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FURTHER BROMINATED BIS- AND TRIS-INDOLE ALKALOIDS FROM THE DEEP-WATER NEW CALEDONIAN MARINE SPONGE *ORINA* SP.

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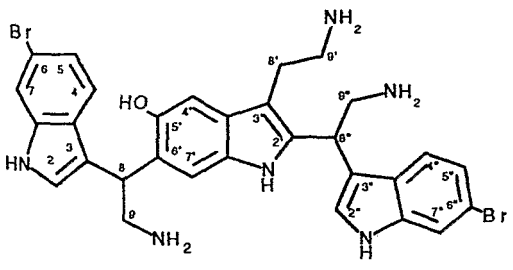
ABSTRACT.—Two tris-indole alkaloids, (±)-gelliusines A and B [**1**], have been isolated for the first time from a marine source, the New Caledonian sponge, *Orina* sp. (or *Gellius* sp.), along with five further indole constituents [**2–6**]. Compound **6** has been identified as 2,2-*bis*-(6′-bromo-3′-indolyl)-ethylamine, previously isolated from the tunicate *Didemnum candidum*, but the remaining four indoles [**2–5**] are novel compounds. These showed anti-serotonin activity and a strong affinity for somatostatin and neuropeptide Y receptors in receptor-binding assays.

In a recent paper we reported the isolation and the structure elucidation of two new diastereomeric brominated tris-indole alkaloids occurring as enantiomeric pairs and designated as (±)-gelliusines A and B [**1**] (1). They represented the major components of a deep-water New Caledonian sponge and constituted the first example of tris-indole metabolites encountered among alkaloids derived from marine sources (2–9). Continuing with the analysis of the less polar extract of this sponge we have isolated four new brominated indole alkaloids [**2–5**], together with the known 2,2-*bis*-(6′-bromo-3′-indolyl)-ethylamine [**6**] (10), and in this paper we report their isolation and structure elucidation. The sponge was originally identified as *Gellius* sp. (Grey) and was later identified by Professor Lévi of the Muséum National d' Histoire Naturelle of Paris as an *Orina* sp. (Grey). *Orina* is a genus closely allied to *Gellius*, sometimes attributed to the family Adicidae Laubenfelds, and sometimes to the family Haliclonidae Laubenfelds. This sponge is probably a new species; a taxonomic description was reported in the previous paper (1). We prefer to continue to name the new metabolites presently described as gelliusines, as they are related to those isolated previously (1).



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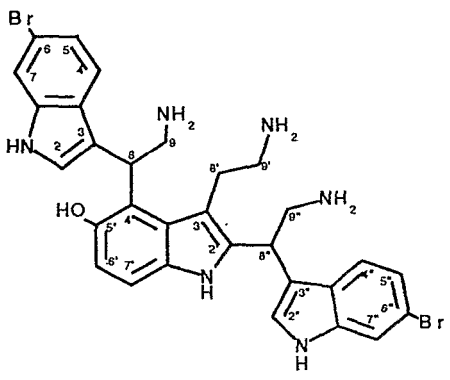

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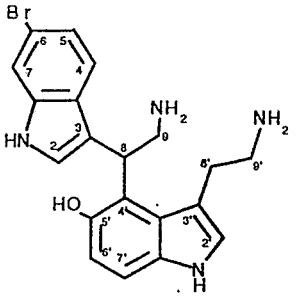

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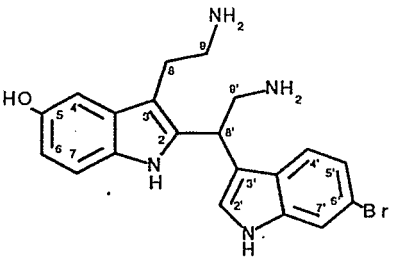

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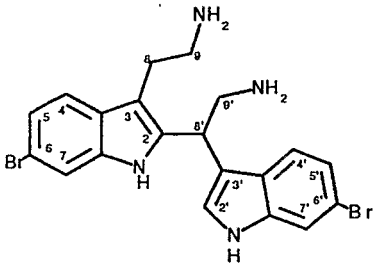

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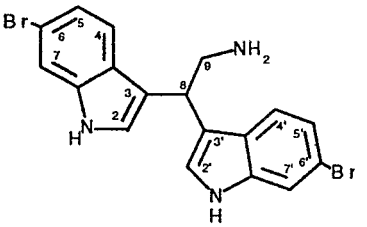

6

The tris-indole, (±)-gelliusine C [**2**], is isomeric with (±)-gelliusines A and B [**1**], bearing one of the two 6-bromotryptamine units at the C-4′ instead of the C-6′ position of the serotonin moiety. (±)-Gelliusines D and E [**3**–**4**] are isomeric bis-indoles, arising from a different attachment between a serotonin and a 6-bromotryptamine unit and represent the left and the right portions of the tris-indole (±)-gelliusines **2** and **1**, respectively. (±)-Gelliusine F [**5**] is a bis-indole containing two 6-bromotryptamine residues. All compounds were isolated by cc on Sephadex LH-20 of a CH_2Cl_2/MeOH extract (ca. 20 g), followed by droplet counter-current chromatography (dccc) and hplc (see Experimental).

The (positive-ion) fabms spectrum of (±)-gelliusine C [**2**], obtained by adding a small amount of CF_3SO_3H, contained an isotopic cluster of pseudomolecular ions at *m/z* 799, 801, and 803 (ratio 1:2:1) $[M+H+CF_3SO_3H]^+$, accounting for molecular ions at *m/z* 648, 650, and 652, thus showing the same mol wt and the same dibrominated isotopic pattern as (±)-gelliusines A and B [**1**]. The ^{1}H-nmr spectrum (Table 1) showed the presence of two aromatic spin-systems (H-4 through H-7 and H-4″ through H-7″) and two aromatic singlets (H-2 and H-2″), similar to those observed in the spectra of (±)-gelliusines A and B [**1**] and assigned to two 6-bromoindol-3-yl residues. Further analogies with the ^{1}H-nmr spectrum of (±)-gelliusines A and B [**1**], although with different pattern and chemical shifts, were three spin-systems consisting of two geminal methylene proton signals coupled with two deshielded methine protons (H-9, H-8 and H-9″, H-8″), and two mutually coupled methylenes (H_2-8′ and H_2-9′) observed in the aliphatic region of the spectrum. The major differences were two coupled proton doublets at δ 6.91 and δ 7.38 (J=8.6 Hz), instead of the two isolated aromatic proton signals assigned, in the ^{1}H-nmr spectrum of (±)-gelliusines A and B [**1**], to H-4′ and H-7′ of the serotonin moi-

TABLE 1. ^{1}H-Nmr Data (500 MHz, CD_3OD) for Compounds **1–5**. [J(Hz) in parentheses].[a]

Proton	Compounds			Proton	Compounds	
	1[b]	**2**	**3**		**4**	**5**
2	7.46 s	7.02 s	7.01 s			
4	7.30 d (8.5)	7.64 d (8.5)	7.62 d (8.5)			
5	7.04 dd (8.5, 1.7)	7.19 dd (8.5, 1.8)	7.18 dd (8.5, 1.7)			
6						
7	7.58 d (1.7)	7.56 d (1.8)	7.53 d (1.7)			
8	5.18 (7.8)	5.35 dd (10.0, 5.2)	5.35 dd (5.2, 10.0)			
9	3.66 dd (7.8, 12.6)	4.25 dd (12.1, 10.0)	4.21 dd (12.3, 10.0)			
	3.68 dd (7.8, 12.6)	3.78 dd (12.1, 5.2)	3.72 dd (12.3, 5.2)			
2′			7.08 s			
4′	7.15 s			4	6.94 d (1.7)	7.51 d (8.5)
5′				5		7.20 dd (8.5, 1.8)
6′		6.91 d (8.6)	6.88 d (8.6)	6	6.74 dd (8.5, 1.7)	
7′	7.06 s	7.38 d (8.6)	7.28 d (8.6)	7	7.26 d (8.5)	7.60 d (1.8)
8′	3.25 t (8)	3.58 m	3.40 m	8	3.30 m	3.27 m
		3.20 m	3.17 m			
9′	3.04 dt (8, 12)	2.88 m	2.90 m	9	3.01 m	3.07 m
	3.11 dt (8, 12)	2.67 m	2.76 m		2.94 m	3.00 m
2″	7.31 s	7.37 s		2′	7.30 s	7.38 s
4″	7.53 d (8.5)	7.75 d (8.5)		4′	7.52 d (8.5)	7.56 d (8.5)
5″	7.13 dd (8.5, 1.7)	7.16 dd (8.5, 1.8)		5′	7.17 dd (8.5, 1.7)	7.17 dd (8.5, 1.8)
7″	7.56 d (1.7)	7.56 d (1.8)		7′	7.57 d (1.7)	7.58 d (1.8)
8″	5.08 dd (7.1, 9.1)	5.16 dd (8.2, 6.8)		8′	5.04 dd (8.5, 6.8)	5.16 dd (8.6, 6.9)
9″	3.61 dd (12.8, 7.1)	3.80 dd (12.7, 6.8)		9′	3.80 dd (12.7, 6.8)	3.84 dd (12.1, 6.9)
	3.74 dd (12.8, 9.1)	3.66 dd (12.7, 8.2)			3.66 dd (12.7, 8.5)	3.69 dd (12.1, 8.6)

[a]Reference ^{1}H-nmr data of serotonin (500 MHz, CD_3OD): δ 7.15 (s, H-2), δ 7.00 (d, J=2.4 Hz, H-4), δ 6.75 (dd, J=2.4 and 8.5 Hz, H-6), δ 7.24 (d, J=8.5 Hz, H-7), δ 3.23 (t, J=7.1 Hz, H_2-8), δ 3.09 (t, J=7.1 Hz, H_2-9).

[b]^{1}H-Nmr data of gelliusine A.

ety. The chemical shifts and the coupling constants of the two doublets were in good agreement with the H-7′ and H-6′ signals of the serotonin nucleus (see Table 1), thus suggesting a different coupling pattern of the two 6-bromoindol-3-yl residues on the 5-hydroxytryptamine moiety. Further support came from nOe experiments performed in DMSO-d_6. Irradiation of the H-7′ signal (δ 7.42 d) resulted in a nOe enhancement of the NH resonance of the serotonin unit (δ 10.70 s). Analysis of the ^{13}C-nmr spectrum (Table 2) confirmed this structural hypothesis. The carbon resonances of the central indole nucleus were in agreement with those expected for a 2,4-disubstituted serotonin unit on the basis of the substitution effects predicted on the serotonin nucleus by the additivity rules utilized in aromatic rings (11) and by comparison with the ^{13}C-nmr spectrum of (±)-gelliusines A and B [**1**]. Hplc analysis of the residue obtained by derivatization of **2** with (+)-α-methoxy-α-trifluoromethylphenylacetic acid (MTPA) chloride gave two diastereomeric peaks in an almost 1:1 ratio, and **2** itself was essentially optically inactive. Compound **2** must thus occur as a mixture of enantiomers. This analysis was extended to the other new compounds described here [**3–5**], and by analogy with data reported for (±)-gelliusines A and B [**1**],

TABLE 2. ^{13}C-Nmr Data (125.76 MHz, CD_3OD) for Compounds **1–5**.[a]

Carbon	Compound			Carbon	Compound	
	1[b]	**2**	**3**		**4**	**5**
2	124.2	126.5	126.6			
3	115.6	114.9	115.4			
3a	139.2	139.1	139.1			
4	121.3	121.0	120.7			
5	123.0	123.5	123.5			
6	116.3	116.1	116.1			
7	115.4	115.5	115.6			
7a	127.1	128.1	127.4			
8	35.8	36.9	36.9			
9	44.7	43.7	43.8			
2′	135.6	137.1	126.5	2	135.3	135.8
3′	107.6	107.9	110.1	3	107.8	108.8
3′a	128.8	126.5	126.5	3a	129.8	138.9
4′	103.4	114.6	114.7	4	103.1	120.2
5′	150.0	151.5	150.5	5	152.0	123.5
6′	124.4	113.1	113.2	6	112.9	116.3
7′	112.4	114.2	114.2	7	112.9	115.2
7′a	132.7	133.5	134.5	7a	132.5	127.9
8′	23.6	25.6	27.3	8	23.6	23.3
9′	41.1	41.7	41.3	9	41.1	41.1
2″	124.7	125.1		2′	124.8	124.8
3″	114.1	114.9		3′	114.2	113.6
3″a	138.8	139.0		3′a	139.0	138.6
4″	120.8	121.1		4′	120.8	120.6
5″	123.4	123.5		5′	123.5	123.6
6″	116.2	116.4		6′	116.5	116.4
7″	115.2	115.5		7′	115.5	115.5
7″a	126.3	126.3		7′a	126.3	126.2
8″	33.7	33.6		8′	34.0	33.8
9″	43.9	43.9		9′	44.1	43.9

[a]Reference ^{13}C-nmr data of serotonin (125.76 MHz, CD_3OD): δ 125.1 C-2, δ 109.4 C-3, δ 128.9 C-3a, δ 103.2 C-4, δ 151.5 C-5, δ 112.9 C-6, δ 113.0 C-7, δ 133.2 C-7a, δ 24.5 C-8, δ 41.1 C-9.

[b]^{13}C-Nmr data of gelliusine A.

all of the new compounds are established to occur as enantiomeric pairs.

(±)-Gelliusine D [**3**] represents the left-hand bis-indole portion of **2**, lacking the 6-bromo-indol-3-yl residue linked to the C-2′ position of the serotonin nucleus. Its positive-ion fabms gave two brominated isotopic pseudomolecular ions at *m/z* 563 and 565 (ratio 1:1) $[M+H+CF_3SO_3H]^+$ accounting for mol wts of 412 and 414. This was 236/238 mass units fewer than the mol wt of **2**, corresponding to a missing 6-bromoindol-3-yl residue. Analysis of the ^{1}H-nmr spectrum showed the presence of six aromatic proton signals (H-2 through H-7 and H-6′, H-7′) and of two aliphatic spin-systems (H-8, H-9 and H-8′, H-9′), nearly superimposable with the corresponding signals in the ^{1}H-nmr spectrum of (±)-gelliusines C [**2**]. The remaining aromatic proton signal at δ 7.08 (s) was thus assigned to H-2′ of the serotonin unit. Further support for structure **3** also came from ^{13}C-nmr data, which contained nine C and seven CH sp^2 resonances in the downfield region. These signals, together with the four sp^3 resonances in the high-field region, could confidently be assigned to a serotonin nucleus bearing a 6-bromotryptamine at the C-4 position by comparison with ^{13}C-nmr spectral data recorded for **2**.

(±)-Gelliusine E [**4**] (fabms spectrum identical with **3**) is structurally related to the right-hand bis-indole moiety of (±)-gelliusines A and B [**1**] and C [**2**], lacking the 6-bromoindol-3-yl residue linked to the C-6 or C-4 position of the serotonin nucleus. The structure elucidation of **4** was thus accomplished by an examination of its spectral properties in direct comparison with those of (±)-gelliusines A and B [**1**] and C [**2**]. The ^{1}H-nmr spectrum (Table 1) showed the presence in the lower-field region of the four aromatic proton resonances (H-2′ through H-7′) characteristic of a 6-bromoindol-3-yl residue. The three other aromatic proton signals at δ 6.94 (d, J=1.7 Hz), δ 6.74 (dd, J=8.5 and 1.7 Hz) and δ 7.26 (d, J=8.5 Hz) were assigned to H-4, H-6, and H-7 of a 2-substituted 5-hydroxytryptamine by comparison of their chemical shifts and coupling constants with ^{1}H-nmr spectral data of serotonin and with the corresponding ^{1}H-nmr signals in the spectra of **1** and **2**. Comparison of the ^{13}C-nmr spectral data, consisting of 16 aromatic and four aliphatic carbon resonances, with those of the corresponding signals in compounds **1** and **2** confirmed structure **4**.

(±)-Gelliusine F [**5**] consists of two 6-bromotryptamine units joined through their C-2, C-8′ positions. The positive-ion fabms contained a dibrominated isotopic cluster of pseudomolecular ions at *m/z* 625, 627, and 629 (ratio 1:2:1) $[M+H^+ +CF_3SO_3H]^+$, accounting for molecular ions at *m/z* 474, 476, and 478. Examination of ^{1}H- and ^{13}C-nmr data indicated the presence of two 6-bromotryptamine units. The type of dimerization was established on the basis of characteristic chemical shifts in the ^{1}H- and ^{13}C-nmr spectra, which contained signals for two coupled methylenes (CH_2-8 δ_C 23.3, δ_H 3.27 m, and CH_2-9 δ_C 41.1, δ_H 3.07 m) linked to an aromatic ring and a nitrogen, respectively, and of the 9′-methylene adjacent to the deshielded 8′-methine, which is the site of attachment to the C-2 position of the second 6-bromotryptamine unit.

In a previous paper (1) we have described the serotonin-like activity of (±)-gelliusine A [**1**] in the range 10–100 μM. The results of in vitro serotoninergic activity assays performed on compounds **2–5** (see Experimental) suggest that these molecules have lost their intrinsic contractile activity and do not act as serotoninergic agonists, whereas weaker antiserotonin activity was shown at lower concentrations (8–50 μM).

The gelliusines were submitted to a series of receptor binding assays. At a concentration of 5 μg/ml, gelliusines A

and B [**1**] totally displaced the radioactive ligand from the somatostatin receptor site. The displacements for compounds **4** and **5** at the same concentration were 87 and 91%, respectively. At the neuropeptide Y receptor site, gelliusines A and B [**1**], and compounds **4** and **5**, at 5 μg/ml each, exhibited 90, 62, 63, and 67% inhibition of the specific ligand binding, respectively. In the human B2 bradykinin receptor-binding assay, these compounds, at the same concentration, exhibited 100, 93, 63, and 89% inhibition of the specific ligand binding, respectively. Although these compounds were not very specific, their activities are interesting for developing immunochemical studies on these neuropeptides. Compounds **1**, **4**, and **5** were also evaluated with the following receptor-binding assays in which they were inactive: senktide-binding assay (NK3 tachykinin receptor site); AMPA-binding assay (quisqualate site of glutamate receptor); calcitonin gene related protein (CGRP)-binding assay; galanin-binding assay with a human cell line (BOWES); glycine-binding assay (regulatory site of NMDA receptor, Gly); neurotensin-binding assay (NT), and vasointestinal polypeptide-binding assay (VIP).

EXPERIMENTAL

GENERAL EXPERIMENTAL PROCEDURES.—Nmr spectra were obtained on a Bruker AMX-500 spectrometer equipped with a Bruker X-32 computer, using the UXNMR software package. Fabms were obtained on a VG ZAB mass spectrometer equipped with a fab source (in glycerol matrix; Xe atoms of energy of 2–6 keV). Optical rotations were determined using a Perkin-Elmer 141 polarimeter, using an Na lamp operating at 589 nm. Uv spectra were taken on a Beckman DU70 spectrometer. Hplc was performed with a Waters model 510 pump with refractive index detection.

ANIMAL MATERIAL.—As reported previously (1).

EXTRACTION AND ISOLATION.—The lyophilized animals (about 900 g) were extracted with petroleum ether (3×3 liters) to give 2.4 g of glassy material, then with CH_2Cl_2-MeOH (8:2, 3×3 liters, 40 g extract), and MeOH-H_2O (8:2, 3×3 liters, 22.5 g extract), and finally with MeOH (100%) (3×3 liters). Half of the CH_2Cl_2:MeOH extract (20 g) containing the bromoindole alkaloids was submitted to chromatography on a Sephadex LH-20 column (4×100 cm) in four runs, with MeOH as eluent. Fractions (6 ml) were collected and analyzed by tlc on SiO_2 with *n*-BuOH-HOAc-H_2O (12:3:5). Medium polarity fractions (70–90, 2 g in total) were then subjected to dccc (two runs) using *n*-BuOH-Me_2CO-H_2O (3:1:5) in the descending mode (the upper phase was the stationary phase, flow rate 15 ml/h; 5 ml fractions were collected and monitored by tlc). Fractions 40–50 (370 mg in total) were combined and the residue was subjected to reversed-phase hplc [μBondapak C_{18} column 8×300 mm, H_2O (100%) as eluent, flow rate 5 ml/min] to collect gelliusine A [**1**] (200 mg), compound **2** (10 mg) [uv (MeOH) λ max 230 (35,324), 284 (13,272) nm], compound **3** (3 mg), compound **4** (13 mg), and compound **5** (12 mg).

(+)-MTPA DERIVATIVES OF COMPOUNDS **2**–**5**.—Each compound (0.5 mg) was treated with freshly distilled (+)-α-methoxy-α-trifluoromethylphenylacetic acid (MTPA) chloride (2 μl) in dry pyridine (30 μl) for 1 h at room temperature. After removal of solvent, the residues were submitted to hplc on a Partisil column 3.9×300 mm (CH_2Cl_2-EtOAc, 84:16 as eluent, flow rate 2 ml/min, uv detector 260 nm). Each residue gave two diastereomeric peaks in an almost 1:1 ratio: *R*(+)-MTPA esters of **2**: *R*, 4.2 and 6.0 min; of **3** *R*, 4.4 and 9.8 min; of **4** *R*, 5.2 and 6.4 min; of **5** *R*, 4.6 and 5.4 min.

IN VITRO ANTISEROTONINERGIC ACTIVITY ASSAY.—The assay was performed on the male guinea pig ileum (weighing 200±50 g). Percentage inhibition was calculated as a response caused by 0.5 μM serotonin in the absence of drug. Six observations were carried out for each sample concentration. The results (IC_{50} values) listed below are reported as the concentration of sample causing 50% inhibition of the submaximal contractions induced by serotonin. The antagonist effect of the tested compounds was compared to the activity of methysergide: IC_{50} values (μM) **2**, 2; **3**, 100; **4**, 50; **5**, 10; methysergide, 0.02.

RECEPTOR-BINDING STUDIES.—In vitro receptor-binding assays were performed with membrane preparations from animal tissues (rats or guinea pigs) or cell lines (cells expressing a human gene), according to established methods (12). The membrane preparations were incubated under optimal conditions of pH, temperature, time, and media, in the presence of the specific radiolabeled ligand with a given concentration of the studied compounds or with a compound possessing a high affinity for the corresponding binding site (nonspecific binding measurement). After equilibrium was reached, the mixtures were filtered through a

glass fiber filter and the radioactivity remaining in the filter was measured with a scintillation counter. The somatostatin and NPY-binding assays (13,14) were performed with membranes from rat cerebral cortices as sources of receptors and [^{125}I]-somatostatin and [^{3}H]-NPY, respectively, as radiolabeled ligands. The bradykinin-binding assay (15) was performed with the membranes from SF21 cells infected by baculovirus expressing B2 bradykinin receptors and [^{3}H]-bradykinin.

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