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Turbotoxins A and B, novel diiodotyramine derivatives from the Japanese gastropod *Turbo marmorata*

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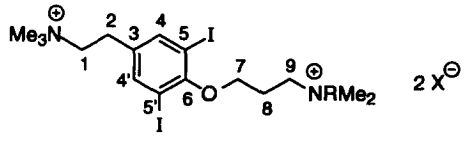
Abstract

Bioassay-guided separation of the aqueous ethanol extract of the viscera of the Japanese gastropod *Turbo marmorata* resulted in the isolation of two toxins, turbotoxins A and B. The structures were determined by spectral analysis and confirmed by organic synthesis to be diiodotyramine derivatives. Turbotoxins A and B exhibited acute toxicity against ddY mice, with LD_{99} values of 1.0 and 4.0 mg/kg, respectively.

Keywords: toxins; ammonium salts; biologically active compounds; marine metabolites.

Human intoxication resulting from the ingestion of shellfish occurs worldwide, and novel compounds have been isolated from toxic shellfish. Toxins from shellfish, such as pinnatoxins,[1] saxitoxin,[2] and neosurugatoxin,[3] have attracted interest not only from pharmacologists but also from biochemists and chemists due to their novel biological activities and structures. In Japan, the gastropod *Turbo marmorata* is eaten after the viscera, which cause intoxication, have been removed. Yasumoto and co-workers studied the toxic components of *T. marmorata* and isolated saxitoxin from the water-soluble fraction.[4] Although they mentioned the presence of other new toxins in this animal,[4,5] not all of these toxins have been identified. We report here the isolation and structures of turbotoxins A and B, marine toxins from the Japanese gastropod *T. marmorata*.



turbotoxin A (1) R = Me X = CF_3COO
turbotoxin B (2) R = H X = CF_3COO

The 75% aqueous ethanol extract of the viscera (4.5 kg, 36 individuals) of *T. marmorata* collected in Okinawa, Japan, was partitioned between ethyl acetate and water. The aqueous layer was chromatographed on TSK G-3000S polystyrene gel (Tosoh Co., Japan) using aqueous ethanol. The eluate of

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Table 1
NMR data of turbotoxins A (1) and B (2) in CD_3OD

position	turbotoxin A		turbotoxin B	
	δ_H[a]	δ_C[b]	δ_H[a]	δ_C[b]
1	3.52 (2H, m)	68.5	3.52 (2H, m)	68.5[c]
1-NMe	3.18 (9H, s)	54.5	3.18 (9H, s)	54.5
2	3.05 (2H, m)	29.1	3.05 (2H, m)	29.1
3		139.1[c]		138.0[c]
4, 4'	7.86 (2H, s)	142.7	7.86 (2H, s)	142.6
5, 5'		92.4		92.5
6		158.0		158.0[c]
7	4.10 (2H, t, J = 5.5 Hz)	71.1	4.10 (2H, t, J = 5.6 Hz)	71.7
8	2.42 (2H, m)	26.0	2.33 (2H, m)	27.1
9	3.78 (2H, m)	66.6	3.55 (2H, m)	58.1
9-NMe	3.22 (9H, s)	54.5	2.98 (6H, s)	44.5

[a] The spectrum was recorded at 600 MHz.
[b] The spectrum was recorded at 100 MHz.
[c] Chemical shifts were determined by HSQC and HMBC experiments.

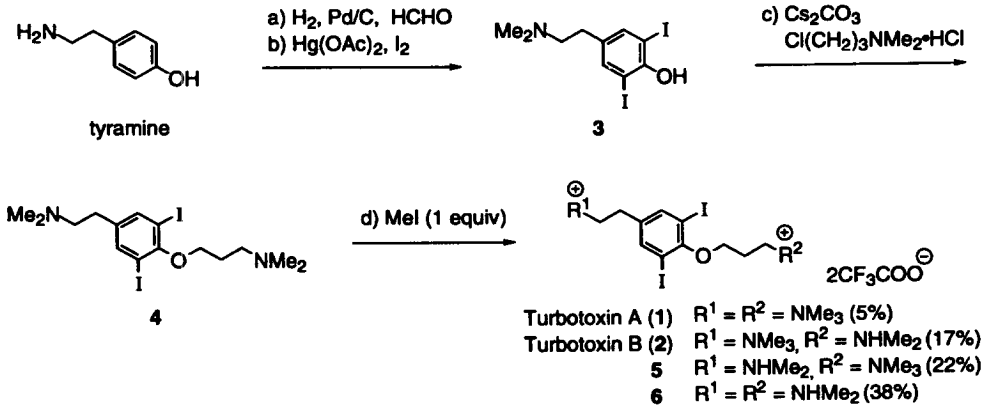
50% aqueous ethanol was collected and concentrated, and the oily residue was chromatographed on repeated reversed-phase MPLC,[6] using bioassay-guided (intraperitoneal mouse lethality) fractionation, to give two toxic fractions. The early toxic fraction was finally purified by HPLC [Develosil ODS HG-5, (1) 20% aqueous methanol containing 0.01 M TFA; (2) 20% aqueous methanol containing 0.01 M TFA] to give turbotoxin A[7] (**1**) (0.5 mg; LD_{99} 1.0 mg/kg), and the late toxic fraction was purified by HPLC [Develosil ODS HG-5, (1) 30 to 40% aqueous methanol containing 0.01 M TFA; (2) 20% aqueous methanol containing 0.01 M TFA] to give turbotoxin B[8] (**2**) (0.9 mg, LD_{99} 4.0 mg/kg). These compounds **1** and **2** were isolated as trifluoroacetate salts because of the solvent system used for chromatographic purification.

The molecular formula of turbotoxin A (**1**) was found to be $C_{17}H_{30}I_2N_2O\cdot(CF_3COO)_2$ (*m/z* 645.0286 $[M-CF_3COO]^+$, Δ −1.2 mmu) by HRFABMS. Resonances in the NMR spectra were assigned based on the COSY, HSQC, and HMBC spectra, as shown in Table 1. Although the carbon signal at C3 was not observed in the ^{13}C NMR spectrum due to the scarcity of the sample, the carbon chemical shift was determined by HMBC (J_{C-H}=6 Hz) experiments.

The ^1H NMR data showed the presence of two trimethylammonium groups. The COSY spectrum allowed -$(CH_2)_2$- and -$(CH_2)_3$- chains to be constructed. The NMR signals at δ_H 7.86 and at δ_C 92.4, 139.1, 142.7, and 158.8 were assigned to a 4-substituted-2,6-diiodophenoxy unit by comparison with those of thyroxine: the unusually high field of the signal at δ_C 92.4 clearly distinguished the sp^2 carbon atoms linked to an iodine atom. The HMBC correlations (H-1/1-NCH_3, 1-NCH_3/C1, H-2/C3, H-2/C4, H-4/C2, H-4/C4′, H-4/C5, H-4/C6, H-9/9-NCH_3, 9-NCH_3/C9) allowed the foregoing partial structures to be connected, giving two partial structures, $Me_3N^+CH_2CH_2C_6H_2I_2O$- and -$CH_2CH_2CH_2N^+Me_3$. Finally, a weak HMBC correlation between H-7 and C6 completed the structure of turbotoxin A, as shown in structure **1**.

Turbotoxin B (**2**) was found to be a demethylated analog of **1** based on its molecular formula, $C_{16}H_{28}I_2N_2O\cdot(CF_3COO)_2$ (HRFABMS *m/z* 517.0209 $[M-CF_3COOH-CF_3COO]^+$, Δ −0.4 mmu). Comparison of the ^1H NMR data of **2** with those of **1** indicated that a trimethylammonium group at C9 in **1** was demethylated to give a dimethylamino group in **2** (Table 1). Furthermore, correlations sufficient to establish the structure of turbotoxin B were found in the HMBC spectrum of **2**.[9] Thus, the structure of turbotoxin B was determined to be **2**.

To confirm the structures of turbotoxins A (**1**) and B (**2**), and to subject **1** and **2** to further biological examinations, **1** and **2** were synthesized (Scheme 1).[10] 2,6-Diiodo-*N*,*N*-dimethyltyramine (**3**) was prepared from tyramine in 2 steps (79%). Alkylation of **3** with chloropropyldimethylamine hydrochloride in the presence of Cs_2CO_3 afforded diamine **4**[11] (48%). Diamine **4** was methylated with 1 molar equivalent of iodomethane to give turbotoxins A (**1**, 5%) and B (**2**, 17%) along with turbotoxin B isomer **5** (22%) and diamine trifluoroacetate salt **6** (38%), which were separated by HPLC [(1) Develosil ODS HG-5, 30% aqueous methanol containing 0.1% TFA; (2) Develosil PhA-5, 30% aqueous methanol containing 0.1% TFA]. The spectral data and biological activities of synthetic turbotoxins A (**1**) and B (**2**) were identical to those of natural **1** and **2**, respectively, which confirmed the structures and toxicity of turbotoxins A and B.



Scheme 1. Conditions: (a) MeOH, rt, 10 h, 93%; (b) EtOH, rt, 30 min, 85%; (c) DMF, 40°C, 20 h, 48%; (d) MeOH, rt, 2 h

The relationships between the structure of turbotoxins and acute toxicity (intraperitoneal mouse lethality) were studied (Table 2). Compound **6** showed the weakest toxicity among the compounds tested, indicating that the quaternary ammonium group is responsible for the toxicity. The toxicity of turbotoxin B (**2**) (4.0 mg/kg) is stronger than that of isomer **5** (8.0 mg/kg). This finding shows that the quaternary ammonium group at C1 is more important for the toxicity than that at C9.

In summary, we isolated two marine toxins, turbotoxins A (**1**) and B (**2**), from the Japanese gastropod *T. marmorata*. These turbotoxins were determined by spectral analysis to be diiodotyramine derivatives, and this was confirmed by organic synthesis. Turbotoxins A (**1**) and B (**2**) are structurally related to dakaramine (**4**)[11] and dibromotyramine derivatives, such as aplysamine-1,[12] moloka'iamine,[13] and ceratinamine,[14] which all were isolated from marine sponges. 3,5-Diiodotyrosine found in corals, sponges, algae, and other marine organisms might be important for biosynthesis of turbotoxins, considering the food-habits of *T. marmorata*. Interestingly, a quaternary ammonium salt, iodomethyltrimethylam-

Table 2
Acute toxicity of turbotoxins A (**1**) and B (**2**) and their analogs

compound	acute toxicity (LD_{99}, mg/kg)[a]
Turbotoxin A (**1**)	1.0
Turbotoxin B (**2**)	4.0
5	8.0
6	100

[a] Upon intraperitoneal injection into ddY mice.

monium chloride, was isolated as a minor toxin from the Japanese gastropod *T. marmorata*[5] and other quaternary ammonium salts have been isolated from *T. argyrostoma*.[15] The mode of action of turbotoxins is currently under investigation.

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6. Chromatography was performed by reversed-phase MPLC on Develosil ODS-T (Nomura Chemical, Japan): (1) 30 to 100% aqueous methanol containing 0.01 M TFA; (2) 10 to 60% aqueous methanol containing 0.01 M TFA; (3) 15% aqueous methanol containing 0.01 M TFA.
7. Compound **1**: UV (MeOH) λ_{max} 226 (ε 19000), 239 (sh, 9500), 275 nm (sh, 2000).
8. Compound **2**: UV (MeOH) λ_{max} 225 (ε 11000), 239 (sh, 5500), 279 nm (sh, 1500).
9. Selected HMBC correlations of **2**: H-1/1-NCH_3, 1-NCH_3/C1, H-2/C3, H-2/C4, H-4/C2, H-4/C4′, H-4/C6, H-9/9-NCH_3, 9-NCH_3/C9.
10. Satisfactory ^{1}H and ^{13}C NMR, mass, and exact mass spectral data were obtained for all new compounds.
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