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The Absolute Stereochemistry of the New Zealand Shellfish Toxin Gymnodimine

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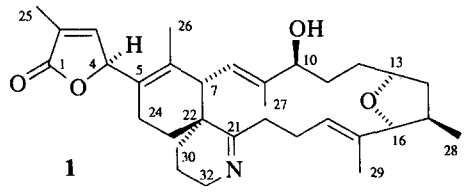
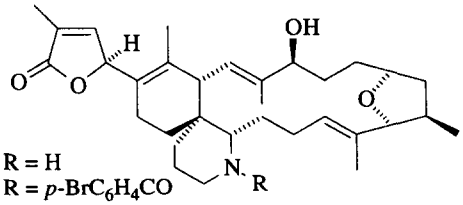
Abstract: The absolute stereochemistry of the New Zealand shellfish toxin gymnodimine **1** has been determined by X-ray crystal structure analysis of the *p*-bromobenzamide derivative **3** of gymnodamine **2**.

Commercial oyster beds in Southland, New Zealand were closed in early 1994 due to high levels in the oysters of a biotoxin showing neurotoxic shellfish poisoning (NSP) features in a mouse bioassay.[1] The toxin, named gymnodimine **1**, was isolated and the structure elucidated by NMR spectroscopy.[2] Gymnodimine **1** is structurally related to the pinnatoxins[3] and the spirolides,[4] with the unusual spirocentre and imine functionalities. The pharmacological action of these compounds has not yet been fully defined, although the cyclic imine functionality has been suggested as the pharmacophore of the spirolides.[4] This suggestion has been supported by our observation of the inactivity of the reduced form of **1**, gymnodamine **2**, in the mouse bioassay.[5] A better understanding of the pharmacology of these compounds might be aided by a knowledge of their shape. The relative stereochemistries of only the distantly related pinnatoxins[3] have been reported, while the absolute stereochemistry has not been elucidated for of any of these compounds. We now report the crystal structure and absolute configuration of the *p*-bromobenzamide derivative **3** of gymnodamine **2**, from which the stereochemistry of gymnodimine **1** follows.

Gymnodimine **1** was reisolated from Foveaux Strait oysters,[6] but all attempts at forming crystalline derivatives of **1** at the C10 hydroxyl were impeded by low reactivity at this position. Furthermore, degradation was observed, possibly due to instability of the imine functionality. After reduction of the imine functionality of gymnodimine **1** the resultant secondary amine gymnodamine **2** was found to be more stable and tractable towards the formation of derivatives. This reduction was achieved stereospecifically with $NaCNBH_3$/MeOH in quantitative yield.[7] Gymnodamine **2** was then converted to the *p*-bromobenzamide **3** by standard methods.[8]



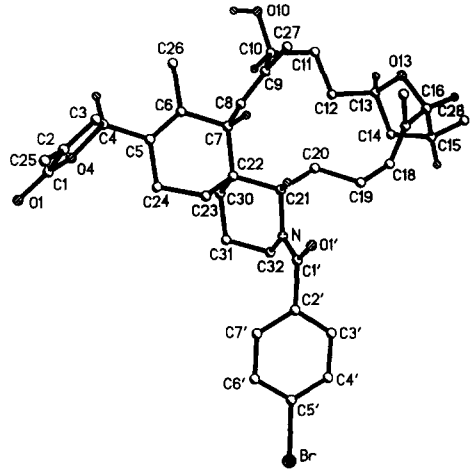
1



2 R = H
3 R = *p*-BrC_6H_4CO

Recrystallisation of the amide **3** from C_6H_6/CH_2Cl_2 gave irregular shaped, transparent crystals suitable for single crystal X-ray structure analysis. The data were collected using a Siemens P4 diffractometer. The structure of **3** was solved by direct methods (SHELXS-96) and refined by a full-matrix least-squares refinement (SHELXL-96) after being corrected for absorption by using the psi-scan method.[9] The absolute configuration was determined from the anomalous scattering of the bromine atom and stereocentres assigned as 4*S*, 7*S*, 10*S*, 13*R*, 15*R*, 16*R*, 21*S*, 22*R*, as shown in the perspective view (see figure). From this it follows that the absolute stereochemistry of gymnodimine **1** is 4*S*, 7*S*, 10*S*, 13*R*, 15*R*, 16*R*, 22*R*.

The ^1H NMR spectrum of the amide **3** showed doubling of various resonances (3:1 ratio), interpreted as arising from two amide conformations. Comparison of the solution conformation of amide **3** with that in the crystalline state showed good agreement, principally by the observation of NOE interactions (%) H4/Me26 (2.1), H7/H21 (5.5), H7/Me27 (2.9), H8/H10 (10.1), H16/H15 (2.8), H16/Me28 (1.5), and H18/H15 (10.0), all of which are expected from the parameters derived from the crystal structure. Except for H7/H21, all of these interactions were also observed for imine **1**, together with interactions for H8/H30, H13/Me28 (0.9) and Me29/H16 (4.1). However, additional interactions for H18/H16 (0.7) and H18/Me27 (0.8) in the NOE spectra of the imine **1** suggest the presence in solution of additional conformations for **1**. This is supported by a large change in the chemical shift for H18 (5.00 for **1**, 5.45 for **2** and 5.38 ppm for **3**).



The reactivity of the amine group in **2** is now being utilised to form haptens for the development of an immunoassay for the more sensitive, quantitative and direct detection of gymnodimine **1** in shellfish.

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REFERENCES AND NOTES

1. Mackenzie, L. *Seafood New Zealand* **1994**, *2*, 47-50.
2. Seki, T.; Satake, M.; Mackenzie, L.; Kaspar, H.F.; Yasumoto, T. *Tetrahedron Lett.* **1995**, *36*, 7093-7096.
3. Chou, T.; Haino, T.; Kuramoto, M.; Uemura, D. *Tetrahedron Lett.* **1996** *37*, 4027-4030, and references cited therein.
4. Hu, T.; Curtis, J.M.; Oshima, Y.; Quilliam, M.A.; Walter, J.A.; Watson-Wright, W.M.; Wright, J.L.C. *J. Chem. Soc. Chem. Commun.* **1995**, 2159-2161. Hu, T.; Curtis, J.M.; Walter, J.A.; Wright, J.L.C. *Tetrahedron Lett.* **1996**, *37*, 7671-7674.
5. Minimum Lethal Doses (MLD) (intraperitoneal): gymnodamine **2**, >4040 μg/kg; gymnodimine **1**, 700 μg/kg.
6. Oysters *Tiostrea chilensis* (5 kg), collected at Foveaux Strait, were extracted with 0.1% AcOH/80% MeOH/20% H_2O. After removal of MeOH the extract was neutralised with NH_3, adjusted to pH 8.5 with $NaHCO_3$ and partitioned against CH_2Cl_2. The organic layer was dried *in vacuo* and partitioned between ether and 0.5% aq. AcOH. The aqueous layer was neutralised with NH_3, adjusted to pH 8.5 with $NaHCO_3$ and partitioned against EtOAc. The organic layer was successively chromatographed on DIOL with 3:1 CH_2Cl_2:pet. ether and on AMINO with MeOH to afford gymnodimine **1** (8.4 mg).
7. Gymnodimine **1** (8 mg) was reacted with $NaCNBH_3$ (2 mg) in MeOH (1 mL) and AcOH (5 μL) for 4.5 hr. All solvents were removed *in vacuo* and reaction mixture filtered through a 0.45 μm filter in CH_2Cl_2 (5 x 1 mL) to give gymnodamine **2** (8 mg, 99%) as a colourless oil. HREIMS: $C_{32}H_{47}NO_4$ (M^+ *m/z* 509.3495 Δ -2.01 ppm). All spectral data were consistent with **2**.
8. Gymnodamine **2** (8 mg) was reacted with *p*-bromobenzoyl chloride (4.4 mg) and DMAP (244 μg) in dry CH_2Cl_2 (1 mL) and diisopropylethylamine (3.47 μL) at 0 °C for 30 min. H_2O (10 drops) was added and all solvents removed *in vacuo*. The reaction mixture was partitioned between CH_2Cl_2 and aq. citric acid (10 % w/v) and the organic phase purified on normal phase HPLC (DIOL) in CH_2Cl_2 to give the *p*-bromobenzamide **3** (4.0 mg, 37 %) which was recrystallised from C_6H_6/CH_2Cl_2 to give irregular shaped crystals; mp 251-254 °C; HRFABMS: $C_{39}H_{51}BrNO_5$ (MH^+ *m/z* 692.2971 Δ 2.90 ppm); δ_H ($CDCl_3$) (major conformation) 7.52, d, *J* = 8.8, 2H, H4' H6'; 7.19, d, *J* = 8.3, 2H, H3' H7'; 6.88, bs, 1H, H3; 5.72, bs, 1H, H4; 5.38, t, *J* = 6.3, 7.8, 1H, H18; 5.20, d, *J* = 10.7, 1H, H8; 4.50, dd, *J* = 3.4, 8.8, 2.9, 1H, H21; 4.15, m, 1H, H13; 3.94, obs, 1H, H10; 3.92, bs, 1H, H16; 3.46, dd, *J* = 5.4, 10.3, 1.4, 1H, H32a; 2.99, d, *J* = 11.2, 1H, H7; 2.95, obs, H32b; 2.48, m, 1H, H15; 2.15, m, H19; 2.00, obs, H11a; 1.94, s, 3H, Me25; 1.77, s, 3H, Me27; 1.67, s, 3H, Me29; 1.60, s, 3H, Me26; 1.08, d, *J* = 7.3, 3H, Me28.
9. *Crystal data*: $C_{39}H_{50}BrNO_5 \cdot C_6H_6$, *F.W.* = 770.82, orthorhombic, space group $P2_12_12_1$, *a* = 10.424(7), *b* = 17.236(12), *c* = 23.642(16) Å, $\alpha = 90$, $\beta = 90$, $\gamma = 90$, $V = 4248(5)$ Å^3, $Z = 4$, $D_c = 1.205$ Mg/m^3, absorption coefficient 1.011 mm^{-1}, minimum and maximum transmission coefficients 0.506, 0.552. Final conventional R factors : [$I>2\sigma(I)$] $R_1 = 0.0360$, $wR_2 = 0.0376$. R_1 for inverse stucture = 0.0574.

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