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Relative Configuration of Yessotoxin and Isolation of Two New Analogs from Toxic Scallops

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Abstract: The relative configuration of yessotoxin (YTX) and ring conformations were determined by NMR experiments. Additionally two new analogs, 45-hydroxyYTX and 45,46,47-trinorYTX were isolated from toxic scallops. Copyright © 1996 Elsevier Science Ltd

Yessotoxin (**1**, YTX) was isolated as one of the causative toxins of diarrhetic shellfish poisoning (DSP). Its planar structure was elucidated by using NMR spectroscopies[1] and by negative FAB MS/MS[2]. Structurally, YTX is closely related to brevetoxin B[3] and ciguatoxin 4B[4] in having a ladder-shape polycyclic ether skeleton and an unsaturated side-chain. Additionally, YTX has two sulfate ester groups. In spite of the structural resemblance with brevetoxin B and ciguatoxin, that are potent activators of voltage-gated sodium channels but are not cytotoxins, YTX does not potentiate those channels and shows cytotoxicity[5] and different toxicological properties.[6] In view of the increasing interest[7,8] in the molecular mechanism of the action of polycyclic ether toxins on sodium channels or cell membranes, we renewed our effort to isolate YTX and compare its stereostructure with that of brevetoxin B. We also searched for its analogs. In this paper we report the relative configuration of **1** and isolation of two new analogs, 45-hydroxyYTX (**2**) and 45,46,47-trinorYTX (**3**).

YTX and its analogs were isolated from the digestive glands (10 kg) of DSP-infested scallops, *Patinopecten yessoensis*, collected in 1993 in Mutsu Bay, Japan, following the previously reported procedures,[1] modifying only the final step of purification; a Develosil ODS-7 column (Nomura Chemicals) with MeOH:MeCN:H_2O (4:2:7) for **1**, and MeOH:MeCN:H_2O (4:2:9) for **2** and **3**. Yields of **1**, **2**, and **3** were 5000, 140, and 100 μg, respectively.

All ether rings in **1** were found to be trans-fused, as in the case of brevetoxin B, on the basis of the typical coupling constants (9-10 Hz) of angular protons for antiperiplanar substitution on oxycarbons.[9] Observed NOEs between angular protons, angular proton and a methyl, and angular methyls on both sides of ether oxygens also supported trans-fusion of rings (Fig. 1). NOEs difficult to detect (H9 and H13, Me49 and Me50) due to close proximity of their chemical shifts in CD_3OD were made discernible by changing the solvent to C_5D_5N.

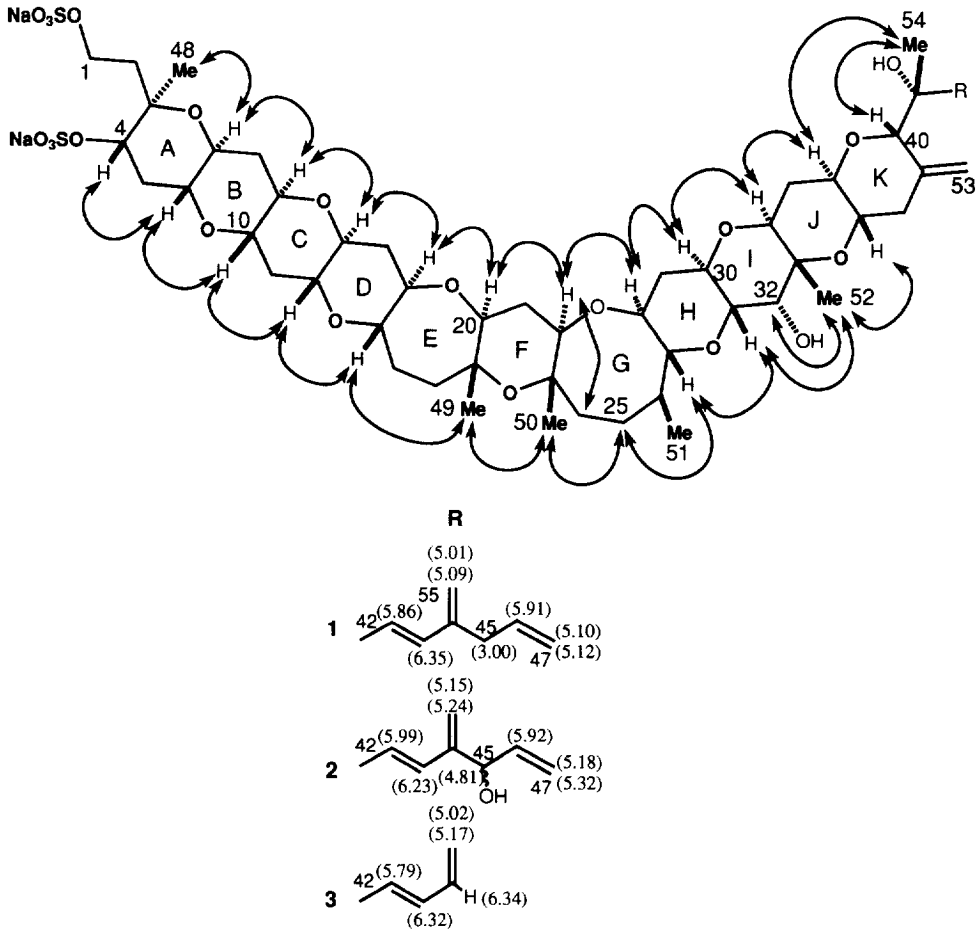

Fig. 1. Structures of yessotoxin (**1**), 45-hydroxy YTX (**2**), and 45,46,47-trinor YTX (**3**). Arrows indicate the significant NOEs confirming the ring conformations of **1** . Chemical shifts of protons on the side-chain measured in CD_3OD (20 °C) are shown in parenthesis.

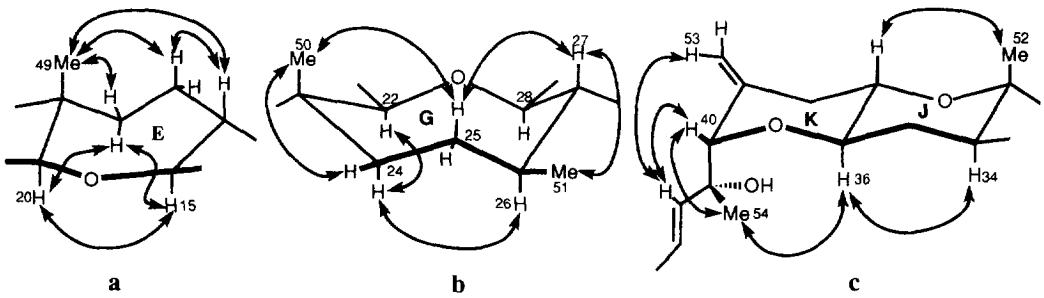

Fig. 2. Conformers and stereostructures around ring E, G, J, and K. Arrows denote the NOEs observed in CD_3OD and /or C_5D_5N.

The configurations of functional groups and ring conformations were clarified by NOEs and coupling constants. The prominent NOE between H4 and H6, and the signal shape of H4 (dd, 4.8 and 9.8 Hz) indicated the sulfate ester at C4 to be equatorial. NOEs observed on H15 versus H18α and H20, and on Me49 versus H16 and H17β, and no NOE between H16 and either of H_2-18 implied that ring E adopts a chair conformation, analogous with ring E in brevetoxin B (Fig. 2a). The β-orientation of Me51 was elucidated in the following manner. Ring G was inferred to adopt a crown conformation, analogous with ring H in catalytically reduced brevetoxin B, based on NOEs from H24α to H22 and H26, and those from Me50 to H24β and H25β (Fig. 2b). The coupling pattern of H24α (t, 10.5 Hz) also supported the crown conformation, as the dihedral angle between H24α and H25α should be close to 90°. The NOE between H27 and Me51, together with the coupling pattern of H27 (t, 9.8 Hz) implies that H26 and H27 are in a 1, 2-diaxial position. Hence Me51 has the β-orientation. The α–orientation of 32-OH was deduced from the NOE between H32 and Me52, and from the coupling constant of H32 (J=2.7 Hz). C41 and H36 are oriented in the same direction, because NOE was observed between H36 and Me54 but not between H36 and H40. As illustrated in Fig. 2c, NOEs between H42 and H40, H42 and H53, H40 and Me54, and Me54 and H36 indicated that the conformation of Me54 and C42 were *gauche* to H40 and that of 41-OH was *anti* to H40. All these data allowed us to assign the relative configuration of YTX as in **1**.

2 was isolated as an amorphous solid: mouse lethality (ip) ca. 0.5 mg/kg; UV λmax 230 nm (ε 15,600, MeOH). The molecular-related ion at m/z 1179, $(M\text{-}Na)^-$ in the negative ESI-MS spectrum suggested **2** to be larger than **1** by one oxygen atom. ^1H NMR chemical shifts and coupling constants of **2** in CD_3OD clearly showed **2** to be identical with **1** except for the side-chain part. The signal of H_2-45 in **1** was replaced in **2** by an oxymethine signal appearing in very lowfield (δ 4.81 ppm). Thus, obviously, the new analog is 45-hydroxyYT X (**2**). The very small amount of **2** (0.14 mg) prevented us from determining the configuration at C45.

3 was obtained as an amorphous solid: mouse lethality (ip) ca. 0.22 mg/kg; UV λmax 226 nm (ε 22,600, MeOH); negative ESI-MS, m/z 1123, $(M\text{-}Na)^-$. ^1H NMR spectra of **3** closely resembled those of **1** in CD_3OD. In particular, connectivities, chemical shifts, and signal shapes from H1 through H40 as well as the side-chain

methyls agreed well with those of the corresponding signals in **1**. Signals arising from protons on C45 through C47 in **1** were absent in the ^1H NMR spectra of **3**. Instead, a new signal due to H44 (6.34 ppm) in a conjugated diene system appeared (Fig. 1). These data together with the MS data (40 mu smaller than **1**) and the hypsochromic shift of the UV maxima by 4 nm allowed us to assign **3** as 45,46,47-trinorYTX.

Structurally, YTX was found to closely resemble brevetoxin B not only in the number (eleven) and type of the cyclic ether rings (six, seven, and eight membered) but also in having the rings fused in trans. The only distinction in the ring conformation was seen in the eight membered rings: ring G in YTX has the crown whilst the corresponding eight membered ring (H) in brevetoxin B has the half-chair. The unsaturated terminal side-chain at ring K is in the axial orientation analogous with that in brevetoxin B. Whether the sulfate esters in **1** are solely responsible for the different mode of action between the two toxins or the different conformation of the eight membered ring also contributes to the difference is an intriguing question to be explored in the future. In the two new analogs, **2** and **3**, the mouse lethality was reduced slightly, perhaps due to reduced affinity to the lipid membrane as the result of increased polarity in the side chain. Though **2** and **3** were minor components in the sample tested, the relative abundance of the three toxins may vary regionally and seasonally, as do other DSP-toxins. Hence analytical methods specific to individual toxins are desired for monitoring shellfish toxicity.

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9. NMR spectra were measured with 500 MHz and 600 MHz instruments in CD_3OD and in C_5D_5N at 20 °C.

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