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

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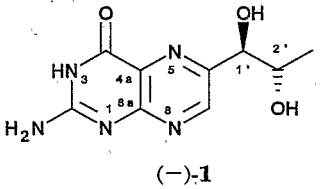
ABSTRACT.—The lithistid sponge *Corallistes undulatus*, which inhabits the base (−510 m) of the New Caledonian coral reef, is shown here to contain the pteridines 1-methylpteridine-2,4-dione [**2**], already known from another *Corallistes*, and the new (1′*R*,2′*S*)-6-(1′,2′-dihydroxypropyl)-1-methylpteridine-2,4-dione [(−)-**8**], together with the steroids 3β-hydroxy-24-methylenecholest-5-en-7-one [**15**], 7α-hydroxysitosterol [**16**],7β-hydroxysitosterol [**17**], and 3β-hydroxystigmast-5-en-7-one [**18**], typical of higher terrestrial plants, and the indole derivatives methyl(2*E*)-3-(indol-3-yl)-2-propenoate [**19**], methyl(2*E*)-3-(6-bromoindol-3-yl)-2-propenoate [**20**], and serotonin [**21**]. The presence of the same compounds in taxonomically, phyletically, and ecologically unrelated organisms is viewed here as resulting from evolutionary convergence toward adaptive products.

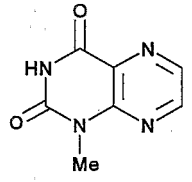
Pteridines have long been known as yellow pigments from insects (1), with biopterin [(−)-**1**] acting also as growth factor in some cases (2).

As far as sea life is concerned, biopterin has been found as a constituent of diatoms (3), while other pteridines known from terrestrial sources were detected in ascidians (4,5) and copepods (6). The lithistid sponge *Corallistes fulvodesmus* of the Coral Sea has recently been shown to contain 1-methyl-pteridine-2,4-dione [**2**] (7), previously known as a synthetic product (8).

Pteridines unknown in terrestrial life have also been found in marine organisms: for example, 3-methylbiopterin [(−)-**3**] in the Mediterranean dendrophylliid coral *Astroides calycularis* (9), leucettidine [(−)-**4**] in the Bermudian calcareous sponge *Leucetta microraphis* (10,11), and the 1′-keto analogue **5** of leucettidine, together with congeners **6** and **7** (12) and analogues with 1′,3′-dioxygenated side chain (13), in the free polychaete *Odontosyllis undecimonta* of Toyama Bay in Japan.



(−)-**1**



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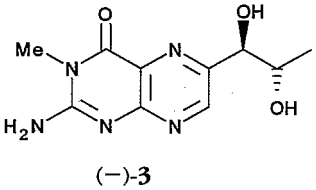
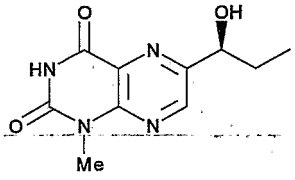
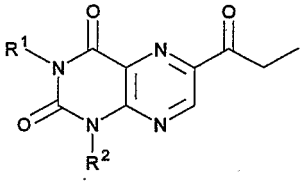


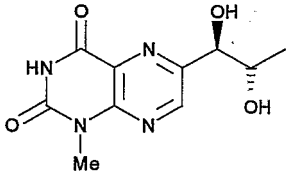
(−)-**3**



(−)-**4**



5 R^1=Me, R^2=H
6 R^1=R^2=Me
7 R^1=R^2=H



(−)-**8**

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 compounds, the known 1-methyl-pteridine-2,4-dione [**2**], previously isolated from a sponge of the same genus of the same area, *C. fulvodesmus,* and known from synthesis (7), and the novel pteridine (−)-**8**.

As far as pteridine **2** is concerned, we have confirmed by $^{n}J_{HC}$ correlation of *N*-Me with both C-2 and C-8a that methylation occurs at N-1 (see Experimental).

For compound (−)-**8** the ^{1}H-nmr (Table 2) and ^{13}C-nmr spectra (Table 1) suggest a methylated biopterin analogue. However, the molecular ion could not be detected in its eims spectra, where extensive fragmentation (highest-mass observable fragment $[M-44]^{+}$) prevented detection of a carbonyl or an amino group at C-2. The problem was circumvented by preparing acetonide **9** from (−)-**8** as indicated in Scheme 1. This gave $[M-Me]^{+}$ as the highest-mass fragment, and the hrms of this fragment established that there is a carbonyl group at C-2.

In order to assign the configurations at the side chain of (−)-**8**, we planned to correlate it chemically with commercially available L-*erythro*-biopterin [(−)-**1**]. How-

TABLE 1. ^{13}C-nmr Data for Pteridines **2** and (−)-**8** of the Sponge *Corallistes undulatus* and (−)-**13** and (−)-**14** Derived from them.

Carbon	Compound			
	2[a]	(−)-**8**[b]	(−)-**13**[c]	(−)-**14**[c]
1-Me	27.97 (q, J=142.1)	28.06 (q)	28.96 (q)	29.00 (q)
C-2	150.06 (q, J=2.8)	150.02 (s)	149.25 (s)	not det.
3-*Me*COO	—	—	—	27.68 (q)
3-Me*C*OO	—	—	—	171.38 (s)
C-4	159.86 (s)	159.95 (s)	158.87 (s)	not det.
C-4a	128.93 (dd, J=11.0, 1.5)	126.66 (s)	126.95 (s)	126.93 (s)
C-6	139.25 (dd, J=186.2, 11.9)	148.28 (s)	147.68 (s)	148.02 (s)
C-7	147.24 (dd, J=189.7, 10.2)	146.46 (d)	147.23 (d)	147.37 (d)
C-8a	149.45 (br dq, J=11.0, 1.5)	152.40 (s)	149.00 (s)	148.25 (s)
C-1′	—	76.45 (d)	75.48 (d)	75.39 (d)
1′-*Me*COO	—	—	20.87 (q)[d]	20.87 (q)[d]
1′-Me*C*OO	—	—	169.83 (s)[e]	169.64 (s)[e]
C-2′	—	69.37 (d)	70.46 (d)	70.43 (d)
2′-*Me*COO	—	—	21.05 (q)[d]	21.06 (q)[d]
2′-Me*C*OO	—	—	169.64 (s)[e]	169.82 (s)[e]
C-3′	—	18.85 (q)	15.82 (q)	15.81 (q)

[a]At 50°, in $(CD_3)_2SO$. J values represent ^{1}H-^{13}C couplings from coupled ^{13}C experiments.
[b]In $(CD_3)_2SO$ at 198.
[c]In $CDCl_3$ at 198.
[d,e]These signals can be interchanged within the same column.

TABLE 2. ^{1}H-nmr Data for Pteridines **2** and (−)-**8** of the Sponge *Corallistes undulatus* and (−)-**13** and (−)-**14** Derived from them.

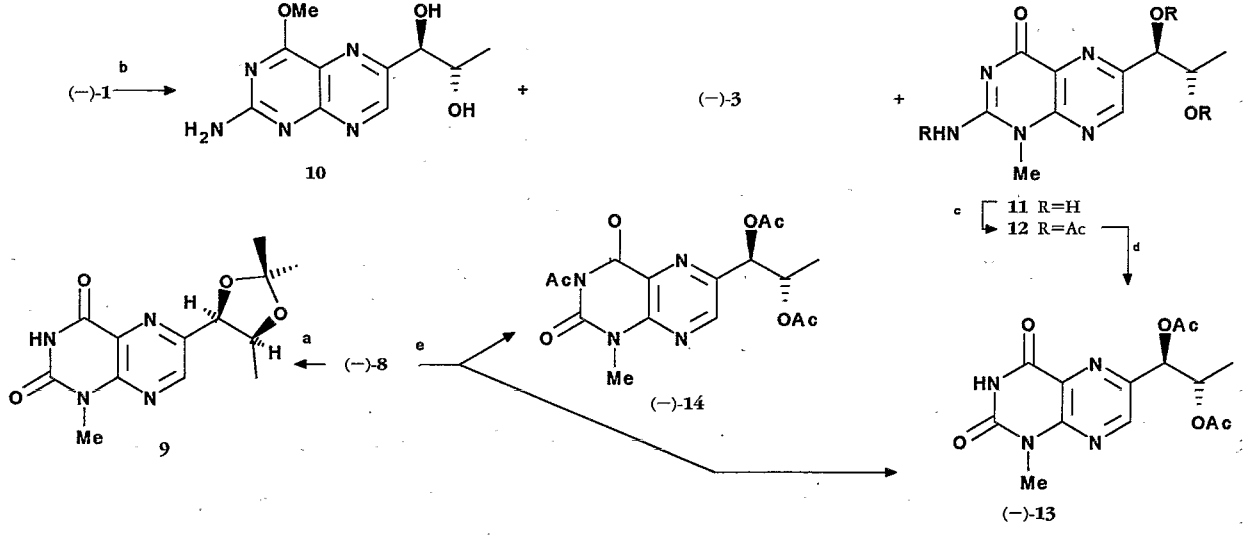
Proton	Compound			
	2[a]	(−)-**8**[b]	(−)-**13**[c]	(−)-**14**[c]
1-Me	3.47 (s)	3.48 (s)	3.68 (s)	3.69 (s)
3-Ac	—	—	—	2.67 (s)
H-6	8.55 (d, $J_{6,7}$=2.4)	—	—	—
H-7	8.72 (d, $J_{7,6}$=2.4)	8.76 (s)	8.73 (s)	8.73 (s)
H-1′	—	4.52 (br t)	6.02 (d, $J_{1',2'}$=4.2)	6.01 (d, $J_{2',3'}$=4.5)
1′-OH	—	5.65 (br s)	—	—
1′-Ac	—	—	2.18 (s)	2.18 (s)
H-2′	—	3.95 (br sext)	5.46 (dq, J=4.2, 6.6)	5.46 (dq, J=4.5, 6.6)
2′-OH	—	4.68 (br s)	—	—
2′-OAc	—	—	2.02 (s)	2.03 (s)
H-3′	—	1.07 (d, $J_{3',2'}$=6.3)	1.30 (d, $J_{3',2'}$=6.6)	1.30 (d, $J_{3',2'}$=6.6)

[a]In $(CD_3)_2SO$ at 50°.
[b]In $(CD_3)_2SO$ at 19°.
[c]In $CDCl_3$ 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_2N_2, only the product of N-3 methylation [(−)-**3**] was isolated (9). Unconvinced that there can be such a high regioselectivity in this CH_2N_2 reaction, we repeated the reaction of (−)-**1** with excess CH_2N_2 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_2O 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 biopterins so far investigated.

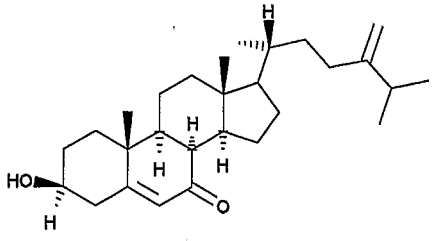
C. undulatus contains also C_{28} (**15**) and C_{29} (**16–18**) steroids, which are typical of higher terrestrial plants. Compounds **15** and **18** have been found in other marine invertebrates as well. The 24-methylenecholestenone **15** was previously isolated from both the higher plant *Entandrophragma 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 phyletically unrelated to *C. undulatus*. Since only the ^{1}H resonances for the methyl groups and deshielded protons have been reported for **15** (18,19), our complete nmr assignments on the basis of ^{1}H-^{1}H and ^{1}H-^{13}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.

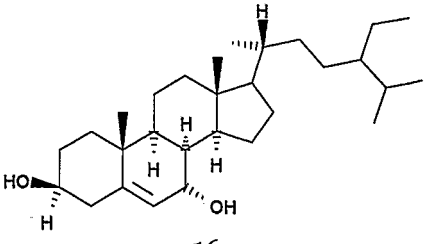


SCHEME 1

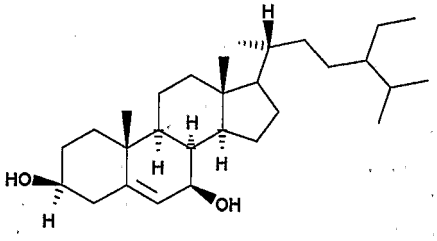
[a](1) (Me_2CO, $CuSO_4$, room temperature, 80 h; (2) tlc.
[b](1) excess CH_2N_2, MeOH, room temperature, 0.5 h; (2) tlc.
[c]excess Ac_2O, pyridine, room temperature, 10 h.
[d](1) CD_3COOD-D_2O (4:1), 55°, 5 h; (2) tlc.
[e]excess Ac_2O, pyridine, room temperature, 18 h; (2) tlc.



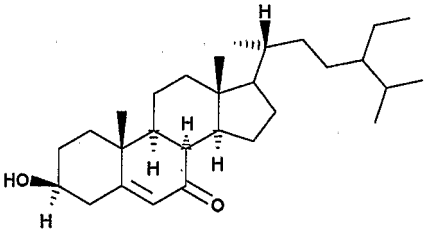
15



16



17



18

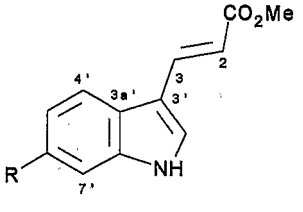
Stigmastanone **18** was previously isolated from the terrestrial plants *Euphorbia fischeriana* (Euphorbiaceae) of Mongolia and Siberia (22) and *T. latifolia* (20). It was also found in the prosobranch mollusc *Patinigera magellanica* of the coasts of Argentina (23). Since the ^{1}H-nmr spectrum was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

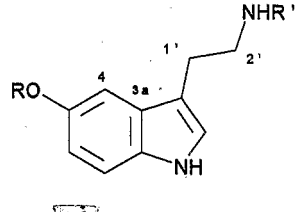
C. undulatus proved to contain also indole derivatives, the 3-indolylpropenoates **19** and **20** (and, as an inseparable trace, the *Z* isomer of **19** and **20**), and serotonin [**21**], which was isolated as the mono- (**22**) and the diacetate (**23**). Their structures are straightforwardly supported 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, hemichordates (25), and bacteria of the genera *Pseudomonas* (26).

Previously compound **20** was isolated from the sponge *Iotrochota* sp. of western Australia waters (27), which, belonging to the order Poecilosclerida, is taxonomically



19 R=H
20 R=Br



21 R=R′=H
22 R=H, R′=Ac
23 R=R′=Ac

and phyletically unrelated to *C. undulatus*. In the Experimental we report ^{13}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, phyletically, and ecologically unrelated sponges, such as the Calcarea 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 $CDCl_3$ if not otherwise stated) [δ values in ppm relative to internal TMS (=0 ppm) and J values in Hz]: Varian XL-300 spectrometer [^{1}H at 300 MHz, ^{13}C at 75.4 MHz, multiplicities and ^{1}H/^{13}C assignments from DEPT (30) and ^{1}H-^{13}C COSY experiments (31)]. Uv spectra (λ max in nm, $\Delta\epsilon$ in mol^{-1} per cm): Perkin-Elmer Lambda-3 spectrophotometer. Polarimetric data: JASCO-DIP-181 polarimeter. Flash-chromatography (fc): Merck Si-60, 15–25 μm. Reversed-phase flash chromatography: Merck LiChrosorb RP18 (7 μm). Tlc: Merck Kieselgel 60 PF_{254} plates. CD: Jasco J-710 spectropolarimeter (λ max in nm, $\Delta\epsilon$ in mol^{-1} per cm). Hplc 25×1 cm column filled with Merck-LiChrosorb Si-60 (7 μm), uv monitoring at λ 254 nm, solvent flux 5 ml·min^{-1}. Tlc: Merck-Si_{F254} 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°53.4′S, 168°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, H_2O added to the residue, and extracted with CH_2Cl_2. Evaporation of the solvent gave a residue (0.26%) that proved to inhibit both KB and P388 tumor cell lines (100% and 34%, respectively, at 10 μg/ml), while H_2O extracts proved inactive. No antibacterial (*Staphylococcus aureus, Escherichia coli, Pseudomonas aeruginosa*), antifungal (*Fusarium oxysporum, Phythophtora hevea, Penicillium digitatum*), toxic (ticks, *Boophilus microplus*; crustaceans, *Artemia salina*), or herbicidal (*Amaranthus caudatus*) activities were observed by either organic or aqueous extracts of the sponge. For the isolation of natural products, the fresh sponge was immediately frozen, freeze-dried (4.5 kg) and extracted first with petroleum ether (bp 40–70°) and then with EtOH. The petroleum ether extract (2 g) was subjected to an fc gradient from petroleum ether to EtOAc. The fraction eluted with petroleum ether-EtOAc (1:1) was evaporated, and the residue was subjected to reversed-phase hplc with MeOH-H_2O (97:3) (λ 210 nm) to give two Ehrlich blue-reacting sterols, the more polar **16** (1.8 mg) and the less polar **17** (2.3 mg). The fc fraction eluted with petroleum ether-EtOAc (3:7), and subjected to reversed-phase hplc with MeOH-H_2O (19:1), gave steroid **15**, Rt 15 min (6.5 mg). The EtOH extract (ca. 30 g containing much inorganic salt) was subjected to an fc, gradient from petroleum ether to 95% EtOH, collecting two fractions, at petroleum ether-EtOH (1:9) (0.23 g) and 95% EtOH (3 g containing much inorganic salt). The above eluate of 0.23 g was subjected to fc, gradient from hexane to Et_2O, and the eluates were subjected to reversed-phase hplc to give the indole derivatives **19**, Rt 6 min with MeOH-H_2O (4:1) (1.2 mg), **20**, Rt 9 min (2.8 mg) with MeOH-H_2O (4:1), and, changing to MeOH-H_2O (19:1), steroid **18**, Rt 18 min (8.5 mg). The above eluate of 3 g was subjected to reversed-phase fc, gradient from H_2O to H_2O-MeOH (1:1). The fraction eluted with H_2O, containing polar compounds, was dried and then treated with Ac_2O/pyridine. Solvent evaporation and tlc with EtOAc-MeOH (9:1) gave **22** (3.5 mg) and **23** (1.2 mg). The fc fraction eluted with

H_2O-MeOH (4:1) was evaporated, and the residue was subjected to reversed-phase hplc with H_2O-MeOH (19:1) to give pteridines **2** (18 mg) and (−)-**8** (11.8 mg).

1-Methylpteridine-2,4-dione [**2**].—Long-range ^{1}H-^{13}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.) $[M]^+$ 178 (100), 135 (32), 107 (51), 80 (78).

(1′R,2′S)-6-(1′,2′-Dihydroxypropyl)-1-methylpteridine-2,4-dione [(−)-**8**].—$[\alpha]^{20}D$ −60.7°, $[\alpha]_{435}^{20}$ −230.5° [*c*=0.4, H_2O-MeOH (3:1)]; cd λ max [Δε, H_2O-MeOH (3:1)] 228 (+4.6), 247 (−3.8), 318 (−1.4); long-range ^{1}H-^{13}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_2O, 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.) $[M-43]^+$ 208 (100), 193 (7), 192 (8), 179 (11).

ACETONIDE PREPARATION FROM (−)-**8**.—Anhydrous $CuSO_4$ (50 mg) was added to a suspension of (−)-**8** (0.5 mg) in Me_2CO (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**].—^{1}H nmr (CD_3OD) 3.63 (s, 1-Me), 8.82 (s, H-7), 5.38 (d, $J_{1',2'}$=6.9, H-1′), 4.79 (dq, *J*=6.9, 6.3, H-2′), 1.66 and 1.49 (2q, *J*=0.6, CMe_2), 0.84 (d, *J*=6.3, H-3′); ms *m/z* (% rel. int.) $[M-Me]^+$ 277 (22), 248 (100), 235 (50), 233 (25), 219 (5), 217 (8), 208 (28), 207 (50); hrms 277.09368 ($C_{12}H_{13}N_4O_4$ requires 277.09367).

SYNTHESIS OF (−)-**13**.—*From (−)-***1**.—A partially solubilized suspension of L-*erythro*-biopterin [(−)-**1**] (5 mg) in 3 ml of MeOH was treated with CH_2N_2 in Et_2O for 0.5 h under stirring at room temperature, whereby all materials were solubilized. The solution was evaporated, and the residue was subjected to tlc with EtOAc-MeOH (2:1) to give, in order of increasing polarity, compounds **10**, **3**, and **11** in 3:4:2 molar ratio. Compound **11**, dissolved in pyridine, was treated with excess Ac_2O to give the triacetate **12**, which, dissolved in CD_3COOD-D_2O (4:1), was heated at 55° in the ^{1}H-nmr probe. After 5 h, the δ 3.86 s [1-Me in **12**] disappeared by 90%, replaced by a δ 3.62 s [1-Me in (−)-**13**]. In fact, the reaction mixture, subjected to tlc with EtOAc-MeOH (9:1) gave compound (−)-**13** (0.35 mg, 5% from biopterin).

*From (−)-***8**.—Compound (−)-**8** (3 mg) was treated with excess Ac_2O in pyridine for 18 h at room temperature, after which the mixture was subjected to tlc with EtOAc-MeOH (9:1) to give, in order of increasing polarity, compounds (−)-**13** (2.8 mg, 70%) and (−)-**14** (0.6 mg, 14%).

(1′R,2′S)-2-Amino-6-(1′,2′-dihydroxypropyl)-4-methoxypteridine [**10**].—^{1}H nmr (CD_3OD) δ (partial data from a mixture with both **3** and **11**) 4.17 (s, OMe), 8.94 (s, H-7), 4.643 (d, H-2′).

(1′R,2′S)-2-Amino-6-(1′,2′-dihydroxypropyl)-3-methylpteridine-4-one [**3**].—^{1}H nmr (CD_3OD) δ (partial data from a mixture with both **10** and **11**) 3.55 (s, 3-Me), 8.821 (s, H-7), 4.638 (d, H-2′).

(1′R,2′S)-2-Amino-6-(1′,2′-dihydroxypropyl)-1-methylpteridine-4-one [**11**].—^{1}H nmr (CD_3OD) δ (partial data from a mixture with both **10** and **3**) 3.78 (s, 1-Me), 8.816 (s, H-7), 4.69 (d, H-2′).

(1′R,2′S)-6-(1′,2′-Diacetoxypropyl)-2-acetylamino-1-methylpteridine-4-one [**12**].—^{1}H nmr ($CDCl_3$) δ 3.82 (s, 1-Me), 2.30 (s, 2-Ac), 8.76 (s, H-7), 6.03 (d, *J*=4.6, H-1′), 2.18 (s, 1′-Ac), 5.46 (dq, *J*=4.6, 6.6, H-2′), 2.02 (s, 2′-Ac), 1.31 (d, *J*=6.6, H-3′).

(1′R,2′S)-6-(1′,2′-Diacetoxypropyl)-1-methylpteridine-2,4-dione [(−)-**13**].—$[\alpha]^{20}D$ −51.5°, $[\alpha]_{435}^{20}$ −108.3° (*c*=0.21, EtOH); cd λ max (Δε, EtOH) 227 (+1.2), 250 (−4.6), 320 (−0.6); uv λ max (EtOH, pH ca. 7) 239 (ε=13600), 254 sh, 334 (ε=6700), (EtOH, pH ca. 12) 247 (ε=19000), 343 (ε=7800); ms *m/z* (% rel. int.) $[M+H]^+$ 337 (0.6), $[M-HOAc]^+$ 276 (4), 250 (23), 234 (44), 208 (100), 43 (65); hrms 276.08622 ($C_{12}H_{12}N_4O_4$ requires 276.08585). Products obtained from either (−)-**1** or (−)-**8** (Scheme 1) had the same spectral and chiroptical data.

(1′R,2′S)-3-Acetyl-6-(1′,2′-diacetoxypropyl)-1-methylperidine-2,4-dione [(−)-**14**].—$[\alpha]^{20}D$ −109°, $[\alpha]_{435}^{20}$ −158° (*c*=0.04, EtOH); uv λ max (EtOH, pH ca. 7) 240 (ε=14600), 254 (ε=15400), 334 (ε=6300); ms *m/z* (% rel. int.) 276 (5), 250 (22), 234 (44), 208 (100), 43 (71).

3β-Hydroxy-24-methylenecholest-5-en-7-one [**15**].—^{13}C nmr δ 36.34 (t, C-1), 31.19 (t, C-2), 70.54 (d, C-3), 41.81 (t, C-4), 165.07 (s, C-5), 126.13 (d, C-6), 202.30 (s, C-7), 45.41 (d, C-8), 49.95 (d, C-9 or C-14), 38.28 (s, C-10), 21.22 (t, C-11), 38.69 (t, C-12), 43.14 (s, C-13), 49.90 (d, C-14 or C-9), 26.31 (t, C-15), 28.54 (t, C-16), 54.63 (d, C-17), 12.00 (q, C-18), 17.33 (q, C-19), 35.66 (d, C-20), 18.87 (q, C-21), 34.68 (t, C-22), 30.97 (t, C-23), 156.79 (s, C-24), 33.76 (d, C-25), 21.87 (q, C-26), 22.01 (q, C-27), 106.03 (t, C-28); ^{1}H nmr δ 1.30 and 2.00 (H_2-1), 1.60 and 1.95 (H_2-2), 3.68 (m, $W_{1/2}$=25 Hz, H-3), 2.40 and 2.50 (H_2-4), 5.70 (br d, *J*=1.5, H-6), 2.30 (H-8), 1.45 (H-9 or H-14), 1.60 (H_2-11), 1.30 and 2.05 (H_2-12), 1.30 (H-14 or H-9), 1.30 and 2.40 (H_2-15), 1.35 and 2.00 (H_2-16), 1.15 (H-17), 0.69 (s, H_3-18), 1.20 (s, H_3-

19), 1.40 (H-20), 0.96 (d, J=6.6, H_3-21), 1.30 (H_2-22), 1.85 and 2.05 (H_2-23), 2.20 (H-25), 1.02 (d, J=6.6, H_3-26), 1.03 (d, J=6.6, H_3-27), 4.66 and 4.72 (two br s, H_2-28); ms *m/z* (% rel. int.) $[M]^+$ 412 (15), 397 (4), 379 (2), 369 (1), 328 (31), 285 (14), 55 (100).

7α-Hydroxysitosterol [**16**].—^{13}C nmr δ 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.) $[M]^+$ 430 (4), 412 (100), 398 (10), 396 (14), 384 (6).

7β-Hydroxysitosterol [**17**].—^{13}C nmr δ 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.) $[M]^+$ 430 (4), 412 (94), 398 (15), 396 (20), 384 (14), 43 (100).

3β-Hydroxystigmast-5-en-7-one [**18**].—^{13}C nmr δ 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); ^{1}H nmr δ 1.19 and 1.94 (H_2-1), 1.61 and 1.92 (H_2-2), 3.67 (H-3), 2.41 and 2.50 (H_2-4), 5.69 (H-6), 2.24 (H-8), 1.50 (H-9 or H-14), 1.56 (H_2-11), 1.10 and 2.03 (H_2-12), 1.28 (H-14 or H-9), 1.29 and 2.40 (H_2-15), 1.24 and 1.89 (H_2-16), 1.09 (H-17), 0.68 (s, H_3-18), 1.20 (s, H_3-19), 1.35 (H-20), 0.93 (d, H_3-21), 0.96 and 1.36 (H_2-22), 1.00 and 1.22 (H_2-23), 0.95 (H-24), 1.66 (H-25), 0.83 (d, H_3-26), 0.81 (d, H_3-27), 1.14 and 1.29 (H_2-28), 0.85 (t, H_3-29); ms *m/z* (% rel. int.) $[M]^+$ 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**].—^{1}H nmr δ 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 δ 3.77 (s, OMe) and 5.84 (d, $J_{1,2}$=12.3). Ms *m/z* (% rel. int.) $[M]^+$ 201 (100), 170 (97), 143 (14), 141 (11), 115 (28).

Methyl(E)-3-(6-bromoindol-3-yl)-2-propenoate [**20**].—^{13}C nmr δ 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′); ^{1}H nmr δ 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 δ 3.76 (s, OMe), 5.85 (d, $J_{2,3}$=12.6, H-2), 7.21 (br d, $J_{3,2}$=12.6, H-3), 7.33 (dd, $J_{5',4'}$=8.4, $J_{5',7'}$=1.8, H-5′). Ms *m/z* (% rel. int.) $[M]^+$ 279/281 (100), 248/250 (34), 223 (13), 221 (22), 219 (6), 169 (96), 141 (25), 140 (18), 114 (15).

Serotonin-N-2′-acetate [**22**].—^{13}C nmr (CD_3OD) δ 125.74 (d, C-2), 114.06 (s, C-3), 131.03 (s, C-3a), 105.02 (d, C-4 or C-6 or C-7), 152.69 (s, C-5), 114.19 (d, C-6 or C-4 or C-7), 113.90 (d, C-7 or C-4 or C-6), 134.67 (s, C-7a), 27.83 (t, C-1′), 43.02 (t, C-2′), 174.82 (s, C=O), 24.15 (q, Me); ^{1}H nmr (CD_3OD) δ 7.01 [br s, $W_{1/2}$=2 Hz (which becomes $W_{1/2}$=1 Hz on irradiation at δ 2.85), H-2], 6.91 (br dd, $J_{4,6}$=2.4, $J_{4,7}$=0.6, H-4), 6.65 (br dd, $J_{6,7}$=8.7, $J_{6,4}$=2.4, H-6), 7.15 (dd, $J_{7,6}$=8.7, $J_{7,4}$=0.6, H-7), 2.85 (br t, J=7.5, H_2-1′), 3.43 (t, J=7.5, H_2-2′), 1.92 (s, Me); ms *m/z* (% rel. int.) $[M]^+$ 218 (18), $[M-AcNH_2]^+$ 159 (100), $[M-CH_2NHAc]^+$ 146 (79).

Serotonin-O,N-2′-diacetate [**23**].—^{13}C nmr (CD_3OD) δ 126.57 (d, C-2), 114.11 (d, C-4 or C-6 or C-7), 146.75 (s, C-5), 118.00 (d, C-6 or C-4 or C-7), 113.00 (d, C-7 or C-4 or C-6), 27.65 (t, C-1′), 43.10 (t, C-2′), 24.15 and 22.59 (q, 2Me) (other singlet C-atoms not detected); ^{1}H nmr (CD_3OD) δ 7.13 (br s, H-2), 7.25 (dd, $J_{4,6}$=2.4, $J_{4,7}$=0.6, H-4), 2.28 (s, OAc-5), 6.81 (br dd, $J_{6,7}$=8.7, $J_{6,4}$=2.4, H-6), 7.31 (dd, $J_{7,6}$=8.7, $J_{7,4}$=0.6, H-7), 2.90 (br t, J=7.5, H_2-1′), 3.44 (t, J=7.5, H_2-2′), 1.90 (s, AcN); ms *m/z* (% rel. int.) $[M]^+$ 260 (9), 218 (3), $[M-AcNH_2]^+$ 201 (51), 188 (16), 159 (100), 147 (72).

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