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 \textit{Zyzza massalis} (DENDY) from the Coral Sea gave the orange alkaloid \textit{zyzzin} (\(=\) 4-(1H-indol-3-yl)-1,5-dihydro-5-thioxo-1H-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\textsubscript{2}O. 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\textsuperscript{\textsuperscript{\textsuperscript{-}}}-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\textsubscript{\textsuperscript{H}}-imidazol-4-one ring, such as alysinopsin (1a) and 2-deimino-2-oxoaliesenisin (1b) ((\textit{E/Z})-mixtures), isolated from sponges and dendrophylliid corals [1], compound 2, obtained from the colonial ascidian \textit{Dendrodoa grossularia} [2], and polyandrocarpamide D (3; Scheme I), isolated from another colonial ascidian, \textit{Polyandrocarpa} sp. [3].

\begin{center}
\begin{tabular}{ll}
1a & R=NH \\
1b & R=O \\
2 & \end{tabular}
\end{center}

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 \textit{Zyzza massalis} (DENDY) (\(=\) \textit{Plocania massalis} DENDY, 1921 = \textit{Zyzza massalis} DE LAUBENFELS, 1936 = \textit{Damirina verticillata} BURTON, 1959), Poecilosclerida, Cornulidae [4] from southeastern New Caledonia in the Coral Sea. \textit{Zyzza} is a genus that has no precedent in the natural-product literature\textsuperscript{1).}

\textsuperscript{1} According to a personal communication from Prof. P.R. Bergquist, University of Auckland, the reported \textit{Zyzyca cf. marshalli} [5] was misspelled and, more important, it cannot belong to the genus \textit{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'-acetyl derivative 9, yielding the molecular formula C_{14}H_{13}N_{3}O_{3}S, in agreement with the $^{13}$C-NMR spectrum. Substitution at C(3') in compound 8 was suggested by $^1$H- and $^{13}$C-NMR spectra (Tables 1 and 2), selective irradiations, $^1$H,$^1$H correlations, and $^1$H,$^{13}$C one-bond and long-range

![Scheme 1](image-url)

(a) Ac$_2$O, pyridine, r.t., overnight.  
(b) MeI, K$_2$CO$_3$, acetone, r.t., overnight.  
c) During RP18-HPLC (MeCN/H$_2$O 1:3).

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

|   | 3 | 8 | 10 | 11 | (+)-12 | 13 | 16 | 19
|---|---|---|----|----|--------|----|----|----
| C(2') | 124.60 (d) | 124.58 (d) | 124.12 (d) | 125.52 (d) | 125.35 (d) | 138.29 (d) | 129.02 (d) | 130.71 (d) | 129.02 (d)
| C(3') | 110.61 (d) | 112.96 (s) | 113.15 (s) | 110.86 (s) | 105.58 (s) | 110.55 (s) | 111.89 (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) | 127.31 (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') | 121.43 (d') | 121.43 (d')
| C(5') | 119.10 (d) | 119.08 (d) | 119.81 (d) | 120.60 (d) | 120.15 (d) | 121.96 (d) | 121.24 (d') | 122.10 (d') | 122.10 (d')
| C(6') | 119.21 (d) | 121.30 (d) | 121.29 (d) | 122.90 (d) | 122.67 (d) | 123.84 (d) | 122.50 (d') | 122.91 (d) | 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) | 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) | 137.36 (s) | 137.36 (s)
| C(2) | 156.17 (s) | 156.25 (s) | 156.01 (s) | 158.80 (s) | 157.59 (s) | 166.64 (s) | 167.87 (s) | 167.87 (s) | 167.87 (s)
| C(4) | 172.52 (s) | 206.09 (s) | 172.98 (s) | 211.45 (s) | 174.69 (s) | 167.87 (s) | 167.87 (s) | 167.87 (s) | 167.87 (s)
| C(5) | 88.53 (s) | 93.85 (s) | 83.91 (s) | 91.48 (s) | 57.16 (d) | 167.87 (s) | 167.87 (s) | 167.87 (s) | 167.87 (s)

$^a$) In CD$_3$OD.  
$^b$) In (CD$_3$)$_2$CO.  
$^c$) Interchangeable data within the same column.  
$^d$) NCO–C(3') at 165.31 (s) and CONH$_3$ at 154.79 (s).  
$^e$) CONH$_3$ at 167.90 (s).  
$^f$) $\delta$H 168.84, 168.18, 167.40 interchangeable in (CD$_3$)$_2$CO.  
$^g$) MeO–C(5) at 50.16 (q).  
$^h$) MeO–C(5) at 49.32 (q).
Table 2. $^1$H-NMR Data for the Alkaloids Isolated from the Sponge Zyzza massalis (in (CD$_3$)$_2$SO, unless otherwise stated)

<table>
<thead>
<tr>
<th></th>
<th>3</th>
<th>8</th>
<th>10</th>
<th>11</th>
<th>(+)-12</th>
<th>13</th>
<th>14</th>
<th>16*</th>
<th>19</th>
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<tr>
<td>H–C(2')</td>
<td>7.35</td>
<td>7.35</td>
<td>7.31</td>
<td>7.31</td>
<td>7.44</td>
<td>8.81</td>
<td>8.83</td>
<td>8.53</td>
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<td>(d, $J$ = 2.7)</td>
<td>(d, $J$ = 2.6)</td>
<td>(d, $J$ = 2.7)</td>
<td>(d, $J$ = 2.7)</td>
<td>(d, $J$ = 2.4)</td>
<td>(d, $J$ = 2.7)</td>
<td>(d, $J$ = 2.4)</td>
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<td>H–C(4')</td>
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<td>7.54</td>
<td>7.53</td>
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<td>8.30</td>
<td>8.28</td>
</tr>
<tr>
<td></td>
<td>(dd, $J$ = 8.1, 0.6)</td>
<td>(br. d, $J$ = 7.6)</td>
<td>(br. d, $J$ = 7.6)</td>
<td>(br. d, $J$ = 7.6)</td>
<td>(br. d, $J$ = 7.8)</td>
<td>(br. d, $J$ = 8.1)</td>
<td>(br. d, $J$ = 8.1)</td>
<td>(br. d, $J$ = 8.1)</td>
<td>(br. d, $J$ = 8.1)</td>
</tr>
<tr>
<td>H–C(5')</td>
<td>7.01</td>
<td>7.01 (ddd, $J$ = 8.1, 7.1, 1.1)</td>
<td>6.98 (br. dd, $J$ = 8.1, 7.2)</td>
<td>6.98 (ddd, $J$ = 8.1, 7.2)</td>
<td>7.01 (ddd, $J$ = 8.1, 7.2)</td>
<td>7.34 (m)</td>
<td>7.34 (m)</td>
<td>7.24 (m)</td>
<td>7.13 (ddd, $J$ = 8.1, 7.2, 1.2)</td>
</tr>
<tr>
<td></td>
<td>(ddd, $J$ = 7.8, 7.2, 0.9)</td>
<td>(J = 7.8, 7.1, 1.1)</td>
<td>(J = 7.8, 7.2)</td>
<td>(J = 7.8, 7.2, 0.9)</td>
<td>(J = 8.1, 7.0, 1.2)</td>
<td>(J = 8.1, 7.0, 1.2)</td>
<td>(J = 8.1, 7.0, 1.2)</td>
<td>(J = 8.1, 7.0, 1.2)</td>
<td>(J = 8.1, 7.0, 1.2)</td>
</tr>
<tr>
<td>H–C(6')</td>
<td>7.11</td>
<td>7.09 (ddd, $J$ = 7.8, 7.2, 1.2)</td>
<td>7.08 (br. dd, $J$ = 8.1, 7.2)</td>
<td>7.08 (br. dd, $J$ = 8.1, 7.2)</td>
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<td>7.34 (m)</td>
<td>7.24 (m)</td>
<td>7.46 (br. d)</td>
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<td>(J = 8.1, 7.2)</td>
<td>(J = 8.1, 7.2)</td>
<td>(J = 8.1, 7.0, 1.2)</td>
<td>(J = 8.1, 7.0, 1.2)</td>
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<tr>
<td>H–C(7')</td>
<td>7.38</td>
<td>7.36 (dd, $J$ = 8.1, 0.9)</td>
<td>7.36</td>
<td>7.36</td>
<td>7.43 (dd, $J$ = 8.1, 1.2)</td>
<td>7.60 (m)</td>
<td>7.61 (m)</td>
<td>7.54 (m)</td>
<td>7.54 (m)</td>
</tr>
<tr>
<td></td>
<td>(dd, $J$ = 7.9, 0.9)</td>
<td>(J = 8.1, 0.9)</td>
<td>(J = 8.1)</td>
<td>(J = 8.1)</td>
<td>(J = 8.1, 1.2)</td>
<td>(J = 8.1, 1.2)</td>
<td>(J = 8.1, 1.2)</td>
<td>(J = 8.1, 1.2)</td>
<td>(J = 8.1, 1.2)</td>
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<tr>
<td>R'</td>
<td>11.27 (br. s)</td>
<td>11.24 (br. d, $J$ = 2.6)</td>
<td>11.15 (br. s)</td>
<td>11.14 (br. s)</td>
<td>10.35 (br. s)</td>
<td>11.57 (br. s)</td>
<td>11.60 (br. s)</td>
<td>11.50 (br. s)</td>
<td>10.71 (br. s)</td>
</tr>
<tr>
<td>R^2</td>
<td>3.23 (s)</td>
<td>3.23 (s)</td>
<td>--</td>
<td>--</td>
<td>--</td>
<td>5.46 (dd, $J$ = 1.2, 0.6)</td>
<td>--</td>
<td>--</td>
<td>9.50 (br. s)</td>
</tr>
<tr>
<td>R^3</td>
<td>9.05 (s)</td>
<td>9.35 (br. s)</td>
<td>8.79 (br. s)</td>
<td>8.79 (br. s)</td>
<td>7.25 (br. s)</td>
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<td></td>
</tr>
<tr>
<td>R^4</td>
<td>11.01 (br. s)</td>
<td>12.71 (br. s)</td>
<td>--</td>
<td>--</td>
<td>--</td>
<td>7.25 (br. s)</td>
<td>--</td>
<td>--</td>
<td>10.70 (br. s)</td>
</tr>
</tbody>
</table>

* In (CD$_3$)$_2$CO.  * In (CD$_3$)$_2$SO or 8.17 in CD$_3$OD.  * In CD$_3$OD.  * $\delta$ 8.50 and 6.40 (2 br. s, CONH$_2$).
heterocorrelations ($^{13}$C-NMR: deshielded at $\delta$ 206.09 (C=S), shielded at $\delta$ 156.25 ppm (imide C=O); low-field NMR signals for MeO–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/K₂CO₃ 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/H₂O, 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/H₂O (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 thioltetone [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](image)

\[
\begin{align*}
\text{a)} & \quad \text{AcCl, pyridine, r.t., or } \text{Ac}_2\text{O, pyridine, 4-(dimethylamino)pyridine, r.t.} \\
\text{b)} & \quad \text{in wet } (\text{CD}_3)_2\text{SO, 14 gave mainly 13, accompanied by minor amounts of 10 and 11.} \\
\text{c)} & \quad \text{(-ROH).} \\
\text{d)} & \quad \text{R.t. (+ROH).}
\end{align*}
\]

\(^2\) 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 $\delta$(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/H₂O, led to both 10 and 3 by addition of either H₂O 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 'H-NMR spectroscopy. A (CD₃)₂SO solution of 3 at room temperature showed only the '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°; 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 H₂O in traces, by which the intensity of the absorption at 445 nm decreased (13 → 10), or addition of 0.1 M 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, λmax 230 nm) which exhibited a reversible ca. 30-nm shift on protonation to the anilinium cation (E-2 band, λmax 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**

\[
\begin{align*}
16 & \quad R^1 = R^2 = H \\
17 & \quad R^1 = R^2 = \text{Me} \\
18 & \quad R^1 = \text{Me}, R^2 = H \\
19 & \quad X = \text{NH}_2 \\
20 & \quad X = \text{OH}
\end{align*}
\]

a) MeI, K₂CO₃, acetone, r.t., overnight.  
b) 1. 1,1'-Carbonylbis(imidazole), DMF, r.t., 0.5 h; 2. NH₃(g), 2 h.

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5) Similarly, heating a solution of 8 in (CD₃)₂SO gave rise to 14; however, on continued heating at 100°, while recording ¹³C-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_{18}H_{19}N_{2}O_{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_{2}CO_{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 1H-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 1H-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_{2}O 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 evaporation 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_{2}O 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; \( \lambda_{max} \) in nm. IR: Perkin-Elmer-337 spectrometer; \( \nu_{max} \) in cm^{-1}. NMR: \( \delta \) values in ppm, in CDCl_{3} rel. to internal SiMe_{4} (=0 ppm) and CDCl_{3} (\( \delta(C) \) 77.00 ppm), in (CD_{3})_{2}SO rel. to the solvent (\( \delta(H) \) 2.49, \( \delta(C) \) 39.50), in (CD_{3})_{2}CO rel. to the solvent (\( \delta(H) \) 2.05, \( \delta(C) \) 29.80), and in CD_{3}OD rel. to the solvent (\( \delta(H) \) 3.30, \( \delta(C) \) 49.30); J in Hz; Varian-XL-300 spectrometer (\( ^{1}H \) at 299.94 MHz; \( ^{13}C \) at 75.43 MHz, multiplicities from DEPT experiments [13]); \( ^{1}H,^{1}H \) and \( ^{1}H,^{13}C \) assignments from one-bond [15a] and long-range \( ^{1}H,^{13}C \) COSY experiments [15b]; HMBC via the heteronuclear multiple-quantum coherence pulse sequence [16a], using a dedicated probe [16b]. EI-MS (m/z; %): Kratos-MS880 mass spectrometer with home-built data system and equipped with a Vaconetics 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-yel-
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 CH₂Cl₂ 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/Et₂O, then hexane/AcOEt, gradient elution, and finally MeOH), collecting 52 fractions of 50 ml each. Frs. 10–12 were evaporated to give 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. 10–12 were evaporated to give 8 (reversed-phase HPLC, MeCN/H₂O 3:7; tᵣ 10.0 min; 35.2 mg). Frs. 13–15 contained 13 and some 14. Frs. 16–18 were evaporated to give 3 (HPLC tᵣ 8.6 min; 39.0 mg). HPLC Purification of Fr. 19–22 gave a colourless, optically inactive product (8.5 mg; tᵣ 7.0 min) whose NMR data are compatible with the general structure of the compounds in Scheme I, although the nature of the R² substituent could not be ascertained⁶).

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 HPLC to give 10 (tᵣ 5.1 min; 11.0 mg) and 11 (tᵣ 7.8 min; 7.0 mg). The eluate with tᵣ 7.3 min was subjected to further reversed-phase HPLC purification (MeCN/H₂O 1:9) to give (+)-12 (tᵣ 21.6 min; 2.0 mg) and 19 (tᵣ 23.4 min; 2.2 mg). The eluate with tᵣ 12.5 min, on evaporation, afforded 16 (3.5 mg), while the yellow eluate with tᵣ 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/H₂O 3:7; tᵣ 19.2 min).

5-((1H-Indol-3-yl)-5-methoxyimidazolidine-2,4-dione (3): [α]₂⁰/D = 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): [α]₂⁰/D = 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 °C (dec.). [α]₂⁰/D = 0.0 (c = 0.13, MeOH). UV (MeOH): 340 (1900), 276 (3200), 269 (3300), 215 (13000). IR (neat): 3270, 1630, 1580.

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).


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⁺), 142 (100), 116 (14), 89 (12), 71 (29), 43 (25). HR-EI-MS: 213.0538 ± 0.0020 ([C₉H₇N₃O]⁺, calc. 213.0538).


N-((1H-Indole-3-carbonyl)urea (16): UV (MeOH): 289 (8100), 235 (8500), 212 (21700). EI-MS: 204 (12, [M + H⁺]), 203 (23, M⁺), 186 (16), 161 (13), 160 (24), 145 (27), 144 (100), 116 (25), 89 (24), 43 (18). HR-EI-MS: 203.0692 ± 0.0030 ([C₉H₇N₃O₂]⁺, calc. 203.0694). FAB-MS (Ar, glycerol): 204.1 (11, [M + H⁺]).


3. 11 → 10 Change During Workup. Compound 11 was subjected to anal. reversed-phase HPLC (MeCN/H₂O 1:3); tᵣ 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 (tᵣ 3.9 min) in a 20:7:1 ratio (from peak-area integration).

⁶ The most notable spectral differences for this compound with respect to 3 and 10 were observed for the ¹³C-NMR s of S(5) which appeared downfield (δ(CDCl₃) 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/K₂CO₃ 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 Ac₂O was stirred for 3 h at r.t. After evaporation, the residue was subjected to FC (CHCl₃/MeOH 9:1); pure 9 (18 mg, 86%). ¹H-NMR ((CD₃)₂SO): 7.46 (d, J = 2.6, H-C(2')); 7.26 (br. d, J = 8.1, 0.9, H-C(4')); 6.99 (dd, J = 8.1, 6.9, 0.9, H-C(5')); 7.32 (dd, 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). ¹³C-NMR (CDCl₃): 125.04 (d, C(2')); 111.66 (s, C(3')); 123.43 (s, C(3')); 118.10, 119.47, 121.18 (3d, 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(202)); 50.02 (q, MeO); 167.37 (s, MeCO); 25.27 (q, MeCO). 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 ([C₁₉H₁₉N₂O₄]⁺, calc. 303.0678; 230.0396 ± 0.0020 ([C₁₇H₁₈N₂O₆]⁺, calc. 230.0388); 186.0464 ± 0.040 ([C₁₇H₁₈N₂O₄]⁺, calc. 186.0469; 144.0494. 2.6. Acylation of 13. To a soln. of 13 (3 mg) in dry pyridine (0.5 ml) were added Ac₂O in excess and 4-(dimethylamino)pyridine in catalytic amount. The mixture was stirred at r.t. overnight, then evaporated under r.t. and subjected to prep. TLC (CHCl₃/MeOH 9:1); pure 15 (2.1 mg, 59%). Similar results with AcCl in pyridine at 0°C. ¹H-NMR (CDCl₃): 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 [M + H]⁺, 212 (2). 5. N-Methyl Derivatives. 5.1. Methylation of 3. To a soln. of 3 (6 mg, 0.024 mmol) in acetonitrile (1 ml) were added Me₂S and an excess of Me₂S (0.15 ml). The mixture was stirred overnight at r.t., filtered, and evaporated, and the residue was subjected to prep. TLC (Et₂O), collecting the band with Rf 0.65 that afforded, after reversed-phase HPLC purification (MeCN/H₂O 1:1), 5 (4.78 mg, 0.05 mmol) gave 4 (12 mg, 79%). ¹H-NMR (CDCl₃): 8.41 (br. s, H-N(1')); 7.49 (d, J = 2.7, H-C(2')); 7.36 (dd, J = 8.0, 1.2, H-C(4')); 7.11 (dd, J = 8.0, 7.0, 1.2, H-C(5')); 7.19 (dd, 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). ¹³C-NMR (CDCl₃): 124.82 (d, C(2')); 113.40 (s, C(3')); 123.27 (d, C(3'a)); 117.89, 120.91, 122.69 (3d, C(4'), C(5'), C(6')); 119.84 (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, MeCO); 167.37 or 168.20 (s, MeCO). EI-MS: 287 (56, M⁺), 256 (2), 244 (2), 214 (100), 201 (26), 186 (40), 144 (33), 134 (24), 116 (14). 4.2. Acetylation of 13. To a soln. of 13 (3 mg) in dry pyridine (0.5 ml) were added Ac₂O in excess and 4-(dimethylamino)pyridine in catalytic amount. The mixture was stirred at r.t. overnight, then evaporated under r.t. and subjected to prep. TLC (CHCl₃/MeOH 9:1); pure 15 (2.1 mg, 59%). Similar results with AcCl in pyridine at 0°C. ¹H-NMR (CDCl₃): 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 [M + H]⁺, 212 (2). 5. N-Methyl Derivatives. 5.1. Methylation of 3. To a soln. of 3 (6 mg, 0.024 mmol) in acetonitrile (1 ml) were added Me₂S and an excess of Me₂S (0.15 ml). The mixture was stirred overnight at r.t., filtered, and evaporated, and the residue was subjected to prep. TLC (Et₂O), collecting the band with Rf 0.65 that afforded, after reversed-phase HPLC purification (MeCN/H₂O 1:1), 5 (4.78 mg, 0.05 mmol) gave 4 (12 mg, 79%). ¹H-NMR (CDCl₃): 8.41 (br. s, H-N(1')); 7.49 (d, J = 2.7, H-C(2')); 7.36 (dd, J = 8.0, 1.2, H-C(4')); 7.11 (dd, J = 8.0, 7.0, 1.2, H-C(5')); 7.19 (dd, 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). ¹³C-NMR (CDCl₃): 124.82 (d, C(2')); 113.40 (s, C(3')); 123.27 (d, C(3'a)); 117.89, 120.91, 122.69 (3d, C(4'), C(5'), C(6')); 119.84 (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, MeCO); 167.37 or 168.20 (s, MeCO). EI-MS: 287 (56, M⁺), 256 (2), 244 (2), 214 (100), 201 (26), 186 (40), 144 (33), 134 (24), 116 (14).
6. 1H-Indole-3-carboxamide (19). A soln. of 1H-indol-3-carboxylic acid (20; 64 mg, 0.40 mmol) and 1,1'-carbonylbis(1H-imidazole) (70 mg, 0.43 mmol) in dry DMF (2 ml) was stirred at r.t. during 30 min. Then dry NH₃ was bubbled through the soln. for 2 h. To the mixture was added H₂O (15 ml). After extraction with AcOEt (3 × 20 ml), the combined org. phases were washed with sat. aq. NaCl soln., dried (Na₂SO₄), 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.

REFERENCES