

PTERIDINES, STEROLS, AND INDOLE DERIVATIVES FROM  
THE LITHISTID SPONGE *CORALLISTES UNDULATUS*  
OF THE CORAL SEA

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87 Ex: unique

We report here on the novel pteridine (–)-**8**, accompanying the known **2**, in a recently identified sponge, *Corallistes undulatus* Lévi and Lévi (14) of the intriguing order Lithistida (15), which inhabits low-light areas at the base of the south New Caledonian coral reef. This sponge also contains steroids typical of higher terrestrial plants, as well as tryptophan derivatives, such as 3-acrylates and serotonin, which were also previously isolated from the Mediterranean gorgonian *Paramuricea chamaeleon* (16).

#### RESULTS AND DISCUSSION

*C. undulatus* is a cup-shaped lamellar sponge with a wide hole at the bottom of the cup. The largest specimens we collected were 16 cm in diameter and brown-reddish in color. Hplc of the EtOH extract from the freeze-dried sponge gave two pale yellow

TABLE 2. <sup>1</sup>H-nmr Data for Pteridines **2** and (–)-**8** of the Sponge *Corallistes undulatus* and (–)-**13** and (–)-**14** Derived from them.

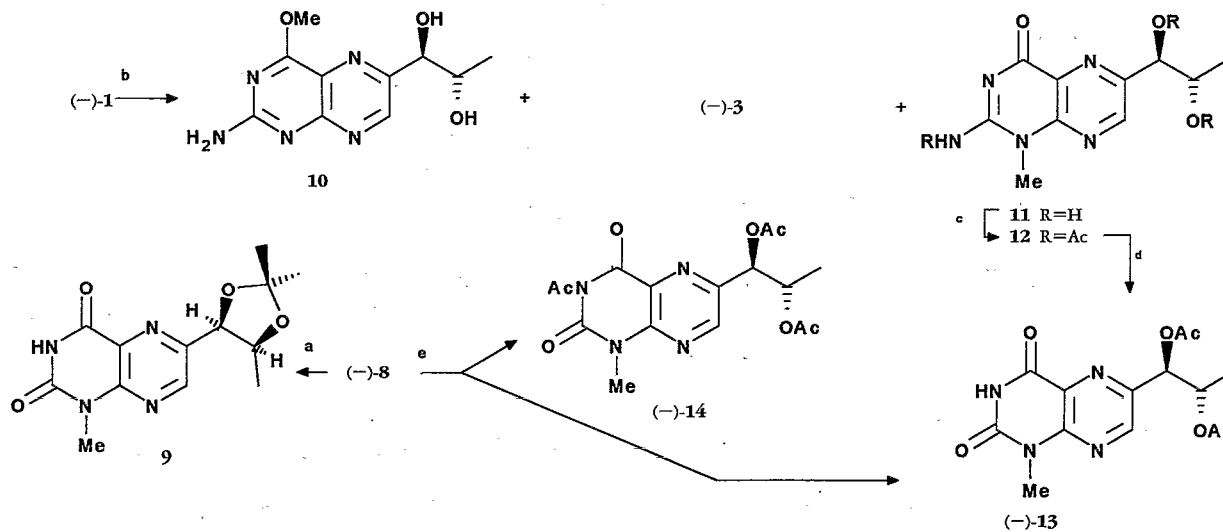
Proton	Compound			
	<b>2</b> <sup>a</sup>	(–)- <b>8</b> <sup>b</sup>	(–)- <b>13</b> <sup>c</sup>	(–)- <b>14</b> <sup>c</sup>
1-Me .....	3.47 (s)	3.48 (s)	3.68 (s)	3.69 (s)
3-Ac .....	—	—	—	2.67 (s)
H-6 .....	8.55 (d, <i>J</i> <sub>6,7</sub> =2.4)	—	—	—
H-7 .....	8.72 (d, <i>J</i> <sub>7,6</sub> =2.4)	8.76 (s)	8.73 (s)	8.73 (s)
H-1' .....	—	4.52 (br t)	6.02 (d, <i>J</i> <sub>1',2'</sub> =4.2)	6.01 (d, <i>J</i> <sub>2',3'</sub> =4.5)
1'-OH .....	—	5.65 (br s)	—	—
1'-Ac .....	—	—	2.18 (s)	2.18 (s)
H-2' .....	—	3.95 (br sext)	5.46 (dq, <i>J</i> =4.2, 6.6)	5.46 (dq, <i>J</i> =4.5, 6.6)
2'-OH .....	—	4.68 (br s)	—	—
2'-OAc .....	—	—	2.02 (s)	2.03 (s)
H-3' .....	—	1.07 (d, <i>J</i> <sub>3',2'</sub> =6.3)	1.30 (d, <i>J</i> <sub>3',2'</sub> =6.6)	1.30 (d, <i>J</i> <sub>3',2'</sub> =6.6)

<sup>a</sup>In (CD<sub>3</sub>)<sub>2</sub>SO at 50°.<sup>b</sup>In (CD<sub>3</sub>)<sub>2</sub>SO at 19°.<sup>c</sup>In CDCl<sub>3</sub> at 19°.

ever, the amino group of (–)-**1** proved exceptionally resistant to hydrolysis. The problem is similar to that of the purine bases, where the transformation of an amino into a keto group requires that the amino group is first acetylated (17). Relying on this example, we needed compound **12**. In previous attempts at methylation of (–)-**1** with CH<sub>2</sub>N<sub>2</sub>, only the product of N-3 methylation [(–)-**3**] was isolated (9). Unconvinced that there can be such a high regioselectivity in this CH<sub>2</sub>N<sub>2</sub> reaction, we repeated the reaction of (–)-**1** with excess CH<sub>2</sub>N<sub>2</sub> in MeOH, isolating, besides (–)-**3** (9) and the product **10** of C=O methylation, the desired product **11** of N-1 methylation, which could be separated (Scheme 1). According to our plans, acetylation of the amino group of **11** to give **12**, followed by hydrolysis, gave (–)-**13** (Scheme 1). In parallel, treatment of (–)-**8** with Ac<sub>2</sub>O in pyridine gave both the product (–)-**13** of diol acetylation and the product (–)-**14** of the further N-3 acetylation, which could be separated (Scheme 1). Identity of (–)-**13** from both routes establishes that the configuration of (–)-**8** is 1'*R*,2'*S*, as in all natural bipterins so far investigated.

*C. undulatus* contains also C<sub>28</sub> (**15**) and C<sub>29</sub> (**16–18**) steroids, which are typical of higher terrestrial plants. Compounds **15** and **18** have been found in other marine invertebrates as well. The 24-methylenecholestenone **15** was previously isolated from both the higher plant *Entandrophragma utile* (Meliaceae) of Cameroon (18) and the sponge *Haliclona oculata* of the Bay of Fundy (Canada) (19). *H. oculata*, belonging to the order Haplosclerida, is taxonomically and phylogenetically unrelated to *C. undulatus*. Since only the <sup>1</sup>H resonances for the methyl groups and deshielded protons have been reported for **15** (18,19), our complete nmr assignments on the basis of <sup>1</sup>H-<sup>1</sup>H and <sup>1</sup>H-<sup>13</sup>C COSY experiments are reported in the Experimental.

7α-Hydroxysitosterol [**16**] and 7β-hydroxysitosterol [**17**] were previously isolated from several higher terrestrial plants, such as the Mediterranean *Typha latifolia* (Typhaceae) (20) and *Urtica dioica* (Urticaceae) (21). Both C-5 and C-15 for **16** were incorrectly assigned (20,21), and our new assignments are reported in the Experimental.



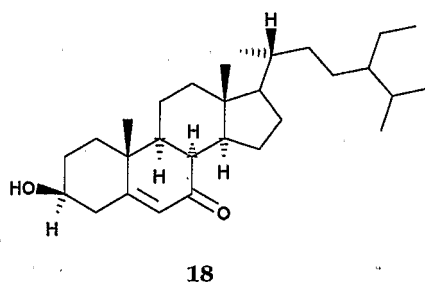
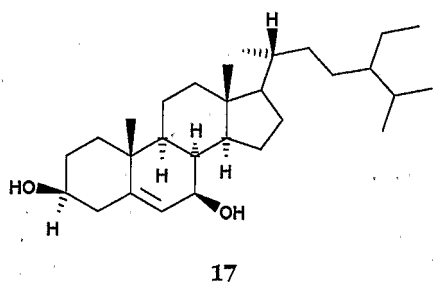
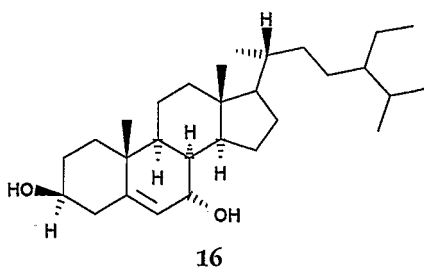
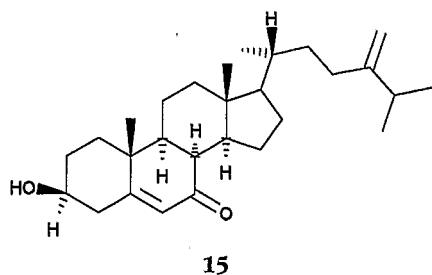
<sup>a</sup>(1) (Me<sub>2</sub>CO, CuSO<sub>4</sub>, room temperature, 80 h; (2) tlc.

<sup>b</sup>(1) excess CH<sub>2</sub>N<sub>2</sub>, MeOH, room temperature, 0.5 h; (2) tlc.

<sup>c</sup>excess Ac<sub>2</sub>O, pyridine, room temperature, 10 h.

<sup>d</sup>(1) CD<sub>3</sub>COOD-D<sub>2</sub>O (4:1), 55°, 5 h; (2) tlc.

<sup>e</sup>excess Ac<sub>2</sub>O, pyridine, room temperature, 18 h; (2) tlc.



Stigmastanone **18** was previously isolated from the terrestrial plants *Euphorbia fischeriana* (Euphorbiaceae) of Mongolia and Siberia (22) and *T. latifolia* (20). It was also found in the prosobranch mollusc *Patinigera magellanica* of the coasts of Argentina (23). Since the  $^1\text{H}$ -nmr spectrum was incompletely interpreted and there are discrepancies with our data as to some  $^{13}\text{C}$  resonances (C-5, C-7, C-12, and C-13), our re-assignments (accurate at  $\pm 0.03$  ppm from  $^1\text{H}$ - $^1\text{H}$  and  $^1\text{H}$ - $^{13}\text{C}$  COSY) are reported in the Experimental.

It should be noticed that C-7 oxidized steroids, such as **15**–**18**, may derive from autoxidation of allylic C-7 methylene precursors via hydroperoxides (24). However, the mild conditions of our quick extraction procedure suggest that sterols **15**–**18** have a natural origin.

*C. undulatus* proved to contain also indole derivatives, the 3-indolylpropenoates **19** and **20** (and, as an inseparable trace, the *Z* isomer of **19** and **20**), and serotonin [**21**], which was isolated as the mono- (**22**) and the diacetate (**23**). Their structures are straightforwardly supported by the spectral data in the Experimental.

Halogenated indole derivatives may derive biogenetically from elaboration of tryptophan and have extremely wide distribution in marine organisms, such as, for example, mollusks, algae, sponges, bryozoans, and bacteria of the genus

and phylogenetically unrelated to *C. undulatus*. In the Experimental we report  $^{13}\text{C}$ -nmr assignments for indole **20**; they have not been previously reported (27). The free acid corresponding to **20** was isolated from the sponge *Penares* sp. of Okinawa (28).

It is interesting that serotonin [**21**], a physiologically important compound in man, affecting blood pressure, promoting intestinal peristalsis, and acting as a neurotransmitter in the brain, is contained in unrelated marine invertebrates like the sponge *C. undulatus*; which lives at  $-510$  m in the New Caledonian coral reef, and the gorgonian *Paramuricea chamaeleon* (16), which lives at shallow depths in the Mediterranean Sea. We link these observations to the suggestion that pteridines in shallow water calcareous sponges may originate from diatoms (10). In the present case, for sponges which, like *C. fulvodesmus* (7) and *C. undulatus*, live at depths below 400 m (14), where photosynthesis is hardly conceivable (29), dietary origin of pteridines from diatoms seems hardly possible, unless these sponges are able to filter-feed on remains of diatoms precipitated from surface water and which still contain pteridines. We prefer to view the presence of identical or similar compounds in taxonomically, phylogenetically, and ecologically unrelated sponges, such as the Calcareia and the Lithistida, and insects, as the result of evolutionary convergence toward serviceable products. The same conclusion can be drawn about the steroid and indole derivatives found in *C. undulatus*, other marine invertebrates, terrestrial plants, and man.

## EXPERIMENTAL

GENERAL EXPERIMENTAL PROCEDURES.—Nmr spectra (in  $\text{CDCl}_3$ , if not otherwise stated) [ $\delta$  values in ppm relative to internal TMS ( $=0$  ppm) and  $J$  values in Hz]: Varian XL-300 spectrometer [ $^1\text{H}$  at 300 MHz,  $^{13}\text{C}$  at 75.4 MHz, multiplicities and  $^1\text{H}/^{13}\text{C}$  assignments from DEPT (30) and  $^1\text{H}-^{13}\text{C}$  COSY experiments (31)]. Uv spectra ( $\lambda$  max in nm,  $\Delta\epsilon$  in  $\text{mol}^{-1}$  per cm): Perkin-Elmer Lambda-3 spectrophotometer. Polarimetric data: JASCO-DIP-181 polarimeter. Flash-chromatography (fc): Merck Si-60, 15–25  $\mu\text{m}$ . Reversed-phase flash chromatography: Merck LiChrosorb RP18 (7  $\mu\text{m}$ ). Tlc: Merck Kieselgel 60  $\text{PF}_{254}$  plates. CD: Jasco J-710 spectropolarimeter ( $\lambda$  max in nm,  $\Delta\epsilon$  in  $\text{mol}^{-1}$  per cm). Hplc 25  $\times$  1 cm column filled with Merck-LiChrosorb Si-60 (7  $\mu\text{m}$ ), uv monitoring at  $\lambda$  254 nm, solvent flux 5  $\text{ml}\cdot\text{min}^{-1}$ . Tlc: Merck-Si $_{\text{F}254}$  plates. Mass spectra (ei) were taken with a Kratos MS80 mass spectrometer with home-built data system.

COLLECTION AND ISOLATION.—The sponge was collected in June 1986 by dredging at 510 m depth south of Noumea ( $24^{\circ}53.4'S$ ,  $168^{\circ}21.7'E$ ) and was identified by Professor C. Lévi. A voucher specimen is deposited at the Muséum National d'Histoire Naturelle, Paris, by Prof. C. Lévi. For biological assays, the freeze-dried sponge was extracted with EtOH, the solvent evaporated,  $\text{H}_2\text{O}$  added to the residue, and extracted with  $\text{CH}_2\text{Cl}_2$ . Evaporation of the solvent gave a residue (0.26%) that proved to inhibit both KB

H<sub>2</sub>O-MeOH (4:1) was evaporated, and the residue was subjected to reversed-phase hplc with H<sub>2</sub>O-MeOH (19:1) to give pteridines **2** (18 mg) and (-)-**8** (11.8 mg).

*1-Methylpteridine-2,4-dione* [**2**].—Long-range <sup>1</sup>H-<sup>13</sup>C-COSY (H-atom→correlated C atoms) 1-Me→C-2, C-8a, H-6→C-4a, C-7, H-7→C-6, C-8a; ms *m/z* (% rel. int.) [M]<sup>+</sup> 178 (100), 135 (32), 107 (51), 80 (78).

*(1'R,2'S)-6-(1',2'-Dihydroxypropyl)-1-methylpteridine-2,4-dione* [(-)-**8**].—[α]<sub>D</sub><sup>20</sup> -60.7°, [α]<sub>D</sub><sup>20</sup> -230.5° [*c*=0.4, H<sub>2</sub>O-MeOH (3:1)]; cd λ max [Δε, H<sub>2</sub>O-MeOH (3:1)] 228 (+4.6), 247 (-3.8), 318 (-1.4); long-range <sup>1</sup>H-<sup>13</sup>C-COSY (H-atom→correlated C atoms) 1-Me→C-2, C-8a, H-7→C-6, C-8a, H-3'→C-2'; uv (H<sub>2</sub>O, pH ca. 6) λ max 234 (ε=12000), 248 sh, 334 (ε=6700), pH ca 12) 245 (ε=16500), 343 (ε=7500); ms *m/z* (% rel. int.) [M-43]<sup>+</sup> 208 (100), 193 (7), 192 (8), 179 (11).

ACETONIDE PREPARATION FROM (-)-**8**.—Anhydrous CuSO<sub>4</sub> (50 mg) was added to a suspension of (-)-**8** (0.5 mg) in Me<sub>2</sub>CO (1 ml) and stirred for 80 h at room temperature, after which the solvent was evaporated and the residue was taken in MeOH and subjected to tlc to get acetonide **9** (0.6 mg, 100%).

*(1'R,2'S)-6-(1',2'-Dihydroxypropyl)-1-methylpteridine-2,4-dione 1',2'-acetonide* [**9**].—<sup>1</sup>H nmr (CD<sub>3</sub>OD)

19), 1.40 (H-20), 0.96 (d,  $J=6.6$ , H<sub>3</sub>-21), 1.30 (H<sub>2</sub>-22), 1.85 and 2.05 (H<sub>2</sub>-23), 2.20 (H-25), 1.02 (d,  $J=6.6$ , H<sub>3</sub>-26), 1.03 (d,  $J=6.6$ , H<sub>3</sub>-27), 4.66 and 4.72 (two br s, H<sub>2</sub>-28); ms  $m/z$  (% rel. int.) [M]<sup>+</sup> 412 (15), 397 (4), 379 (2), 369 (1), 328 (31), 285 (14), 55 (100).

**7 $\alpha$ -Hydroxysitosterol [16].**—<sup>13</sup>C nmr  $\delta$  37.01 (t, C-1), 31.37 (t, C-2), 71.35 (d, C-3), 42.01 (t, C-4), 146.26 (s, C-5), 123.86 (d, C-6), 65.37 (d, C-7), 37.52 (d, C-8), 42.25 (d, C-9), 37.40 (s, C-10), 20.71 (t, C-11), 39.16 (t, C-12), 42.14 (s, C-13), 49.42 (d, C-14), 24.32 (t, C-15), 28.28 (t, C-16), 55.66 (d, C-17), 11.65 (q, C-18), 18.26 (q, C-19), 36.23 (d, C-20), 18.86 (q, C-21), 33.89 (t, C-22), 26.17 (t, C-23), 46.04 (d; C-24), 28.91 (d, C-25), 19.58 (q, C-26), 19.01 (q, C-27), 23.01 (t, C-28), 12.33 (q, C-29); ms  $m/z$  (% rel. int.) [M]<sup>+</sup> 430 (4), 412 (100), 398 (10), 396 (14), 384 (6).

**7 $\beta$ -Hydroxysitosterol [17].**—<sup>13</sup>C nmr  $\delta$  36.91 (t, C-1), 31.52 (t, C-2), 71.38 (d, C-3), 41.69 (t, C-4), 143.43 (s, C-5), 125.41 (d, C-6), 73.31 (d, C-7), 40.86 (d, C-8), 48.22 (d, C-9), 36.40 (s, C-10), 21.04 (t, C-11), 39.51 (t, C-12), 42.89 (s, C-13), 55.30 (d, C-14), 26.36 (t, C-15 or C-23), 28.51 (t, C-16), 55.91 (d, C-17), 11.79 (q, C-18), 19.13 (q, C-19), 36.19 (d, C-20), 18.86 (q, C-21), 33.89 (t, C-22), 26.34 (t, C-23 or C-15), 46.01 (d, C-24), 28.87 (d, C-25), 19.57 (q, C-26), 18.93 (q, C-27), 22.95 (t, C-28), 12.30 (q, C-29); ms  $m/z$  (% rel. int.) [M]<sup>+</sup> 430 (4), 412 (94), 398 (15), 396 (20), 384 (14), 43 (100).

**3 $\beta$ -Hydroxystigmast-5-en-7-one [18].**—<sup>13</sup>C nmr  $\delta$  36.37 (t, C-1), 31.21 (t, C-2), 70.53 (d, C-3), 41.83 (t, C-4), 165.04 (s, C-5), 126.12 (d, C-6), 202.25 (s, C-7), 45.43 (d, C-8), 49.98 (d, C-9 or C-14), 38.72 (s, C-10), 21.23 (t, C-11), 38.29 (t, C-12), 43.11 (s, C-13), 49.96 (d, C-14 or C-9), 26.40 (t, C-15), 28.54 (t, C-16), 54.69 (d, C-17), 11.97 (q, C-18), 17.31 (q, C-19), 36.19 (d, C-20), 18.99 (q, C-21), 33.94 (t, C-22), 26.33 (t, C-23), 46.07 (d, C-24), 28.96 (d, C-25), 19.58 (q, C-26), 18.99 (q, C-27), 23.03 (t, C-28), 12.30 (q, C-29); <sup>1</sup>H nmr  $\delta$  1.19 and 1.94 (H<sub>2</sub>-1), 1.61 and 1.92 (H<sub>2</sub>-2), 3.67 (H-3), 2.41 and 2.50 (H<sub>2</sub>-4), 5.69 (H-6), 2.24 (H-8), 1.50 (H-9 or H-14), 1.56 (H<sub>2</sub>-11), 1.10 and 2.03 (H<sub>2</sub>-12), 1.28 (H-14 or H-9), 1.29 and 2.40 (H<sub>2</sub>-15), 1.24 and 1.89 (H<sub>2</sub>-16), 1.09 (H-17), 0.68 (s, H<sub>3</sub>-18), 1.20 (s, H<sub>3</sub>-19), 1.35 (H-20), 0.93 (d, H<sub>3</sub>-21), 0.96 and 1.36 (H<sub>2</sub>-22), 1.00 and 1.22 (H<sub>2</sub>-23), 0.95 (H-24), 1.66 (H-25), 0.83 (d, H<sub>3</sub>-26), 0.81 (d, H<sub>3</sub>-27), 1.14 and 1.29 (H<sub>2</sub>-28), 0.85 (t, H<sub>3</sub>-29); ms  $m/z$  (% rel. int.) [M]<sup>+</sup> 428 (100), 413 (3), 410 (7), 395 (16), 287 (15), 269 (5), 205 (14), 192 (36).

**Methyl (E)-3-(indol-3-yl)-2-propenoate [19].**—<sup>1</sup>H nmr  $\delta$  3.82 (s, OMe), 6.48 (d,  $J_{2,3}=15.9$ , H-2), 7.93 (d,  $J_{3,2}=15.9$ , H-3), 8.45 (br s, H-1'), 7.51 (br d,  $J_{2',1'}=2.6$ , 7.93 (m, H-4'), 7.24–7.32 (m, H-5' and H-6'), 7.43 (m, H-7'). That **19** is accompanied by ca. 17% of the *Z* isomer is indicated by the signals  $\delta$  3.77 (s, OMe) and 5.84 (d,  $J_{1,2}=12.3$ ). Ms  $m/z$  (% rel. int.) [M]<sup>+</sup> 201 (100), 170 (97), 143 (14), 141 (11), 115 (28).

**Methyl (E)-3-(6-bromoindol-3-yl)-2-propenoate [20].**—<sup>13</sup>C nmr  $\delta$  51.47 (q, OMe), 168.34 (s, C-1), 113.87 (d, C-2), 137.65 (d, C-3), 128.88 (d, C-2'), 113.69 (s, C-3'), 124.27 (s, C-3a'), 121.56 (d, C-4'), 124.74 (d, C-5'), 116.81 (s, C-6'), 114.76 (d, C-7'), 137.69 (s, C-7a'); <sup>1</sup>H nmr  $\delta$  3.81 (s, OMe), 6.42 (d,  $J_{2,3}=16.2$ , H-2), 7.88 (dd,  $J_{3,2}=16.2$ ,  $J_{3,4'}=0.5$ , H-3), 8.55 (br s, H-1'), 7.48 (br d,  $J_{2',1'}=2.8$ ), 7.77 (br d,  $J_{4',5'}=8.4$ ,  $J_{4',3'}=J_{4',7'}=0.5$ , H-4'), 7.36 (dd,  $J_{5',4'}=8.4$ ,  $J_{5',7'}=1.8$ , H-5'), 7.58 (dd,  $J_{7',5'}=1.8$ ,  $J_{7',4'}=0.5$ , H-7'). That **20** is accompanied by ca. 20% of the *Z* isomer is indicated by the signals  $\delta$  3.76 (s, OMe), 5.85 (d,  $J_{1,2}=12.3$ ).



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