Three New Brominated and Iodinated Tyrosine Derivatives from Iotrochota birotulata, a Non-Verongida Sponge

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THREE NEW BROMINATED AND IODINATED TYROSINE DERIVATIVES FROM *IOTROCHOTA BIROTULATA*, A NON-VERONGIDA SPONGE

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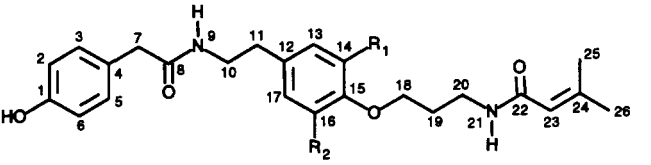
ABSTRACT.—Three novel halogenated metabolites [**1–3**], derived from tyrosine, have been isolated from the Caribbean sponge *Iotrochota birotulata*, and their structures determined by spectroscopic means. Brominated tyrosine-derived metabolites such as **1** have previously been considered to be characteristic of species in the order Verongida, to which *I. birotulata* does not belong.

The frequent occurrence of halogenated, and particularly brominated, metabolites is a unique feature of marine natural product chemistry. They are not uniformly distributed among the marine plant and invertebrate taxa, and are most commonly found in red algae and sponges. Even in this latter phylum they are present in a relatively small number of species, and are mainly associated with horny sponges, which are distributed in the three orders Dictyoceratida, Dendroceratida, and Verongida. In particular, Verongida are characterized by their ability to synthesize bromotyrosine derivatives, which seem to be peculiar to these sponges, and have been suggested as chemical markers for taxonomic studies (1). In our continuing studies on the secondary metabolites from marine invertebrates, we have now isolated three new, quite unusual compounds [**1–3**] from *Iotrochota birotulata* (Higgin, 1877) (Esperiopsidae; Poecilosclerida).

Iotrochota birotulata is a tropical marine sponge which forms ramose, bushy aggregates of thick branches (1.5 cm across) with a conulose surface; the sponge is purplish black to black in color, and occasionally green in life. It gives off a dark exudate when squeezed. The sponge is widely distributed, coming from reefs, but also from deeper waters, and even from hidden positions in shallow bays. Its distribution ranges all over the Caribbean, although the conspecificity of Indo-Pacific specimens requires verification. In 1979, from a sample of *I. birotulata* collected along the coasts of Miami, Florida, Baden and Corbett (2) isolated a peroxidase, which showed an activity characteristic of halogenating enzymes. The haloperoxidases are known to play a key role in the biosynthesis of the halogenated naturally occurring compounds (3). However, until now no halometabolites have been isolated from this sponge.

Compounds **1–3** appear to be inter-



1 R_1=Br, R_2=Br
2 R_1=I, R_2=Br
3 R_1=I, R_2=I

esting in several ways. Each of these mixed-biogenesis metabolites contains a halogenated partial structure, very probably arising from tyrosine, which for compound **1** closely resembles bromo- compounds from sponges classified in the order Verongida. It is noteworthy that this brominated metabolite derived from tyrosine has been isolated in this study in a species taxonomically far removed from Verongida; to our knowledge, *I. birotulata* is the first sponge that elaborates such metabolites not belonging to the order Verongida. The existence of a mevalonate-derived moiety in the molecule is also very unusual for a halogenated derivative of tyrosine.

The presence of iodine atoms in compounds **2** and **3** is another interesting issue. Iodo- compounds are relatively rare in marine chemistry and particularly in sponges, even if all known haloperoxidases are effective in oxidizing iodide (3). The biosynthesis of iodinated metabolites seems to be related to the capability of organisms to concentrate iodide from sea water, rather than to the presence of a specific peroxidase; most of iodo- metabolites have been isolated from red algae, which are known to contain iodine concentrations as high as 0.5% of wet wt. Interestingly, Kaestner reported that significant amounts of iodine (0.12–1.21%), together with comparable quantities of bromine (0.16–2.66%), are present in the spicule tracts of *I. birotulata*, cemented with variable amounts of spongin (4). This further confirms the relationship between the presence of iodo- metabolites and high concentrations of iodine in the sponge tissues.

Specimens of *I. birotulata* (160 g dry wt), collected along the coast of Little San Salvador Island, Bahamas, during the summer of 1992 and stored frozen, were extracted first with a 3:1 mixture of MeOH-toluene and then with $CHCl_3$. The EtOAc-soluble material from the extracts was subjected to mplc on Si gel. A fraction eluted with EtOAc was mainly composed of a mixture of **1–3**. Hplc chromatography on SiO_2 of the mixture gave **1–3** as pure compounds. Due to the close similarity of compounds **1–3**, as testified by the respective ^{1}H-nmr spectra, structural determination was mainly performed on the compound in highest yield, **1**. Most nmr experiments were performed both in $CDCl_3$ and Me_2CO-d_6 solution, and provided complementary data. In the following discussion, nmr data are referred to those experiments performed in Me_2CO-d_6 solution, unless otherwise stated.

The fabms of **1** showed a 1:2:1 triplet for the pseudomolecular ion peak $[M+H]^+$ at *m/z* 571, 569, and 567, indicative of the presence of two bromine atoms in the molecule, which was appropriate for the molecular formula, $C_{24}H_{28}Br_2N_2O_4$. Analysis of the ^{1}H- and COSY nmr spectra allowed the identification of one C_3- [2H multiplets at δ 4.04 (H_2-18), δ 2.06 (H_2-19), and δ 3.45 (H_2-20)] and one C_2- [2H multiplets at δ 3.42 (H_2-10) and δ 2.74 (H_2-11)] methylene chain; one terminal methylene group of each chain (δ 3.45 and 3.42) was also coupled with two D_2O-exchangeable protons at δ 7.10 and 6.94, respectively. These signals, together with the two ^{13}C-nmr resonances at δ 167.5 and 171.5 and their absorption band at 1635 cm^{-1}, pointed to the presence of two secondary amide functions in the molecule. An isobutenyl part structure was also readily recognizable from the ^{1}H-nmr spectrum, due to the presence of two broad methyl singlets at δ 2.13 and 1.96, both long-range coupled with an olefinic proton resonating at δ 5.70.

The ^{1}H-nmr spectrum of **1** also contained a 4H AB system at δ 7.04 and 6.75 in the aromatic region, suggesting a para disubstituted benzene ring; the corresponding carbon atoms resonated at δ 130.9 and 116.1, respectively, as indicated by an HMQC experiment, while an HMBC experiment allowed the identification of the remaining two quaternary

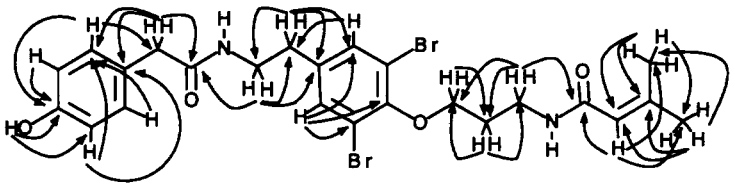


FIGURE 1. ^{1}H-^{13}C long-range couplings of compound **1** detected by the HMBC experiment in Me_2CO-d_6 solution.

carbon atoms (δ 127.5, C-4, and 157.1, C-1) of the ring. The latter atom also showed an HMBC correlation peak with a 1H deuterium-exchangeable singlet at δ 8.37, thus suggesting the phenolic nature of the benzene ring. The C-4 aromatic carbon was linked to a methylene group, whose protons resonated as a singlet at δ 3.33 (H_2-7), as evidenced by a nOe difference experiment, performed in $CDCl_3$ by saturation of H_2-7, which displayed enhancement of H-3, H-5, and by the HMBC correlation peaks of H_2-7 with C-4 and C-3, C-5, and of H-3, H-5 with C-7.

The long-range coupling of the amidic carbon atom resonating at δ 171.5 with H_2-7 allowed us to connect the CH_2-CH_2-NH-CO group to C-7, thus extending the determined structure up to C-11. The nOe enhancement exhibited in $CDCl_3$ by a 2H singlet at δ 7.18 on saturation of H_2-11 suggested that this methylene group was linked to a second, symmetrically tetrasubstituted benzene ring. Several HMBC correlation peaks (see Figure 1) confirmed this hypothesis, and allowed the identification of the quaternary carbon atoms of the ring. The high-field chemical shift of the quaternary sp^2 carbon atoms C-14, C-16 (δ 118.4) clearly showed that they were linked to the two bromine atoms present in the molecule, while the deshielded C-15 (δ 152.3) must be attached to the sole oxygen atom implied by the molecular formula and not yet assigned.

For the structural determination of compound **1** to be completed, only the -CH_2-CH_2-CH_2-NH-CO- and the isobutenyl partial structures, which accounted for all the remaining atoms of the molecule, had to be located. They are sequentially linked to the rest of the molecule as indicated in structure **1**, on the basis of the following evidence. In the HMBC spectrum performed in $CDCl_3$, a correlation peak of C-15 with H_2-18 was present, which established the ether linkage between C-15 and C-18. Furthermore, the location of the isobutenyl group was confirmed by the nOe enhancement of H-23 on saturation of the amidic proton H-21, and by the HMBC correlation of H-23 with C-22.

Compound **2** showed $[M+H]^+$ ion peaks in the fabms at *m/z* 617 and 615, corresponding to the molecular formula, $C_{24}H_{28}BrIN_2O_4$. Its ^{1}H-nmr spectrum was almost identical to that of compound **1**, but the 2H singlet at δ 7.18 (H-13, H-17) was replaced by two 1H doublets at δ 7.65 and 7.44. This data led us to the conclusion that compound **2** differs from **1** only in that one iodine atom is linked to C-14 in place of one bromine atom.

Compound **3** showed a molecular formula of $C_{24}H_{28}I_2N_2O_4$ from its fabms spectrum, and, in its ^{1}H-nmr spectrum, also very similar to that of **1**, the singlet due to H-13, H-17 was shifted downfield to δ 7.68. Therefore structure **3** is identical with **1**, but the atoms linked to the tetrasubstituted benzene ring are two iodine atoms.

EXPERIMENTAL

GENERAL EXPERIMENTAL METHODS.—Fabms were obtained on a VG ZAB mass spectrometer in a glycerol/thioglycerol matrix. ^{1}H- and ^{13}C-nmr spectra were determined on a Bruker AMX-500 spectrometer in Me_2CO-d_6 or $CDCl_3$ solution. ^{1}H-Nmr chemical shifts were referenced to the re-

sidual solvent signal (δ 2.05 and 7.26, respectively). ^{13}C-Nmr spectra were referenced to the center peak of the solvent at δ 29.8 and 77.0, respectively. The multiplicities of ^{13}C resonances were determined by DEPT experiments. Proton-detected multiple-quantum heteronuclear correlation (HMQC) spectra were recorded using the pulse sequence developed by Bax and Subramanian (5), with a BIRD pulse 0.50 sec before each scan to suppress the signal from protons not directly bonded to ^{13}C. The interpulse delays were adjusted for an average $^{1}J_{CH}$ of 140 Hz. During the acquisition time, ^{13}C broadband decoupling was performed using the GARP sequence (6). Proton-detected multiple-bond heteronuclear chemical shift correlation (HMBC) spectra (7) were performed with no ^{13}C decoupling, and optimized for a $^{2,3}J_{CH}$ of 9 Hz. Mplc was performed on a Büchi 861 apparatus using an SiO_2 (230–400 mesh) column. Hplc was performed on a Varian 2050 apparatus equipped with an RI-3 refractive index detector, using Hibar columns.

ANIMAL MATERIAL.—The sponge *I. birotulata* was collected (depth 20 m) in the summer of 1990, along the coast of Little San Salvador Island, Bahamas, and was stored frozen at −20°. Reference specimens are deposited at the Istituto di Zoologia dell'Università di Genova under the number PSS 21-01.

ISOLATION AND EXTRACTION.—Freshly collected animals (161 g dry wt after extraction) were homogenized and successively extracted with MeOH-toluene, 3:1 (5×1 liter) and with $CHCl_3$ (3×1 liter). After evaporation of the solvent, the MeOH extracts were partitioned between EtOAc (4×500 ml) and H_2O (500 ml). The combined EtOAc and $CHCl_3$ extracts were dried (Na_2SO_4) and concentrated *in vacuo* to afford 6.3 g of a dark brown oil, which was chromatographed by mplc on an SiO_2 column using a solvent gradient system from *n*-hexane to EtOAc and then to MeOH. Fractions eluted with EtOAc afforded a mixture containing compounds **1**, **2**, and **3** (19 mg). Hplc on a Hibar LiChrospher Si60 (10×250 mm) column with a mobile phase of EtOAc yielded pure **1** (4.3 mg), **2** (1.4 mg), and **3** (3.0 mg).

1,3-Dibromo-5-{2-[(p-hydroxyphenyl)-acetamido]ethyl}-2-[3-(3-methyl-2-butenamido)-propoxy]benzene [**1**].—Fabms, positive-ion mode *m/z* 571, 569, 567 ([M+H]$^+$); ir (neat) ν max 3286, 1635, 1542, 1515, 1455, 1256 cm^{-1}; ^{1}H- and ^{13}C-nmr data are reported in Table 1.

1-Bromo-3-iodo-5-{2-[(p-hydroxyphenyl)-

TABLE 1. ^{1}H- and ^{13}C-Nmr Data of Compound **1**.

Position	$CDCl_3$		Me_2CO-d_6	
	^{1}H [mult., *J* (Hz)]	^{13}C (mult.)	^{1}H [mult., *J* (Hz)]	^{13}C (mult.)
1		156.4 (C)	8.37 (s)[a]	157.1 (C)
2, 6	6.77 (d, 8.1)	115.9 (CH)	6.75 (d, 8.5)	116.1 (CH)
3, 5	6.92 (d, 8.1)	130.7 (CH)	7.04 (d, 8.5)	130.9 (CH)
4		124.6 (C)		127.5 (C)
7	3.47 (s)	42.9 (CH_2)	3.33 (s)	43.1 (CH_2)
8		171.7 (C)		171.5 (C)
9	5.01 (br s)		6.94 (br s)	
10	3.39 (q, 6.2)	40.0 (CH_2)	3.42 (t, 6.5)	40.8 (CH_2)
11	2.68 (t, 6.2)	33.6 (CH_2)	2.74 (t, 6.5)	34.8 (CH_2)
12		137.5 (C)		139.9 (C)
13, 17	7.18 (s)	133.0 (CH)	7.42 (s)	134.1 (CH)
14, 16		124.8 (C)		118.4 (C)
15		151.8 (C)		152.3 (C)
18	4.04 (t, 7.5)	71.9 (CH_2)	4.04 (t, 6.4)	72.3 (CH_2)
19	2.13 (m)	31.9 (CH_2)	2.06[b]	32.2 (CH_2)
20	3.54 (q, 6.2)	36.1 (CH_2)	3.45 (t, 6.5)	36.6 (CH_2)
21	5.80 (br s)		7.10 (br s)	
22		167.9 (C)		167.5 (C)
23	5.62 (br s)	124.8 (CH)	5.70 (br s)	119.9 (CH)
24		151.8 (C)		149.8 (C)
25	1.85 (br s)	27.2 (CH_3)	1.96 (br s)	26.5 (CH_3)
26	2.13 (br s)	20.1 (CH_3)	2.13 (br s)	19.6 (CH_3)

[a]Resonance of the hydroxyl proton.
[b]Submerged by other signals.

acetamido]ethyl}-2-[3-(3-methyl-2-butenamido)-propoxy]benzene [**2**].—Fabms, positive-ion mode *m/z* 617, 615 ($[M+H]^+$); ir (neat) ν max 3283, 1628, 1537, 1514, 1443, 1260 cm^{-1}; ^1H nmr ($CDCl_3$) δ 7.42 (1H, d, J=1.8 Hz, H-13), 7.21 (1H, d, J=1.8 Hz, H-17), 6.92 (2H, d, J=8.1 Hz, H-3, H-5), 6.77 (2H, d, J=8.1 Hz, H-4, H-6), 5.81 (1H, br s, H-21), 5.63 (1H, br s, H-23), 5.01 (1H, br s, H-9), 4.02 (2H, t, J=7.5 Hz, H_2-18), 3.55 (2H, q, J=6.2 Hz, H_2-20), 3.47 (2H, s, H_2-7), 3.39 (2H, q, J=6.2 Hz, H_2-10), 2.67 (2H, t, J=6.2 Hz, H_2-11), 2.15 (2H, m, H_2-19), 2.13 (3H, s, H_3-26), 1.85 (3H, s, H_3-25).

1,3-Diiodo-5-{2-[(p-hydroxyphenyl)-acetamido]ethyl}-2-[3-(3-methyl-2-butenamido)-propoxy]benzene [**3**].—Fabms, positive ion mode *m/z* 663 ($[M+H]^+$); ir (neat) ν max 3276, 1629, 1538, 1514, 1435, 1256 cm^{-1}; ^1H nmr ($CDCl_3$) δ 7.45 (2H, s, H-13, H-17), 6.92 (2H, d, J=8.1 Hz, H-3, H-5), 6.77 (2H, d, J=8.1 Hz, H-4, H-6), 5.82 (1H, br s, H-21), 5.64 (1H, br s, H-23), 5.01 (1H, br s, H-9), 4.00 (2H, t, J=7.5 Hz, H_2-18), 3.56 (2H, q, J=6.2 Hz, H_2-20), 3.47 (2H, s, H_2-7), 3.39 (2H, q, J=6.2 Hz, H_2-10), 2.65 (2H, t, J=6.2 Hz, H_2-11), 2.16 (2H, m, H_2-19), 2.13 (3H, s, H_3-26), 1.85 (3H, s, H_3-25).

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