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ISOLATION AND STRUCTURE DETERMINATION OF A NEW MARINE TOXIN, NEOSURUGATOXIN, FROM THE JAPANESE IVORY SHELL, BABYLONIA JAPONICA.

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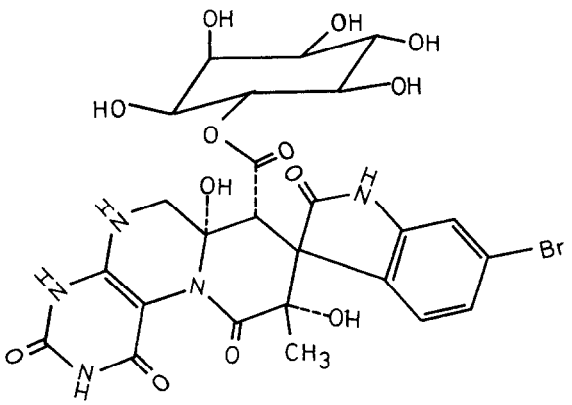
Summary: A new toxin, named neosurugatoxin, was isolated from the toxic japanese Ivory Shell and its structure was determined by X-ray crystallographic analysis.

We previously reported[1] that surugatoxin(1) was the causative agent of intoxication resulting from ingestion of the toxic Japanese Ivory Shell. Subsequently pharmacological studies on the toxin showed that surugatoxin specifically inhibits nicotinic receptors in autonomic ganglia.[2-7] This paper describes the isolation and structure determination of a new toxin isolated from the Japanese Ivory Shell, which has about one hundred times greater antinicotinic activity than does surugatoxin.



surugatoxin(1)

Shellfish containing surugatoxin was used as a source of material, and purification was followed by measuring activity to evoke mydriasis in mice. The mid-gut gland of the shellfish was extracted with 1% AcOH and proteins and fats were removed by the procedures described previously.[1] The extract was concentrated under reduced pressure at 30°C and subjected to gel filtration on a Sephadex G-25 column using water acidified to pH 3.9 with AcOH as eluant. Active fractions were pooled and lyophilized. The material was then applied to a CM-

Sephadex ion exchange column in the same solvent. Two fractions with activity were obtained. Surugatoxin was isolated from the fraction eluted first, and the new toxin was eluted in second fraction. The new toxin was further purified by gel filtration on Bio-Gel P-2 in water acidified to pH 3.9 and ion exchange column chromatography on CM-Sephadex C-25 eluted with a linear gradient of 10mM NH_4OAc, pH 5.0 to 100mM NH_4OAc, pH 5.0 and then gel filtration of Sephadex G-15 in 10mM NH_4OAc, pH 5.0. Final purification was achieved by reverse phase HPLC. The toxic principle, obtained as a colorless powder, was crystallized from water and designated as neosurugatoxin. Approximately 4mg of neosurugatoxin was obtained from 20kg of shellfish. Full details of the isolation procedure will be published elsewhere.

The molecular weight could not be determined by mass spectroscopic techniques(EI-MS, FD-MS).[8] The molecular structure and chemical formula, $C_{30}H_{34}N_5O_{15}Br \cdot H_2O$, were determined by X-ray crystallographic analysis. The space group of the crystal is orthorhombic $P2_12_12_1$ with four molecules in a unit cell. The cell dimensions are a=21.595(10), b=13.964(7), c=10.659(5)Å. A number of 2476 reflexions were measured on a Philips 4-circle diffractometer using CuKα radiation.

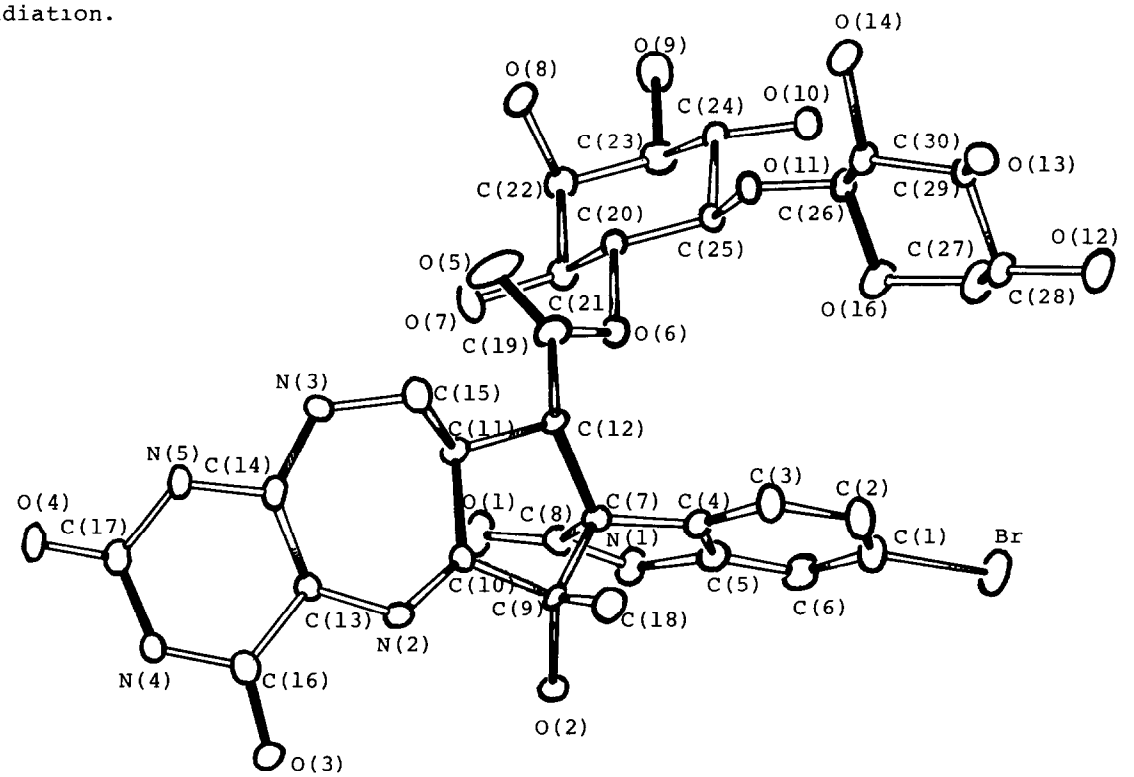


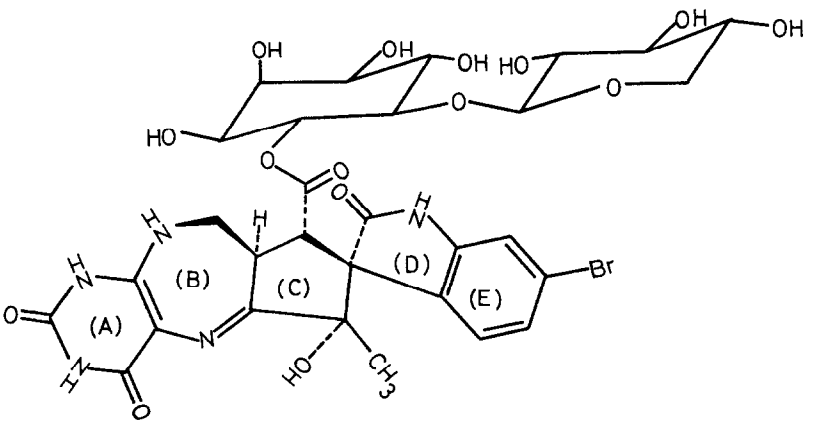
Fig. Molecular structure of neosurugatoxin(2)

The crystal structure was determined by the heavy atom method and refined by the block-diagonal least-squares method. The final R value was 0.051 including hydrogen atoms. The molecular structure and bond lengths are shown in Fig. and Table I. For additional crystallographic details consult reference 9.

Table I. Bond Lengths(Å) of neosurugatoxin.

Br	- C(1)	1.911	C(13)	- C(14)	1.401	C(21)	- C(22)	1.538
C(1)	- C(2)	1.365	C(13)	- C(16)	1.450	C(22)	- O(8)	1.426
C(2)	- C(3)	1.405	C(16)	- O(3)	1.254	C(22)	- C(23)	1.510
C(3)	- C(4)	1.363	C(16)	- N(4)	1.382	C(23)	- O(9)	1.438
C(4)	- C(5)	1.400	N(4)	- C(17)	1.354	C(23)	- C(24)	1.507
C(5)	- C(6)	1.318	C(17)	- O(4)	1.234	C(24)	- O(10)	1.427
C(6)	- C(1)	1.404	C(17)	- N(5)	1.367	C(24)	- C(25)	1.544
C(4)	- C(7)	1.510	N(5)	- C(14)	1.395	C(25)	- O(11)	1.428
C(7)	- C(8)	1.540	C(14)	- N(3)	1.326	C(25)	- C(20)	1.521
C(8)	- O(1)	1.239	N(3)	- C(15)	1.461			
C(8)	- N(1)	1.364	C(15)	- C(11)	1.535	O(11)	- C(26)	1.405
N(1)	- C(5)	1.399	C(11)	- C(12)	1.568	C(26)	- O(16)	1.429
			C(12)	- C(7)	1.553	O(16)	- C(27)	1.455
C(7)	- C(9)	1.575	C(12)	- C(19)	1.515	C(27)	- C(28)	1.527
C(9)	- C(18)	1.542	C(19)	- O(5)	1.206	C(28)	- O(12)	1.441
C(9)	- O(2)	1.420	C(19)	- O(6)	1.328	C(28)	- C(29)	1.504
C(9)	- C(10)	1.504	O(6)	- C(20)	1.478	C(29)	- O(13)	1.438
C(10)	- C(11)	1.409				C(29)	- C(30)	1.529
C(10)	- N(2)	1.275	C(20)	- C(21)	1.522	C(30)	- O(14)	1.431
N(2)	- C(13)	1.404	C(21)	- O(7)	1.441	C(30)	- C(26)	1.508

The molecule is a glycoside with a pentacyclic aglycone, which is constructed from two planar parts, 6-bromo-2-oxindole(D,E ring) and a heterocyclic system(B,C ring) including a dioxopyrimidine ring (A ring). The saccaride is O-β-D-xylopyranosyl(1→5)myo-inositol. The planar configuration of C(13) strongly suggests the conjugated nature of the A-B juncture. Protonation of N(3) is suggested by the difference electron density map, which in conjunction with the bond lengths of C(14)-N(3) and C(13)-C(14), supports the present structure.



neosurugatoxin(2)

Several structural features common to both neosurugatoxin and surugatoxin are noted: both have an oxindole, a dioxopyrimidine, myo-inositol, and other functional groups. The most striking difference is that neosurugatoxin has a ring system with a six-seven heterocyclic system (A,B ring) instead of the six-six system in surugatoxin.

Neosurugatoxin is extremely unstable in alkaline medium and fairly heat-labile. Neosurugatoxin at a concentration of 1×10^{-9}g/ml inhibited the contractile response of isolated guinea pig ileum to 3×10^{-5}g/ml of nicotine and evoked mydriasis in mice at a minimum dose of 0.003μg.

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References and Notes

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8) The physical data of neosurugatoxin are as follows: colorless prisms, mp 331-335°C dec; UV:$\lambda_{max}^{H_2O}$ nm(logε) 220(4.65), 282(4.18), 310(sh 3.96), 325(sh 3.80); IR:ν_{max}^{KBr} 3300, 1700, 1600, 1380, 1030cm^{-1}; ^{1}H-NMR(DMSO-d_6, 360MHz):δ 1.21 (3H,s), 2.90-2.98(2H,m), 2.99-3.08(2H,m), 3.53-3.62(2H,m), 3.69(1H,s), 3.96 (1H,d,J=12Hz), 4.18(1H,d,J=7.2Hz), 4.36(1H,d,J=6.8Hz), 4.62(1H,d,J=6.0Hz), 4.77(1H,d,J=3.2Hz), 4.83(1H,t,J=9.5Hz), 4.95(1H,d,J=8.0Hz), 4.96(1H,s), 5.14 (1H,br s), 5.27(1H,br s), 5.60(1H,br s), 6.66(1H,br s), 6.88(1H,s), 7.10 (2H,s), 10.12(1H,br s), 10.18(1H,s), 10.53(1H,br s);
(D_2O, 360MHz):δ 1.24(3H,s), 2.98(1H,t,J=11Hz), 3.06(1H,t,J=9.1Hz), 3.11 (1H,t,J=9.1Hz), 3.20-3.28(3H,m), 3.34(1H,t,J=5.4Hz), 3.38(2H,t,J=9.0Hz), 3.52(1H,dd,J=11, 4.5Hz), 3.59(1H,t,J=10Hz), 3.65(1H,td,J=9.0, 1.8Hz), 3.77-3.86(2H,m), 4.32(1H,d,J=7.2Hz), 7.03(1H,d,J=1.8Hz), 7.05(1H,d,J=7.9Hz), 7,12 (1H,dd,J=7.9, 1.8Hz).

9) Crystallographic coordinates have been deposited with the Cambrige Crystallographic Data Centre.

(Structure factors have been deposited with the British Library at Boston Spa, Wetherby, Yorkshire. Supplementary Publication No: SUP.45,056)

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