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(±)-GELLIUSINES A AND B, TWO DIASTEREOMERIC BROMINATED TRIS-INDOLE ALKALOIDS FROM A DEEP WATER NEW CALEDONIAN MARINE SPONGE (*GELLIUS* OR *ORINA* SP.)

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Abstract.—Two new diastereomeric brominated tris-indole alkaloids occurring as enantiomeric pairs, (±)-gelliusines A [1] and B [2], have been isolated from a deep water New Caledonian sponge (*Gellius* or *Orina* sp.), whose crude extract exhibited cytotoxicity against KB cells. Their structures were elucidated by spectroscopic methods including one- and two-dimensional nmr spectroscopy. The major compound, (±) gelliusine A [1], which showed very weak cytotoxicity, proved to be active at the serotonin receptor.

Several simple and bis-indole alkaloids having either 6-bromotryptophan or 6-bromotryptamine as the basic unit have been isolated from a variety of marine invertebrates including sponges, coelenterates, tunicates, and a bryozoan (1–11). 6-Bromotryptamine itself has been found as a natural product in the tunicate *Didemnum candidum* (7). The first examples of brominated bis-indole alkaloids possessing unusual novel structures and interesting biological activities were the topsentins isolated from the sponges *Topsentia genitrix* (12,13), *Spongorites* spp. (14), and a *Hexadella* sp. (15). Related compounds differing in the mode of coupling between the indole units were described later from a *Dragmacidon* sp. (16), a *Hexadella* sp. (17), *Spongorites* spp. (10,18), and also from the tunicate *Didemnum candidum* (7). Non-brominated bis-indole alkaloids have been reported from the sponge *Fascaplysinopsis* sp. (19) and the tunicate *Dendroda grossularia* (20). Tris-indole alkaloids have not previously been described from marine sources and, as part of our ongoing studies on bioactive metabolites from New Caledonian marine species, we wish to report the first

isolation and characterization of two diastereomeric compounds made up by the coupling of three indole units: two 6-bromotryptamines linked through their aliphatic chains to the C-2 and C-6 positions of a central serotonin. The coupling of the indole units appears to be non-stereoselective and two enantiomeric pairs having different relative configurations at C-8 and C-8″ have been isolated and named (±)-gelliusines A [1] and B [2].

Gellius sp. Gray (or *Orina* sp. Gray) (Demospongiae, Haplosclerida) was collected at a depth of about 300 m off the north coast of New Caledonia (Grand Passage). The identification was performed by C. Levi of the Museum
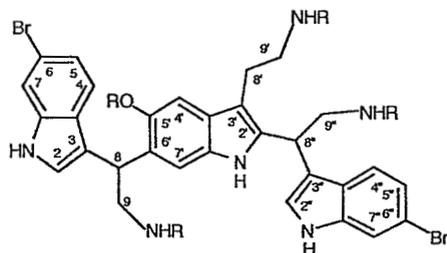
1 and **2** R=H, (±)-Gelliusines A and B
3 R=Ac

Nationale d'Histoire Naturelle, Paris, France. A voucher specimen is preserved at the ORSTOM Centre in Nouméa, reference no. R1519. *Gellius* Gray is a genus close to *Orina* Gray, sometimes attributed to the family Adoclidae Laubenfels, and sometimes to the family Haliclonidae Laubenfels. A taxonomic description of this species, which is probably new, is provided in the Experimental.

The lyophilized animals (900 g) were sequentially extracted with petroleum ether, CH_2Cl_2-MeOH (8:2), and MeOH, and MeOH-H_2O (8:2). Purification of half of the CH_2Cl_2/MeOH extract (ca. 20 g) by sequential application of Sephadex LH-20 (eluent MeOH), droplet counter-current chromatography (dccc) (*n*-BuOH-Me_2CO-H_2O (3:1:5 descending mode) and reversed-phase hplc (μ-Bondapak C-18, eluents MeOH-H_2O, 5:95 and H_2O 100%) afforded (±)-gelliusine A [1] (200 mg) and (±)-gelliusine B [2] (48 mg). Lesser amounts were also recovered from the MeOH extract.

(±)-Gelliusine A [1] showed uv (MeOH) λ max at 226 (52000) and 285 (15000) nm, and the positive ion fabms, performed by adding a small amount of CF_3SO_3H, contained an isotopic cluster of pseudomolecular ions at *m/z* 799, 801, 803 (ratio 1:2:1) $[M+H+CF_3SO_3H]^+$, characteristic of a dibrominated compound and accounting for molecular ions at *m/z* 648, 650, and 652. The composition $C_{30}H_{30}Br_2N_6O$ was deduced by combining mass spectral data with the following spectroscopic evidence. The lower-field region of the ^{1}H-nmr spectrum (Table 1) contained, in addition to two isolated aromatic proton signals at δ 7.15 s and 7.06 s (H-4' and H-7'), two similar sets of four aromatic proton resonances (H-2 through H-7 and H-2″ through H-7″) that could be confidently assigned to 6-bromoindol-3-yl residues by their characteristic chemical shifts and coupling constants in comparison with those reported for reference compounds (7,15).

The region of the spectrum from δ 3.60 to 5.20 showed the presence of two very similar aliphatic spin systems (H-8, H-9 and H-8″, H-9″) consisting of two geminal methylene protons adjacent to a deshielded methine proton and appropriate for 2,2-disubstituted ethylamine moieties linked to deshielding aromatic rings. The last spin system, appearing slightly upfield, consisted of two coupled methylenes (H_2-8' and H_2-9') that, considering also their carbon shifts (23.6 and 41.1 ppm), had to be linked to an aromatic ring and a nitrogen, respectively. When measured in DMSO-d_6, the ^{1}H-nmr spectrum exhibited signals for 10 exchangeable protons [δ 11.40 s, 11.32 s and 10.70 s (1H each, H-1 of indole rings); 9.25 s (1H, -OH); 8.16 br, 7.98 br (4H and 2H, respectively, -NH_2)]. The downfield region of the ^{13}C-nmr spectrum (Table 1) contained 14 C and 10 CH sp^2 resonances, while six more sp^3 resonances (2 CH and 4 CH_2) appeared in the high-field region. Among these, the three signals at δ 41.1, 43.9, and 44.7 ppm (C-9', C-9″ and C-9, respectively) were appropriate for methylenes bearing nitrogen. Two sets of similar ^{13}C-nmr signals (C-2 through C-9 and C-2″ through C-9″, Table 1) were assigned to two 6-bromotryptamine moieties as a result of ^{1}H-^{13}C HETCOR measurements, long-range C-H connectivities deduced through a COLOC nmr experiment, nOe difference experiments, and comparison with reference data, thus definitely proving the early structural hypothesis. The remaining eight sp^2 carbon signals in the ^{13}C-nmr spectrum (C-2' through C-7'), together with the 8' and 9' methylene signals, could be accounted for by the presence of a serotonin unit, a hypothesis fully supported by COLOC and nOe data and by the C-H coupling constants of the C-4'–C-7' signals (C-4' and C-7' showed J_1 couplings of ca. 156 Hz, C-5' and C-6' showed endocyclic J_2 and J_3 couplings of ca. 3.1 and 7.9 Hz, respectively), in agreement with those expected for carbons in

TABLE 1.   Nmr Data (500 MHz, CD$_3$OD) for **1–3**.

	Compound							
	(±)-Gelliusine A [1]				(±)-Gelliusine B [2]			**3**
	^{13}C	^{1}H	COLOC[a]	^{1}H-^{1}H nOes	^{13}C	^{1}H	COLOC[a]	^{1}H
2	124.2	7.46 s	—		124.6	7.45 s	—	7.26 s
3	115.6	—	H-8		115.2	—	H-2	—
3a	139.2	—	H-2		139.2	—	H-2, H-4	—
4	121.3	7.30 d (8.5)	—		121.3	7.38 d (8.5)	—	7.31 d (8.6)
5	123.0	7.04 dd (8.5, 1.7)	H-7		123.0	7.05 dd (8.5, 1.7)	H-7	6.99 dd (8.6, 1.7)
6	116.3	—	H-4		116.1	—	H-7, H-5	—
7	115.4	7.58 d (1.7)	—		115.2	7.58 d (1.7)	H-7	7.51 d (1.7)
7a	127.1	—	H-2		127.1	—	—	—
8	35.8	5.18 t (7.8)	H-7′	H-7′, H-2, H-4	36.9	5.08 t (7.8)	—	4.75 dd (8.5, 6.0)
9	44.7	3.66 dd (7.8, 12.6)	H-8	H-7′, H-2	44.5	3.75 dd (7.8, 10.2)	—	3.57 dd (6.0, 13.2)
		3.68 dd (7.8, 12.6)				3.73 dd (7.8, 10.2)		4.15 dd (8.5, 13.2)
2′	135.6	—	H-8″		135.8	—	—	—
3′	107.6	—	H-8′		107.4	—	—	—
3′a	128.8	—	H-7′		129.0	—	H-7′	—
4′	103.4	7.15 s	—		103.5	7.05 s	H-7′	7.18 s
5′	150.0	—	H-7′		150.0	—	H-7′	—
6′	124.4	—	H-8, H-4′		124.4	—	H-4′	—
7′	112.4	7.06 s	—		112.7	7.15 s	—	7.16 s
7′a	132.7	—	H-4′		132.5	—	H-7′	—
8′	23.6	3.25 t (8.0)	—	H-4′, H-8″	23.6	3.19 t (8.0)	—	3.38 t (7.1)
9′	41.1	3.04 dt (12.0, 8.0)	—		41.1	2.98 m	—	2.97 t (7.1)
		3.11 dt (12.0, 8.0)						
2″	124.7	7.31 s	—		124.8	7.30 s	—	7.07 s
3″	114.1	—	H-2″, H-8″		114.1	—	H-2″	—
3″a	138.8	—	H-2″, H-4″		138.8	—	H-2″	—
4″	120.8	7.53 d (8.5)	—		120.8	7.55 d (8.5)	—	7.38 d (8.6)
5″	123.4	7.13 dd (8.5, 1.7)	H-7″		123.4	7.15 dd (8.5, 1.7)	H-7″	7.06 dd (8.6, 1.7)
6″	116.2	—	—		116.3	—	H-7″	—
7″	115.2	7.56 d (1.7)	—		115.4	7.55 d (1.7)	H-5″	7.48 d (1.7)
7″a	126.3	—	H-2″, H-7″		126.4	—	—	—
8″	33.7	5.08 dd (7.1, 9.1)	—	H-2″, H-4″	34.0	5.04 t (8.5)	—	4.70 t (7.5)
9″	43.9	3.61 dd (7.1, 12.8)	H-8″	H-2″	44.1	3.62 dd (8.5, 12.5)	—	3.61 dd (7.5, 13.2)
		3.74 dd (9.1, 12.8)				3.66 dd (8.5, 12.5)		3.97 dd (7.5, 13.2)
								1.76 s CH$_3$CONH-
								1.86 s CH$_3$CONH-
								1.87 s CH$_3$CONH-
								2.36 s CH$_3$COO-

[a]Pulse sequence was optimized for 7 Hz coupling constant.

the six-membered ring of an indole nucleus (21). COLOC and nOe data also provided connectivities between C-8 of one of the 6-bromotryptamine units and C-6′ of the serotonin moiety, on one side, and between C-2′ of the serotonin and C-8″ of the second 6-bromotryptamine unit on the other, thus leading to structure **1**. (±)-Gelliusine A [**1**] was converted to a tetracetate [**3**] by treatment with Ac_2O in pyridine [fabms *m/z* 817, 819, 821 $[M+H]^+$; ^1H nmr (CD_3OD) in Table 1]. In accordance with the proposed structure the ^1H-nmr spectrum exhibited six exchangeable proton signals when measured in DMSO-d_6, the three indole NH's at δ 10.48 s, 11.07 s, and 11.10 s and three acetamido protons at δ 7.89 t (5.8 Hz), 7.92 t (5.8 Hz) and 7.95 t (5.8 Hz), coupled with the H_2-9, H_2-9′, and H_2-9″ methylene signals at δ 3.91 m, 2.80 m, and 3.66 m, respectively. Although having two chiral centers, (±)-gelliusine A [**1**] had a nearly zero optical rotation, and derivatization with (+)- and (−)-α-methoxy-α-trifluoromethyl-phenylacetic acid (MTPA) chlorides showed that it was indeed a mixture of two enantiomers. Residue from each of the two derivatizations with (+)- and (−)-MTPA chlorides in pyridine gave identical hplc profiles (Partisil, CH_2Cl_2-EtOAc, 84:16) with two prominent peaks in an almost 1:1 ratio. When individual peaks were collected and ^1H-nmr spectra recorded, the expected enantiomeric relationship between the two pairs of derivatives having identical retention times [R(+)- and S(−)-MTPA derivatives with R_t 6.6 min and R(+)- and S(−)-MTPA derivatives with R_t 17.4 min] was substantiated by identical ^1H-nmr spectra and opposite rotations (see Experimental). These results eliminate any doubt about the possibility that either chemical degradation products or conformationally locked isomers, resulting from restricted rotation about a single bond, might arise during the derivatization with (+)- and (−)-MTPA chlorides.

(±)-Gelliusine B [**2**] showed uv (MeOH) λ max at 226 (53000) and 285 (15000) nm and fabms *m/z* 799, 801, 803 (ratio 1:2:1) $[M+H+CF_3SO_3H]^+$. The spectroscopic analysis of (±)-gelliusine B [**2**], which exhibited ^1H- and ^{13}C-nmr spectra (Table 1) very similar to those of (±)-gelliusine A [**1**], followed the same steps as described above and proved (±)-gelliusine B [**2**] to be a mixture of two more enantiomers with different relative configurations at C-8 and C-8″ from (±)-gelliusine A [**1**].

The preliminary results, showing the crude extract to exhibit cytotoxicity against KB cells, were not confirmed by assays done on the major (±)-gelliusine A [**1**]. We found only very weak cytotoxic responses in the KB, P388, P388/dox, HT29 and NSCLCN6 cell lines (10 μg/ml<IC_{50}<20 μg/ml for each test system). Structural features of gelliusine A [**1**] prompted us to investigate a possible interaction with the serotoninergic system and (±)-gelliusine A proved to act as a partial agonist for serotonin receptor(s). With the male guinea pig ileum, gelliusine A [**1**] showed at high concentration (5–70 μg/ml) a contractile activity inhibited only by methysergide. This result clearly indicated that it acts as a serotonin-like compound. At lower concentration it does not have a contractile effect per se, but it is able to antagonize serotonin-induced contraction. In conclusion, the compound is interesting for its pharmacological profile since it seems to block the serotoninergic receptor(s) at low concentration by acting as a serotonin antagonist, whereas at higher concentration it causes a serotonin-like and methysergide-sensitive contraction, suggesting a possible physiological role as a modulator of the serotoninergic system.

EXPERIMENTAL

ANIMAL MATERIAL.—The sponge was collected off New Caledonia, stations DW 114–115, at a depth of 255–285 m. It is a lobate massive sponge (width 60×30 mm; height 40 mm). Its color in life is cream and in EtOH reddish brown.

The texture is firm but brittle, the surface is smooth, with some obtuse processes 5–10 mm high. The skeleton is a dense subisodictyal network of oxeas with a tangential reticulation in the dermal membrane. It shows oxeas megascleres, slightly curved (220–320 $\mu m \times 10$–12 μm) and sigmas angulate microscleres, more numerous in the choanosome (30–45 $\mu m \times 1.5$ μm).

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 of extract), with MeOH-H_2O, 8:2 (3×3 liters, 22.5 of extract) and finally with MeOH 100% (3×3 liters). The MeOH extract was evaporated and residual H_2O was partitioned between EtOAc and BuOH to give 1.67 g and 25.9 g of glassy material, respectively. 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).

The next dccc fractions (50–60, 120 mg) were similarly combined and subjected to reversed-phase hplc with MeOH-H_2O (5:95) to collect gelliusine B [2] (48 mg in total).

ACETYLATION OF GELLIUSINE A [1].— Gelliusine A [1] (10 mg) was treated with Ac_2O (60 μl) and pyridine (500 μl). After removal of solvent, the residue was submitted to hplc (μBondapak C-18 column (3.9×300 mm), MeOH-H_2O (60:40) as eluent, flow rate 2 ml/min). ^1H nmr (CD_3OD), see Table 1.

(+)- AND (−)-MTPA DERIVATIVES OF GELLIUSINE A [1].—Gelliusine A [1] (5 mg) was treated with freshly distilled (+)-methoxytrifluoromethylphenyl acetyl chloride (4 μl) in dry pyridine (150 μl) for 1 h at room temperature. After removal of solvent, the residue was submitted to hplc [Partisil (3.9×300 mm), CH_2Cl_2-EtOAc (84:16) as eluent, flow rate 2 ml/min, uv detector 260 nm] giving two diastereomeric peaks (R_t 6.6 min and 17.4 min, respectively). Another sample of 1 (5 mg) was similarly treated with (−)-MTPA chloride. The residue, purified by hplc under the same conditions, gave two peaks which co-eluted with the two (+)-MTPA derivatives of 1.

$R(+)$-MTPA esters of gelliusine A [1] (2): (a) R_t 6.6 min; [α]D (MeOH)+62.4°; (b) R_t 17.4 min; [α]D (MeOH) −29.9°; $S(−)$-MTPA esters of gelliusine A [1] (2): (a) R_t 6.6 min; [α]D (MeOH) −61.5°; (b) R_t 17.4 min; [α]D (MeOH) +30.8°.

The ^1H-nmr spectra of the (+)- and (−)-MTPA derivatives with the same R_ts proved to be identical.

^1H-Nmr data of $R(+)$- and $S(−)$-MTPA derivatives of gelliusine A [1] with R_t 6.6 min ($CDCl_3$) δ 7.6–6.6 aromatic protons, 4.79 (1H, t, J=8 Hz, H-8), 3.73 (1H, m, H-9α), 4.12 (1H, m, H-9β), 4.31 (1H, dd, J=6.5 and 8 Hz, H-8″), 3.42 (1H, m, H-9″α), 4.05 (1H, m, H-9″β), 3.36 (2-H, m, H_2-8′), 2.89 (2-H, m, H_2-9′).

^1H-Nmr data of $R(+)$- and $S(−)$-MTPA derivatives of gelliusine A [1] with R_t 17.4 min ($CDCl_3$) δ 7.6–6.6 aromatic protons, 4.84 (1H, m, H-8), 3.80 (1H, m, H-9α), 4.15 (1H, m, H-9β), 4.33 (1H, m, H-8″), 3.79 (2H, m, H_2-9″), 3.25 (2H, m, H_2-8′), 2.97 (2H, m, H_2-9′).

(+)- AND (−)-MTPA DERIVATIVES OF GELLIUSINE B [2].—Samples of gelliusine B [2] (5 mg) were treated with freshly distilled (+)- and (−)-methoxytrifluoromethylphenyl acetyl chloride (4 μl) in dry pyridine (150 μl) as above. After removal of solvent, the residues were submitted to hplc (conditions given above) giving two peaks each (R_t, respectively, 8.4 min and 9.6 min) which were analyzed by ^1H-nmr spectroscopy.

$R(+)$-MTPA esters of gelliusine B [2]: (a) R_t 8.4 min; [α]D (MeOH) +20.6°; (b) R_t 9.6 min; [α]D (MeOH) +15.6°; $S(−)$-MTPA esters of gelliusine B [2]: (a) R_t 8.4 min; [α]D (MeOH) −19.0°; (b) R_t 9.6 min; [α]D (MeOH) −14.6°.

^1H-Nmr data of $R(+)$- and $S(−)$-MTPA derivatives of gelliusine B [2] with R_t 8.4 min ($CDCl_3$) δ 7.6–6.6 aromatic protons, 4.67 (1H, m, H-8), 3.52 (1H, m, H-9α), 4.13 (1H, m, H-9β), 4.32 (1H, m, H-8″), 3.83 (2H, m, H_2-9″), 3.42 (2H, m, H_2-8′), 2.88 (2H, m, H_2-9′).

^1H-Nmr data of $R(+)$- and $S(−)$-MTPA derivatives of gelliusine B [2] with R_t 9.6 min ($CDCl_3$) δ 7.6–6.6 aromatic protons, 4.81 (1H, dd, J=5.5 and 9.5 Hz, H-8), 3.83 (1H, m, H-9α), 4.13 (1H, m, H-9β), 4.35 (1H, dd, J=5.5 and 9.5 Hz, H-8″), 3.48 (1H, m, H-9″α), 4.03 (1H, m, H-9″β), 3.15 (1H, m, H-8′α), 3.23 (1H, m, H-8′β), 2.91 (2H, m, H_2-9′).

IN VITRO SEROTONINERGIC ACTIVITY ASSAY.— Male guinea pigs (200±50 g body weight) were killed by exanguation. According to Magnus (22) the ileum was rapidly dissected and placed in warm (37°) Krebs solution gassed with 95% O_2-5% CO_2 and connected to an isotonic transducer (U. Basile, Italy). Methysergide was obtained from Sandoz, mepyramine maleate from Rhone-Poulenc, and all other drugs were obtained from Sigma (St. Louis, MO). The results are expressed as means±SEM.

After 30 min of equilibration, the compound under trial was added to the organ bath in cumulative concentration. A minimal contractile activity of the compound was obtained at 6.5 ± 0.5 µg/ml (5% of the maximum contraction evoked by 10^{-6} M acetylcholine $n=6$), whereas the maximal contractile activity (37% of the maximal concentration caused by ACh) was observed at 70 ± 0.3 µg/ml ($n=6$). The contractile action was abolished only by methysergide (0.1 µg/ml), whereas atropine (0.1 µg/ml), mepyramine (0.1 µg/ml), propanolol (0.2 µg/ml), and phenoxybenzamine (0.5 µg/ml) were ineffective. The compound was also tested at a concentration which per se was without contractile effect against serotonin-induced contraction. The compound at a concentration of 4 µg/ml had no contractile activity per se, but was able to reduce by 50% the submaximal contraction caused by serotonin (IC_{50}).

ACKNOWLEDGMENTS

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