The Absolute Configuration of Ciguatoxin

Masayuki Satake,† Akio Morohashi,† Hiroki Oguri,‡ Tohru Oishi,‡ Masahiro Hirama,*,‡ Nobuyuki Harada,§ and Takeshi Yasumoto*,†

Faculty of Agriculture, Tohoku University Tsutsumidori-Amamiya, Aoba-ku, Sendai 981, Japan Graduate School of Science, Tohoku University Aramaki Azaaoba, Aoba-ku, Sendai 980-77, Japan Institute for Chemical Reaction Science Tohoku University Katahira, Aoba-ku, Sendai 980-77, Japan

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Ciguatera is the name of human intoxication that is caused by eating certain tropical reef fishes.[1] Ciguatoxin (CTX) is the principal toxin which was first isolated in Hawaii from moray eel livers. Its structure (**1**), except for the absolute configuration and C2 stereochemistry, was later determined by the Yasumoto group.[2] Two ciguatoxin precursors coded CTX4A (**2**) and CTX4B (formerly GT4b, **3**), were also isolated from epiphytic dinoflagellate microalgae *Gambierdiscus toxicus*, and their relative stereostructures were elucidated (see Figure 1 for structures).[3] Further chemical studies to determine the absolute configuration of these intriguing toxins were hampered because of extremely limited availability of the toxins: 0.35 mg of CTX from 4000 kg of fish and 0.45 mg of **2** from 1100 L of dinoflagellate cultures. We had assigned *R* configuration to C5 of CTX4B by comparing its CD spectrum with that of a synthetic fragment composed of the butadienyl side chain and AB rings of CTX4B.[4] However, uncertainty remained due to the small Cotton effects observed.

The 2*S* configuration assigned on the basis of the CD exciton chirality data of tetrakis-*p*-bromobenzoates of **1** and tris-*p*-bromobenzoates of AB fragments also needed further confirmation because of the difficulty to assign the position of tetrakis-*p*-bromobenzoates of **1**.[5] In view of the importance of knowing the absolute configuration, we renewed our efforts to eliminate the above ambiguities. When a new chiral fluorescent reagent was used in conjunction with the CD exciton chirality method, the absolute configurations of CTX and CTX4A were successfully determined with very small amounts of toxins.

As shown in Scheme 1, hydroxyl groups of **1** (5 μg) were protected as (benzyloxy)methyl (BOM) ethers (**5**). Compound **5** was cleaved at the C3, C4-double bond with OsO_4/$NaIO_4$, and the resultant aldehyde (**6**) was immediately reduced with $NaBH_4$ yielding a glycerol derivative (**7**).[5b] Alcohol **7** was esterified with chiral reagent (*S*)-2-*tert*-butyl-2-methyl-1,3-benzodioxole-4-carboxylic acid [(*S*)-TBMB-carboxylic acid] resulting in a fluorescent derivative (**8**).[6] Reference (2*S*)- and (2*R*)-TBMB esters (**8**) were prepared from (*R*)- and (*S*)-2,2-dimethyl-1,3-dioxolane-4-methanol (**9**) as shown in Scheme 2. Structures of the fluorescent esters (**8**) thus prepared were confirmed by FABMS (MNa^+, *m/z* 573) and NMR spectra. The two diastereomers have clearly different retention times on both normal and reversed phase columns.[7] The retention times (normal phase, 17 min; reversed phase, 54 min) of the derivative of **1** agreed well with those of the TBMB ester having a 2*R* configuration derived from the 2*S*-standard. Consequently, C2 configuration of **1** was unambiguously determined to be *S*.

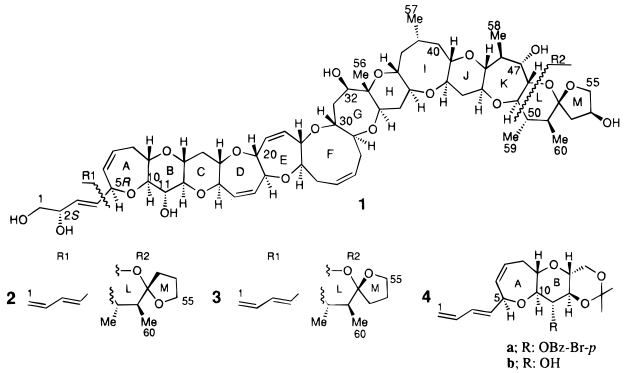
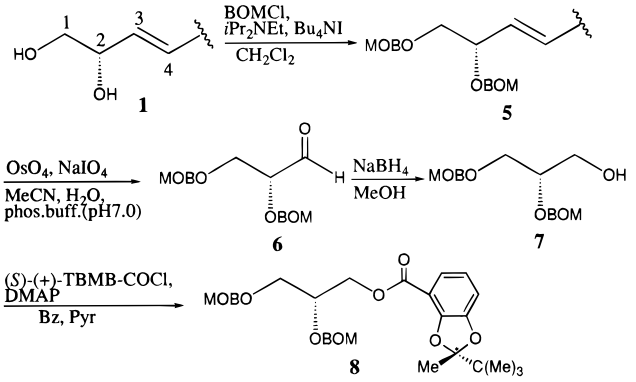
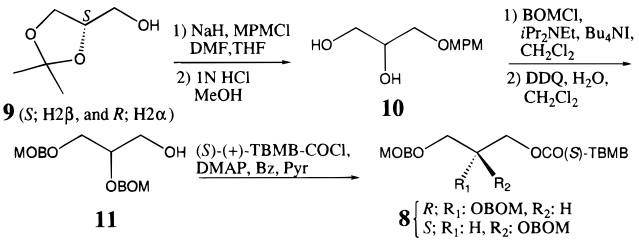


Figure 1. Structures of CTX (**1**), CTX4A (**2**), CTX4B (**3**), *p*-bromobenzoate of CTX4A AB ring fragment (**4a**), and CTX4A AB ring fragment (**4b**).

Scheme 1



Scheme 2



The configuration of C5 in **2** was determined by comparing CD spectra of 11-*p*-bromobenzoate of the AB ring fragment of CTX4A having 5*R* configuration (**4a**) and 11,32,47-tris(*p*-bromobenzoyl)-CTX4A. *p*-Bromobenzoate **4a** was stereoselectively synthesized from **12**[5] as shown in Scheme 3. The CD spectrum of **4a** clearly exhibits a split Cotton effect with a positive followed by a negative extremum, which is caused by the expected interaction between the 1,3-diene and *p*-bromobenzoate (MeOH, λ_{ext} 242 nm, $\Delta\epsilon$ +25; λ_{ext} 225 nm, $\Delta\epsilon$ −14).[8]

(1) Scheuer, P. J.; Takahashi, W.; Tsutsumi, J.; Yoshida, T. *Science* **1976**, *155*, 1267−1268.

(2) Murata, M.; Legrand, A.-M.; Ishibashi, Y.; Fukui, M.; Yasumoto, T. *J. Am. Chem. Soc*. **1990**, *112*, 4380−4386.

(3) Satake, M.; Ishibashi, Y.; Legrand, A.-M.; Yasumoto, T. *Biosci. Biochem. Biotech*. **1996**, *60*, 2103−2105.

(4) Suzuki, T.; Sato, O.; Hirama, M.; Yamamoto, Y.; Murata, M.; Yasumoto, T.; Harada, N. *Tetrahedron Lett.* **1991**, *32*, 4505−4508.

(5) (a) Oguri, H.; Hishiyama, S.; Oishi, T.; Hirama, M. *Synlett* **1995**, 1252−1254. (b) Oguri, H.; Hishiyama, S.; Sato, O.; Oishi, T.; Hirama, M.; Murata, M.; Yasumoto, T.; Harada, N. *Tetrahedron* **1997**, *53*, 3057−3072.

(6) (a) Nishida, Y.; Ohrui, H.; Meguro, H. *Tetrahedron Lett*. **1989**, *30*, 5277−5280. (b) Kim, J.-H.; Uzawa, H.; Nishida, Y.; Ohrui, H.; Meguro, H. *J. Chromatogr*. **1994**, *677*, 35−43.

(7) Normal phase HPLC analysis was done under the following conditions: column, Develosil 60−3 (4.6 × 250 mm, Nomura Chemical) solvent, hexane/*t*-BuOH 150:1; flow, 1.7 mL/min; detection, excitation 310 nm and emission 370 nm T = 25 °C Reversed phase HPLC was done under the following conditions: column, Cosmosil 5C18 AR (4.6 × 250 mm, Nakarai Tesch); solvent, MeOH/H_2O 75:25; flow, 1.1 ml/min; detection, excitation 310 nm and emission 370 nm T = 40 °C.

(8) Unlike that of *p*-bromobenzoate **4a** exhibiting intense dual Cotton effects, the CD spectrum of alcohol **4b** shows a single weak Cotton effect: **4b**, CD (MeCN) λ_{ext} 224 nm ($\Delta\epsilon$ +6.8). Therefore, as a first approximation, the contribution of 1,3-diene helicity can be neglected. See also the CD spectra of related compounds in ref 4.

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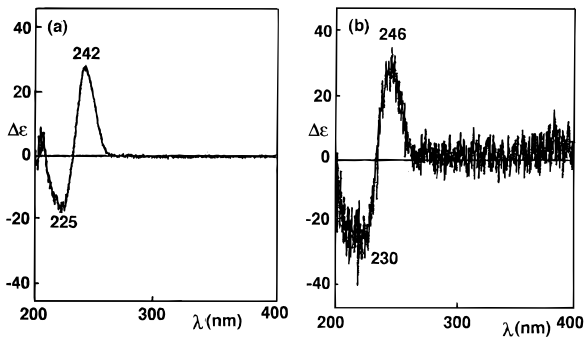
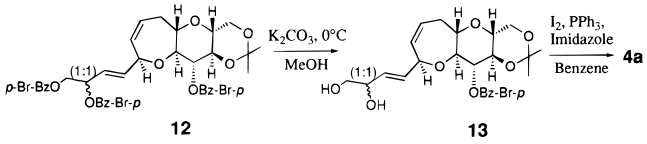


Figure 2. CD spectra of **4a** (a) and tris-*p*-bromobenzoate of **2** (b).

Scheme 3



Reaction of **2** (100 μg) with *p*-bromobenzoyl chloride for 24 h in the presence of Et_3N and 4-(dimethylamino)pyridine afforded tris-*p*-bromobenzoate of **2**, which was purified on an Asahipak ODP-50 column (4.6 × 150 mm) with MeOH and confirmed by FABMS [MH^+ *m/z* 1607, 1609, 1611, 1613]. The CD spectrum of the tris-*p*-bromobenzoate of **2** (MeOH, λ_{ext} 246 nm, $\Delta\epsilon$ +32; λ_{ext} 230 nm, $\Delta\epsilon$ −28) was virtually identical with that of **4a** (Figure 2), thereby implying that the split Cotton effect was mainly due to the coupling between the 1,3-diene and 11-α benzoate.[8,9] The contribution due to the chiral exciton coupling between the 11-α and 32-β benzoate chromophores should be insignificant because of their large separation (approximately 13 Å). Similarly, coupling between the C32-equatorial and C47-axial benzoate chromophores is predictably small because the two chromophores are approximately 10 Å apart. On the basis of these results, the configuration of C5 in **2** was concluded to be *R*. Since **1** is an oxidized metabolite of **2** and was confirmed to have the same relative configurations at C5 and C11,[2,3] **1** must have 5*R* configuration. Thus, **1** has the absolute configuration as shown in **1**.

Because of the extremely limited availability of **1** and **2**, it was imperative to carry out the stereochemical determination with the minimum amount of toxins. In this study, we successfully determined their absolute configuration using only 5 μg of **1** and 100 μg of **2**. The stereochemical information obtained in this study should greatly enhance synthetic efforts toward these intriguing molecules, which will eventually allow immunological, toxicological, and pharmacological studies of ciguatera to be undertaken.

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Supporting Information Available: HPLC chromatograms of CTX derivative and CD spectrum of CTX4A AB ring fragment (3 pages). See any current masthead page for ordering and Internet access instructions.

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(9) The exciton coupling between 1,3-diene and *p*-bromobenzoate chromophores was confirmed by the molecular orbital calculation of CD spectra. The calculation gave dual Cotton effects of positive exciton chirality in agreement with the observed CD spectra of **4a** and tris-*p*-bromobenzoate of **2** (Harada, N. et al., to be published).