 97.55 (*s*, C(5)); 50.46 (*q*, MeO); 32.52 (*q*, Me–N(1′)); 24.45, 24.32 (2*q*, Me–N(1), Me–N(3)). EI-MS: 287 (24, M^+), 256 (100), 171 (33), 156 (15), 158 (8), 115 (6).

*5-Hydroxy-1,3-dimethyl-5-(1-methyl-1*H*-indol-3-yl)imidazolidine-2,4-dione* (**6**): ^{1}H-NMR ($(CD_3)_2SO$): 7.55 (*s*, H–C(2′)); 7.45 (br. *d*, *J* = 8.1, H–C(4′)); 7.03, 7.17 (2*ddd*, *J* = 8.1, 7.0, 1.2, H–C(5′), H–C(6′)); 7.10 (br. *d*, *J* = 8.1, H–C(7′)); 3.78 (*s*, Me–N(1′)); 2.59, 2.97 (2*s*, Me–N(1), Me–N(3)). ^{13}C-NMR ($(CD_3)_2SO$): 129.44 (*d*, C(2′)); 108.71 (*s*, C(3′)); 124.06 (*s*, C(3′a)); 118.10, 119.69, 121.55 (3*d*, C(4′), C(5′), C(6′)); 110.28 (*d*, C(7′)); 136.92 (*s*, C(7′a)); 32.08 (*q*, Me–N(1′)); 24.81, 24.38 (2*q*, Me–N(1), Me–N(3)); no signals for the other C-atoms. EI-MS: 256 (100), 171 (29), 156 (5).

*5-(1*H*-Indol-3-yl)-5-methoxy-1,3-dimethylimidazolidine-2,4-dione* (**7**): ^{1}H-NMR ($CDCl_3$): 7.45 (*d*, *J* = 2.7, H–C(2′)); 7.36–7.10 (series of *m*, H–C(4′)–H–C(7′)); 8.32 (br. *s*, H–N(1′)); 3.33 (*s*, MeO); 3.15 (*s*, Me–N(1)); 2.81 (*s*, Me–N(3)). EI-MS: 273 (26, M^+), 242 (100), 157 (40), 144 (9), 142 (14), 116 (6).

5.2. *Methylation of* **16**. Compound **16** (2.9 mg, 0.014 mmol) was treated as described in *5.1*. The residue was subjected to HPLC (MeCN/H_2O 3:7): **17** (t_R 16.5 min; 1.6 mg, 50%) and **18** (t_R 14.1 min; 1.4 mg, 46%).

N-*Methyl*-N-*(1-methyl-1*H*-indole-3-carbonyl)urea* (**17**): ^{1}H-NMR ($(CD_3)_2CO$): 8.01 (*s*, H–C(2′)); 8.03 (br. *d*, *J* = 8.1, H–C(4′)); 7.30, 7.23 (2*ddd*, *J* = 7.8, 7.2, 1.2, H–C(5′), H–C(6′)); 7.52 (*dd*, *J* = 8.1, 0.9, H–C(7′)); 3.96 (*s*, Me–N(1′)); 3.47 (*s*, MeNCO). ^{13}C-NMR (CD_3OD, detectable signals): 135.83 (*d*, C(2′)); 106.12 (*s*, C(3′)); 122.62, 123.11 (2*d*, C(4′), C(5′)); 124.40 (*d*, C(6′)); 111.47 (*d*, C(7′)); 154.81 (*s*, $CONH_2$); 33.82 (*q*, Me–N(1′)); 35.88 (*q*, MeNCO). EI-MS: 231 (5, M^+), 188 (6), 158 (32), 130 (5), 103 (4). HR-EI-MS: 188.0946 ± 0.0020 ($[C_{11}H_{12}N_2O]^+$, calc. 188.0949). FAB-MS (Ar, 3-nitrobenzyl alcohol): 232 (22, $[M + H]^+$).

N-*(1-Methyl-1*H*-indole-3-carbonyl)urea* (**18**): ^{1}H-NMR ($(CD_3)_2CO$): 8.40 (*s*, H–C(2′)); 8.28 (br. *d*, *J* = 7.6, H–C(4′)); 7.32, 7.25 (2*ddd*, *J* = 8.1, 7.2, 0.9, H–C(5′), H–C(6′)); 7.53 (br. *d*, *J* = 7.6, H–C(7′)); 8.96, 8.38, 6.36 (3 br. *s*, NH); 3.95 (*s*, Me–N(1′)). FAB-MS (Ar, glycerol): 218 (58, $[M + H]^+$), 158 (100).

6. *1H-Indole-3-carboxamide* (**19**). A soln. of 1*H*-indol-3-carboxylic acid (**20**; 64 mg, 0.40 mmol) and 1,1′-carbonylbis(1*H*-imidazole) (70 mg, 0.43 mmol) in dry DMF (2 ml) was stirred at r.t. during 30 min. Then dry NH_3 was bubbled through the soln. for 2 h. To the mixture was added H_2O (15 ml). After extraction with AcOEt (3 × 20 ml), the combined org. phases were washed with sat. aq. NaCl soln., dried (Na_2SO_4), and evaporated: **19** (48 mg), identical in all respects to natural **19**. Some **20** (20 mg) was recovered from the aq. phase, giving a 92% yield of **19**.

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