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ISOLATION AND STRUCTURE OF YESSOTOXIN, A NOVEL POLYETHER COMPOUND IMPLICATED IN DIARRHETIC SHELLFISH POISONING

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Summary: A novel polyether toxin, yessotoxin, was isolated from scallops implicated in diarrhetic shellfish poisoning, and its planar structrure 1 was proposed by means of modern NMR techniques.

Diarrhetic shellfish poisoning is a gastroenteritis caused by ingestion of shellfishes infested with certain dinoflagellate toxins.[1] We previously isolated the causative agents and determined their structures to be okadaic acid[2] and its derivatives,[3,4] and novel polyether lactones named pectenotoxin.[4,5] We now report the isolation and structural determination of a new toxic constituent named yessotoxin (YTX, 1).[6]

Digestive glands of the scallops Patinopecten yessoensis (84 kg) collected at Mutsu Bay, Japan, in 1986 were homogenized, extracted with acetone, and the extract was partitioned between petroleum ether and CH_3OH-H_2O (4:1). The concentrated methanolic layer was suspended in H_2O and extracted with 1-BuOH. The toxic extract was loaded on an aluminum oxide (Merck, Act II-III) column and eluted successively with CH_2Cl_2-CH_3OH (1:1) and 1% aqueous NH_3-CH_3OH (1:1) to obtain pectenotoxin in the lipid, YTX and okadaic acid derivatives in the aqueous eluate. Further purification was carried out by successive chromatography with the following columns and solvents. 1) ODS-Q3 (Fuji gel) and CH_3OH-H_2O (1:1); 2) Develosil® ODS (Nomura Chem.) and CH_3OH-H_2O (13:7); 3) Toyopearl® HW-40 (Toyo Soda) and CH_3OH-H_2O (1:1); 4) Develosil® ODS and CH_3CN-CH_3OH-H_2O (2:1:2). The eluates were monitored by mouse lethality tests.[1-a] The purified toxin (60 mg) was obtained as an amorphous solid, $[\alpha]_D^{20}$ +3.01° (c 0.45, CH_3OH); UV max (CH_3OH) 230 nm (ε 10600); IR (KBr): 3400, 1240, 1220, 1070 and 820 cm^{-1}; FABMS (negative), m/z 1163 $(M-Na)^-$ and 1061 $(M-SO_3Na+H-Na)^-$. The toxin killed mice at a dose of 100 μg/kg (i.p.) but caused no fluid accumulation in suckling mice intestines even at the fatal dose. ^{13}C NMR revealed 55 carbons consisting of 6 methyls, 18 methylenes, 24 methines and 7 quaternary carbons.

The connectivities of the protons were established as shown in Fig.1-a by detailed analyses of ^1H-^1H COSY, ^1H-^1H-RELAY[7] and ^{13}C-^1H COSY. Except for the methines in ring C, and the methylenes in rings E and G, all vicinal and allylic couplings were observed as off-diagonal cross peaks in the ^1H-^1H COSY. Assignments of the heavily overlapping protons on rings C, E and G were effected by the RELAY. Most of the interproton connectivities across four bonds as well as three bonds were detectable in this pulse sequence. Thus, correlation of vicinal protons having close chemical shifts like those on C9/C10, C12/C13, C17/C18 or C25/C26 was detected by a set of off-diagonal peaks due to another vicinal coupling. The six partial structures in Fig.1-a (C1-C2, C4-C18, C20-C22, C24-C32, C34-C40 and C42-C47) involve 45 carbons but leave ten carbons consisting of the five singlet oxycarbons (δ 78.38, 76.74,

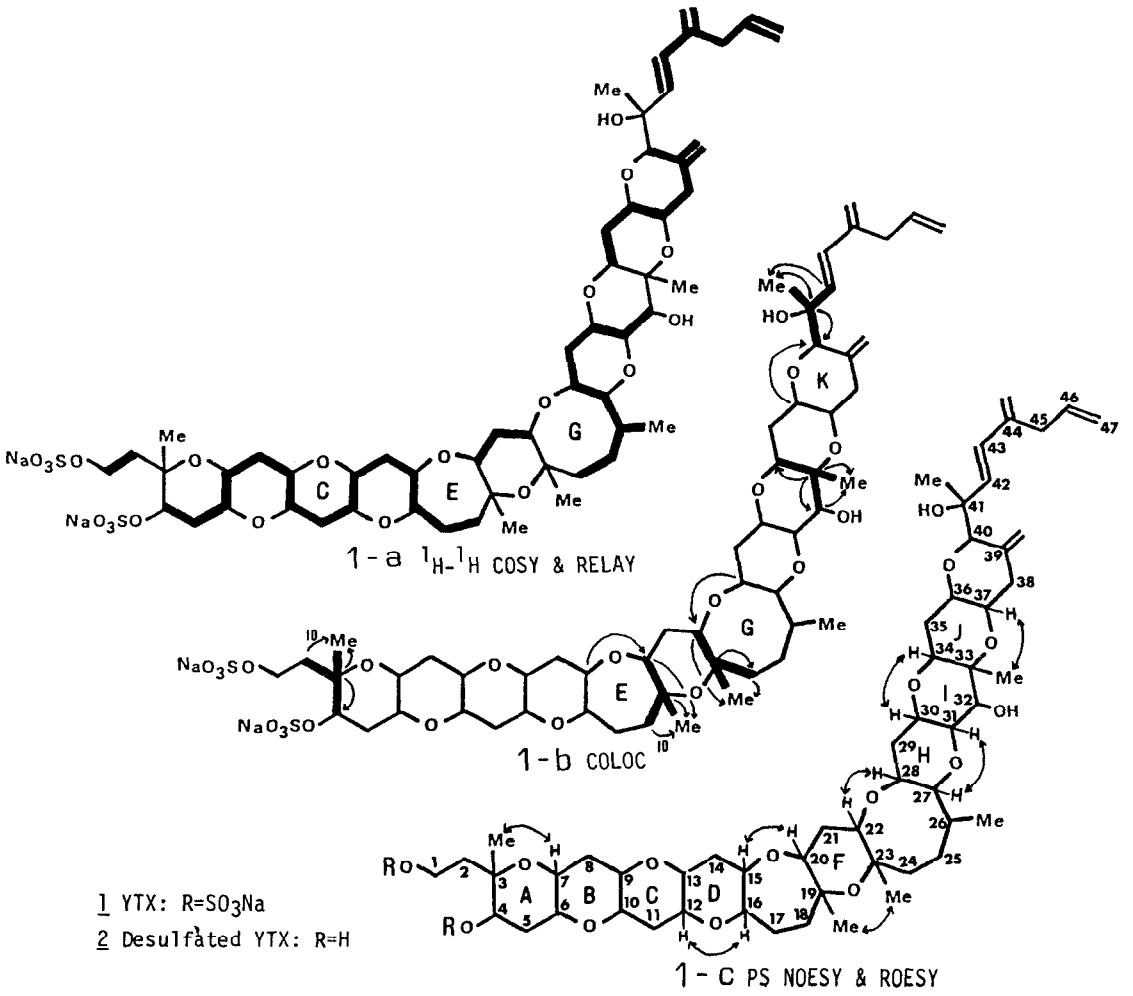

Fig.1. NMR Techniques Used for Structural Elucidation of YTX (1). 1-a) Broad lines indicate the partial structures assigned by ^{1}H-^{1}H COSY and RELAY. 1-b) Arrows denote the correlations between ^{13}C (root) and ^{1}H (point) observed in the COLOC. '10' means the COLOC at 10 Hz and the others at 7 Hz. The other correlations are omitted for clarity. 1-c) The NOE's around ether linkages were measured by phase sensitive (PS) NOESY and ROESY. '$\longleftrightarrow$' indicates the protons giving cross peaks by both of the techniques. All spectra were measured in CD_3OD on an AM 500 (500 MHz, Bruker) spectrometer.

76.98, 76.74 and 78.38) and their adjacent methyls (δ 16.42, 23.90, 20.72, 15.40, 26.20) unassigned. The COLOC sequence[8] (7 and 10 Hz) designed to detect ^{13}C-^{1}H two- and three-bond coupling enabled us to assemble the six partial structures into a single chain. As shown in Fig.1-b, the connectivities among the fragments were clearly established on the basis of ^{13}C-^{1}H couplings between the terminal protons and/or carbons of the fragments, and the

Table 1. ^{1}H and ^{13}C NMR Assignments (δ) of Yessotoxin (1) in CD_3OD

Position	H	C	Position	H	C	Position	H	C	Position	H	C
1	4.24	65.1	13	3.12	78.1	25	1.51	32.8	38	2.47	39.0
	4.24		14	1.47	38.0		1.75			2.75	
2	1.99	40.1		2.34		26	1.74	40.8	39	-	143.1
	2.21		15	3.37	81.1	CH_3-26	1.07	22.4	CH_2=39	4.84	115.7
3	-	76.5	16	3.26	82.2	27	2.81	89.4		5.05	
CH_3-3	1.31	16.4	17	1.84	30.3	28	3.34	84.1	40	3.92	85.1
4	4.26	78.4		1.99		29	1.58	40.0	41	-	78.4
5	1.77	32.8	18	1.83	41.1		2.32		CH_3-41	1.43	26.2
	2.60			1.89		30	3.64	73.2	42	5.86	136.7
6	3.09	78.4	19	-	78.4	31	3.22	79.6	43	6.35	130.6
7	3.36	70.6	CH_3-19	1.29	23.9	32	3.89	73.8	44	-	145.4
8	1.44	36.5	20	3.46	82.4	33	-	76.7	CH_2=44	5.01	116.6
	2.22		21	1.79	33.2	CH_3-33	1.25	15.4		5.09	
9	3.18	78.3		1.97		34	3.80	73.2	45	3.00	37.8
10	3.16	78.3	22	3.53	87.3	35	1.53	31.7		3.00	
11	1.45	36.2	23	-	77.0		2.14		46	5.91	137.5
	2.30		CH_3-23	1.20	20.7	36	4.09	73.1	47	5.10	116.6
12	3.06	77.6	24	1.54	47.0	37	3.43	73.0		5.12	
				1.77							

quaternary carbons and/or the adjacent methyls. Hydroxy-bearing carbons were assigned by isotope-shifts of ^{13}C NMR signals, as observed by the difference of chemical shifts between CD_3OD and CD_3OH solution. Significant shifts of C32, C41 and CH_3-C41 (12.1,12.3 and 8.2 Hz) revealed that YTX has two hydroxyls at C32 and C41. Other signals remained superimposable within 2 Hz between the two solvents.

Positions of ether bonds were determined mainly by COLOC (7Hz) and NOE measurements with use of phase sensitive NOESY[9] and ROESY.[10] Three-bond couplings across ether linkages detected between C15 and H-20, C28 and H-22, and, C36 and H-40 by the COLOC (Fig.1-b) indicated the presence of seven, eight and six membered rings (rings E, G, and K), respectively. The other ether rings except rings B and C were determined by the NOE's observed on the protons or the methyls on the ring juncture carbons, which took 2,6-diaxial configuration in a tetrahydropyran system. Clear cross peaks due to CH_3-C3/H-7, H-12/H-16, CH_3-C19/CH_3-C23, H-27/H-31, H-30/H-34, and CH_3-C33/H-37 allowed us to assign rings A, D, F, H, I and J (Fig.1-c). On rings B and C, overlapping signals of oxymethines prevented NOE analyses. The protons on C8 and C11, however, showed approximately equal chemical shifts (axial/equatorial proton: 1.44/2.22 and 1.45/2.30) to those of the methylenes on rings D, H and J. Moreover, the signal widths of H_2-8 and -11 (37/19 and 34/19 Hz) determined by the cross peaks of the ^{1}H-^{1}H COSY (data points, 2k x 0.5k) agreed well with those for H_2-35 (36/19 Hz; axial H: J=12.5, 11.5, 11.0; equatorial H: J=11.0, 4.5, 4.0 Hz), confirming rings B and C.

The presence of sulfate ester(s) suggested by the IR bands at 1240, 1220 and 820 cm^{-1} was confirmed by elemental analysis for sulfur (5.57%) and by ion chromatography[11] of sulfate ions liberated by solvolysis. Both analyses indicated the presence of two sulfate esters. The position of the esters was determined by comparison between ^{1}H NMR spectra of desulfated YTX (2)[12] and intact toxin. In the spectrum of 2, H_2-1, H_2-2, CH_3-C3, H-4 and H_2-5 were shielded by 0.50/0.50, 0.20/0.29, 0.06, 0.65 and 0.31/0.83 ppm, compared with those of YTX. These changes can be explained by hydrolytic desulfations on C1 and C4.

All these data allowed us to assign the planar structure of YTX to be 1, with a molecular formula of $C_{55}H_{80}O_{21}S_2Na_2$. Table 1 shows the assingments of protons and carbons. The structure partly resembles those of the brevetoxins,[13] isolated from Ptychodiscus brevis, the dinoflagellate which causes massive fish kills along the Florida coast. Yet, YTX is distinct from the brevetoxins in having a longer backbone of 47 carbons (brevetoxins, C42 or C44), a terminal side chain of nine carbons, two sulfate esters, and in lacking carbonyl groups. Thus, its biosynthesis poses an intriguing problem.

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