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Isolation of a New Toxin, Prosurugatoxin, from the Toxic Japanese Ivory Shell, *Babylonia japonica*

TAKUO KOSUGE,* KUNIRO TSUJI, KOICHI HIRAI, TOSHINORI FUKUYAMA, HARUO NUKAYA and HITOSHI ISHIDA

Shizuoka College of Pharmacy, 2-2-1 Oshika, Shizuoka 422, Japan

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A new toxin, named prosurugatoxin because it formed surugatoxin on decomposition, was isolated from the toxicated Japanese ivory shell (*Babylonia japonica*). Prosurugatoxin evoked mydriasis in mice at a minimum effective intraperitoneal dose of 15 ng/g body weight, and inhibited the contractile response of isolated guinea pig ileum induced by 3×10^{-5} g/ml of nicotine at a concentration of 5×10^{-9} g/ml. In this mollusc the prosurugatoxin content was about 5 times that of neosurugatoxin. Prosurugatoxin was deduced on the basis of physical and chemical data to be des-xylopyranosyl-neosurugatoxin.

Keywords——prosurugatoxin; marine toxin; mydriasis-evoking; anti-nicotinic activity; brominated compound; Japanese ivory shell; *Babylonia japonica*; surugatoxin; des-xylopyranosyl-neosurugatoxin

Previously we reported the isolation of neosurugatoxin,[1] one of the toxins causing intoxication on ingestion of the toxicated Japanese ivory shell, *Babylonia japonica*, and the determination of its chemical structure by X-ray crystallography.[2] Pharmacological studies suggested that neosurugatoxin has selective affinity for ganglionic nicotine receptors, its affinity constant for these receptors being more than three orders of magnitude greater than that of hexamethonium:[3] neosurugatoxin could therefore be useful in studies on nicotinic cholinergic receptors.

As mentioned before,[1] neosurugatoxin was responsible for half the total toxicity of the mollusc. Thus, we continued our studies in an attempt to identify the remaining toxic components. We would like now to report the isolation and structure study of a new marine toxin, prosurugatoxin, which is responsible for the remaining toxicity.

All the purification procedures were carried out in a cold room and each step of purification was followed by monitoring the ultraviolet (UV) absorbance at 280 nm and the mydriasis-evoking activity. A 1% acetic acid extract of the mid-gut gland of *B. japonica* was prepared as described previously[1] and applied to a Sephadex G-25 column. The activity emerged in a yellow-colored fraction, which was collected and applied to a CM-Sephadex C-25 column. Much nontoxic material was removed on this column.[4] Then, all the fractions showing toxicity were combined and applied to a Bio-Gel P-2 column. As shown in Fig. 1, two toxic components were clearly separated on this column. High-performance liquid chromatography (HPLC) and tests of toxic activity showed that the toxic activity in the first peak was due to neosurugatoxin. On HPLC, 5 mg of neosurugatoxin was isolated from the first peak in fractions No. 171—180 (28 mg), while fractions No. 281—290 (71 mg), which contained the second peak of activity, yielded 39 mg of a highly hygroscopic amorphous powder. This material evoked mydriasis in all mice tested at a dose of 0.7 μg/mouse.

Like neosurugatoxin, this newly isolated toxin was very unstable in an alkaline medium, and its was also fairly unstable under neutral and weakly acidic conditions (Table I). Figure 2

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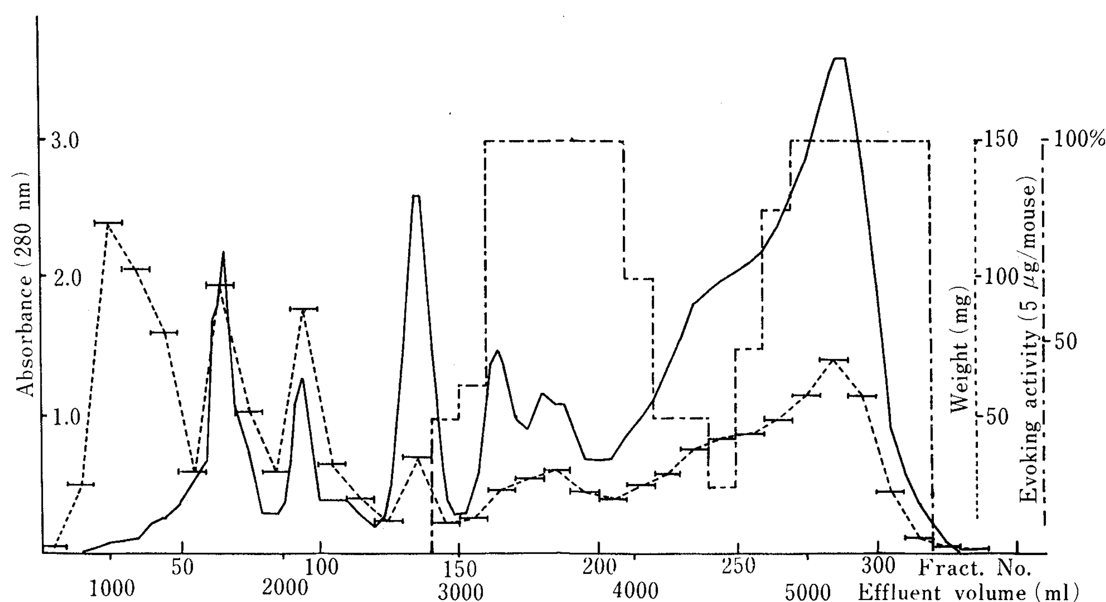

Fig. 1. Bio-Gel P-2 Chromatography of Active Fractions Obtained from a CM-Sephadex C-25 Column

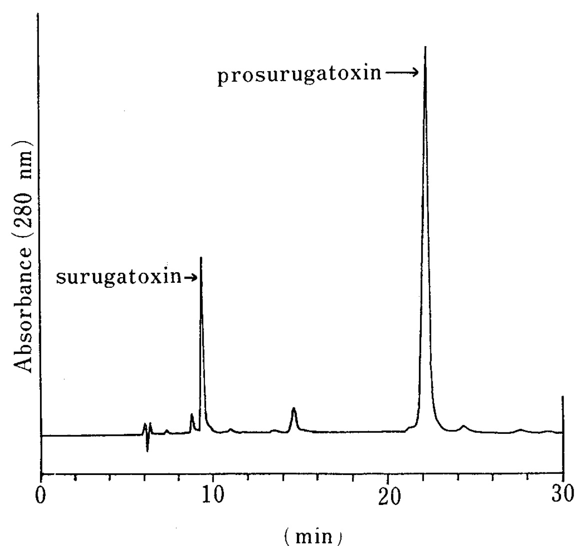

Fig. 2. HPLC of the Degradation Products Formed from Prosurugatoxin

TABLE I. Stability of Prosurugatoxin (Recovery %)

	0 °C		40 °C	
	24 h	48 h	24 h	48 h
H_2O	96.3	93.5	80.4	60.0
0.01 N HCl	83.6	72.2	57.3	42.8
0.1% AcOH	88.8	74.8	80.0	61.0
5.0% AcOH	81.5	64.7	24.4	—

Prosurugatoxin (50 ng) was dissolved in 0.5 ml of medium. Remaining prosurugatoxin was determined by HPLC.

shows the result of HPLC of the degradation products formed from the new toxin in a solution of 5% AcOH. The chief degradation product was identified as surugatoxin by direct comparison with an authentic sample.[5] Thus, the new compound was named prosurugatoxin. Prosurugatoxin was soluble in water and dimethylsulfoxide (DMSO). In DMSO it was unstable, forming one main bioactive product, an isomerized prosurugatoxin, that was converted to prosurugatoxin in very weak alkaline media.

The mydriasis-evoking and anti-nicotinic activities of prosurugatoxin and the isomerized product in DMSO are shown in Figs. 3 and 4. Prosurugatoxin evoked mydriasis in mice at a minimum effective intraperitoneal dose of 15 ng/g body weight, and at a concentration of 5×10^{-9} g/ml it inhibited the contractile response of isolated guinea pig ileum to nicotine at 3×10^{-5} g/ml. These activities are about one-fifth of those of neosurugatoxin, but the content of prosurugatoxin in the mollusc was 5 to 6 times that of neosurugatoxin. The exact

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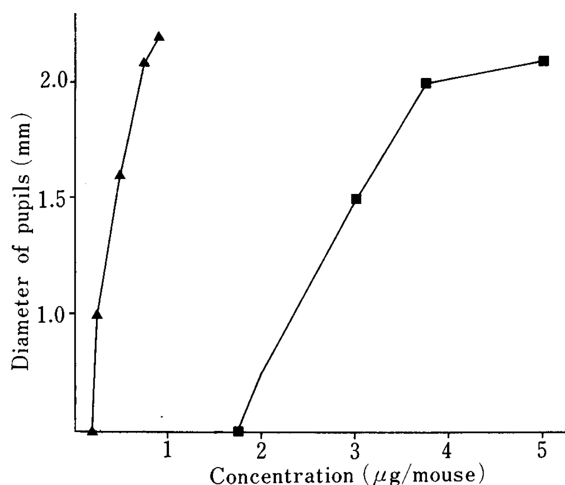

Fig. 3. Mydriatic Effects of Prosurugatoxin (▲—▲) and Isomerized Prosurugatoxin in DMSO (■—■)

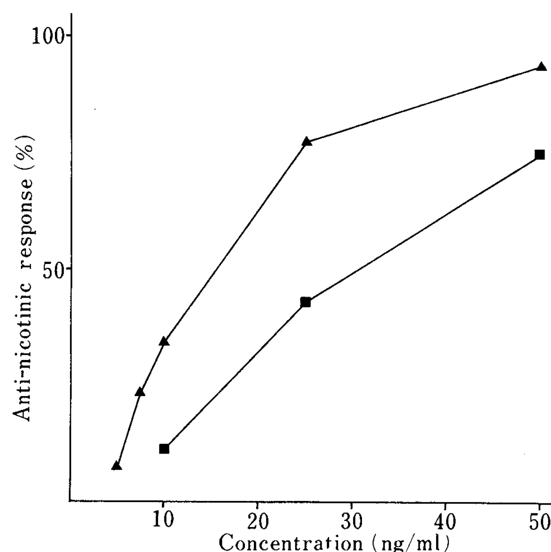

Fig. 4. Inhibitory Effects of Prosurugatoxin (▲—▲) and Isomerized Prosurugatoxin in DMSO (■—■) on the Contractile Response of Isolated Guinea Pig Ileum to Nicotine

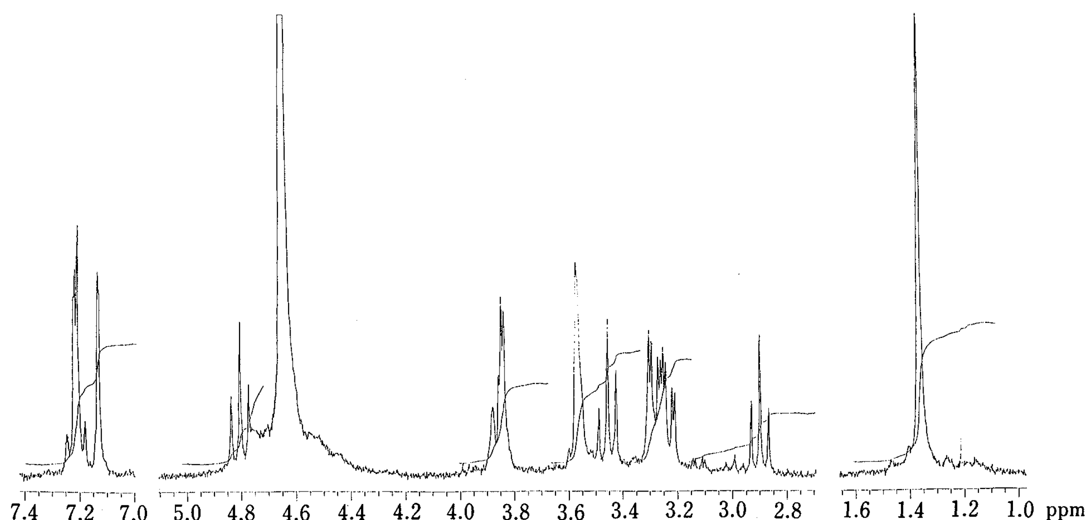

Fig. 5. ^{1}H-NMR Spectrum of Prosurugatoxin at 300 MHz in D_2O

mydriasis-evoking and anti-nicotinic activities of prosurugatoxin could not be determined because of its instability in alkaline media such as the body fluid of mice and Tyrode's solution.

Prosurugatoxin has not yet been crystallized. The secondary ionization mass spectrum (SIMS) of prosurugatoxin showed two molecular ion peaks at m/z 652 and 654 with the same relative intensities, indicating the presence of a bromine atom in the molecule. The molecular weight of prosurugatoxin was 132 daltons less than that of neosurugatoxin. The proton nuclear magnetic resonance (^{1}H-NMR) spectrum and two-dimensional ^{1}H shift correlated (2D-COSY) NMR spectrum of prosurugatoxin are shown in Figs. 5 and 6. At higher field in the ^{1}H-NMR spectrum of prosurugatoxin in d_6-DMSO, AMX type signals at δ 7.20 (1H, d), 7.11 (1H, dd) and 6.92 (1H, d) were ascribable to C_5-H, C_4-H and C_--H of the benzene ring of a 6-bromooxindole moiety by comparison with those of neosurugatoxin and surugatoxin. In the H^1- and 2D-COSY-NMR spectra in D_2O, six signals at δ 2.90 (1H, t), 3.23 (1H, dd), 3.26

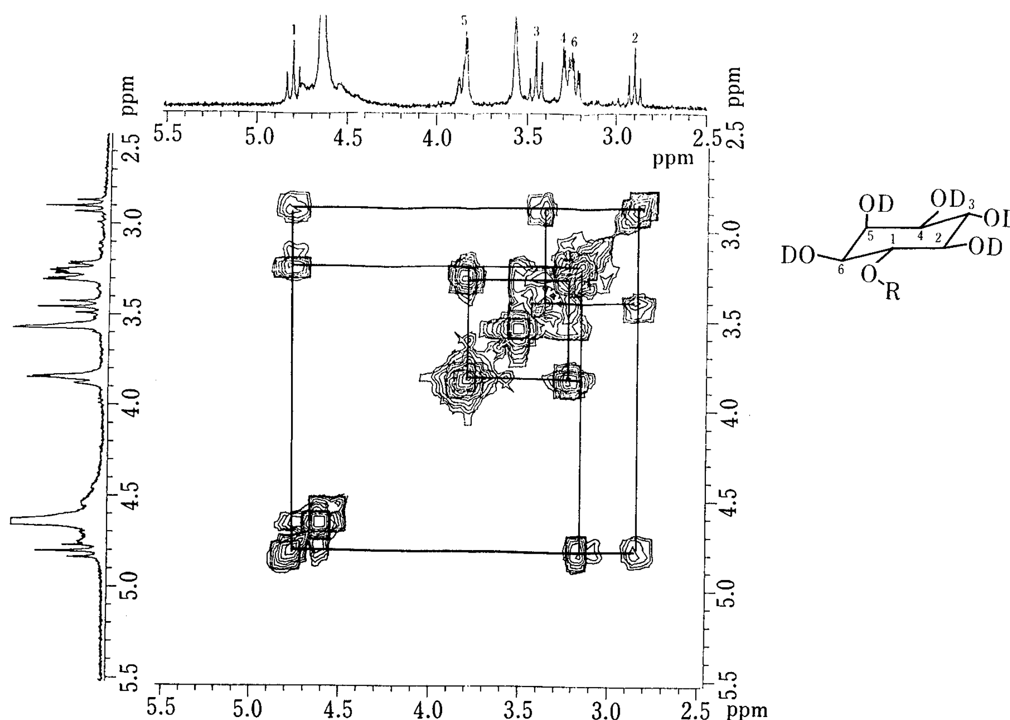

Fig. 6. Contour Plot of the COSY Map of Prosurugatoxin (300 MHz, 2 mg in 0.5 ml of D_2O)

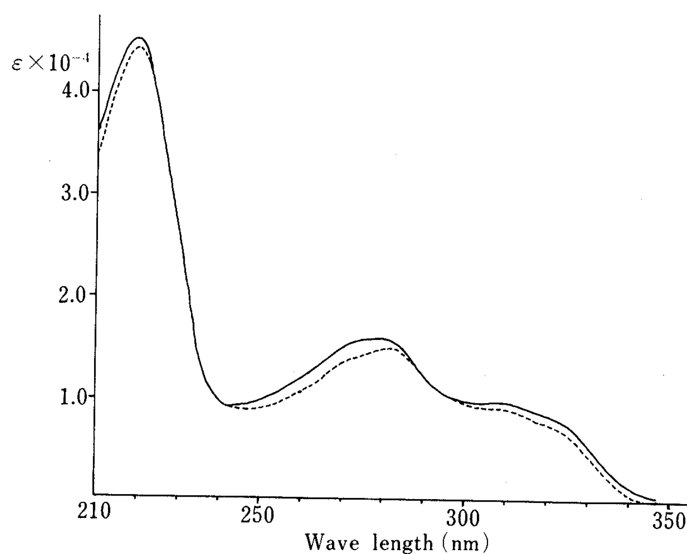

Fig. 7. UV Spectra of Prosurugatoxin (——) and Neosurugatoxin (------) in H_2O

(1H, dd), 3.46 (1H, t), 3.80—3.90 (1H, m) and 4.80 (1H, t) were assignable to C_2-H, C_6-H, C_4-H, C_3-H, C_5-H and C_1-H of myoinisitol, and the signal at δ 1.35 (s, 3H) to the protons of a methyl group. The ^{1}H-NMR spectrum indicated that no xylopyranose ring is present in prosurugatoxin. The UV spectrum of prosurugatoxin (Fig. 7) is almost identical with that of neosurugatoxin. Further, prosurugatoxin has the same biological activities as neosurugatoxin. These results suggest that prosurugatoxin has the same chromophore as neosurugatoxin.

The above analyses of spectral data of prosurugatoxin and the finding that prosurugatoxin was converted into surugatoxin in the presence of acetic acid suggested that

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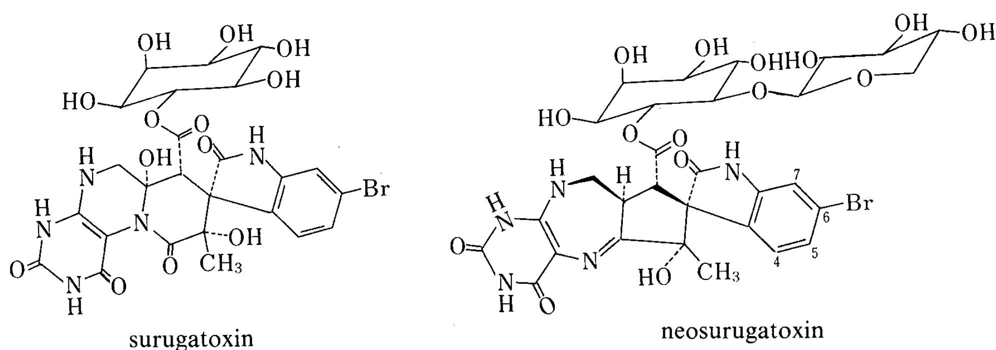

Fig. 8. Structures of Surugatoxin and Neosurugatoxin

prosurugatoxin is a des-xylopyranosyl-neosurugatoxin. Further studies on the chemical structure and stereochemistry of this compound are in progress.

Our studies showed that neosurugatoxin and prosurugatoxin are responsible for all the toxicity of *B. japonica*. Fortunately, this kind of intoxication with mydriasis has so far been caused only by shellfish from the Ganyudo area of Suruga Bay, Shizuoka Prefecture, but similar intoxication may occur elsewhere, and so the mechanism of toxication should be clarified. The facts that *B. japonica* becomes toxic when cultivated in the Ganyudo area and that the toxicity of shellfish grown in this area disappears when they are cultivated in another area[6] suggest that the toxicity originates from material in the Ganyudo area. The bromine content of toxicated shellfish was found to be far greater than that of normal shellfish. We are now studying the mechanism of development of toxicity in relation to microorganisms in the Ganyudo area with ability to concentrate bromine from the environment.

Experimental

Material——Toxicated specimens of *B. japonica* collected in 1973 in the Ganyudo area of Suruga Bay and stored at $-20\,^{\circ}C$ were used as a source of material.

Isolation of Prosurugatoxin——The mid-gut gland (1.0 kg) was homogenized in 2 l of 1% AcOH and allowed to stand for 9 h at 2 °C. The mixture was then treated as described previously.[1] Chromatography of this extract on a column of Sephadex G-25 (4.7 × 94 cm) with 0.5% AcOH as the eluent gave a yellow material that evoked mydriasis at a dose of 20 μg/mouse in all mice tested. A total of 6.0 g of active material was obtained from four parallel columns of Sephadex. A portion (3 g) of the active material was dissolved in 65 ml of 0.5% AcOH and a small amount of insoluble material was removed by centrifugation. The supernatant was subjected to chromatography on a CM-Sephadex C-25 ion exchange column (3.5 × 96 cm) with 0.5% AcOH as the eluent. The fractions with activity were pooled and lyophilized. In all, 1567 mg of active material, evoking mydriasis at a dose of 10 μg/mouse in all mice tested, was obtained from two parallel columns. This active material was applied to a Bio-Gel P-2 column (4.6 × 91 cm) and developed with 0.1% AcOH (Fig. 1). Fractions of 16 ml were collected and their UV absorbance at 280 nm was measured. Purification of the lyophilized eluate in fractions No. 171 to 180 by HPLC yielded 5.0 mg of neosurugatoxin, while 39 mg of prosurugatoxin was obtained by purification of the eluate in fractions No. 281 to 290 (71 mg) by HPLC. The conditions for HPLC were as follows: column, YMC-Pack A-324 (ODS) 10 × 300 mm; flow rate, 3 ml/min; mobile phase, MeOH : H_2O = 25 : 75; detector, UV (280 nm).

Physical Data for the Chief Degradation Product Formed from Prosurugatoxin in AcOH——The ^{1}H-NMR spectrum was recorded with a Nicolet NT 300 spectrometer. ^{1}H-NMR (in d_6-DMSO): 10.70 (2H, br), 10.21 (1H, br s), 7.10 (1H, d, J=8.8 Hz), 7.04 (1H, dd, J=8.8, 1.5 Hz), 6.86 (1H, d, J=1.5 Hz), 6.33 (1H, br), 5.74 (1H, br s), 5.11 (1H, s), 4.84 (1H, d, J=4.3 Hz), 4.75 (1H, t, J=9.2 Hz), 4.71 (1H, d, J=3.5 Hz), 4.45 (1H, d, J=6.1 Hz), 4.32 (1H, d, J=5.3 Hz), 4.15 (1H, m), 4.12 (1H, d, J=7.5 Hz) and 1.32 (3H, s). Other signals of the product and the signals of the solvent overlapped. UV $\lambda_{max}^{H_2O}$ nm (log ε): 276 (4.19) and 217 (4.57).

Physical Data for Prosurugatoxin——The ^{1}H-NMR spectrum was recorded with a Nicolet NT 300 spectrometer. ^{1}H-NMR (in D_2O): 7.18—7.26 (2H, m), 7.13 (1H, d, J=1.8 Hz), 4.80 (1H, t, J=11.5 Hz), 3.80—3.90 (2H, m), 3.57 (2H, br s), 3.46 (1H, t, J=11.5 Hz), 3.26 (1H, dd, J=3.5, 11.5 Hz), 3.23 (1H, dd, J=3.5, 11.5 Hz), 3.20—3.32 (1H, m), 2.90 (1H, t, J=11.5 Hz), 1.35 (3H, s). ^{1}H-NMR (in d_6-DMSO): 10.45 (1H, br), 10.25 (1H, br), 7.20 (1H, d, J=8.8 Hz), 7.11 (1H, dd, J=8.8, 1.5 Hz), 6.92 (1H, d, J=1.5 Hz), 5.08 (1H, br), 4.45 (1H, br), 4.17 (1H, d, J=7.2 Hz), 3.92 (1H, br), 3.69 (1H, br s), 1.26 (3H, s). Other signals of prosurugatoxin and the signals of the solvent overlapped. SIMS was

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recorded with a Hitachi M-80 mass spectrometer under the following conditions; primary ion, $[Xe]^+$; acceleration voltage of primary and secondary ions, 9 and 3 kV, respectively: glycerol matrix. SIMS m/z: 652, 654 $(M+H)^+$. UV $\lambda_{max}^{H_2O}$ nm ($\log \varepsilon$): 309 (sh) (3.99), 325 (sh) (3.87), 280 (4.20) and 219 (4.65).

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References

1) T. Kosuge, K. Tsuji and K. Hirai, *Chem. Pharm. Bull.*, **30**, 3255 (1982).
2) T. Kosuge, K. Tsuji, K. Hirai, K. Yamaguchi, T. Okamoto and Y. Iitaka, *Tetrahedron Lett.*, **1981**, 3417.
3) E. Hayashi, M. Isogai, Y. Kagawa, N. Takayanagi and S. Yamada, *J. Neurochem.*, **42**, 1491 (1984).
4) T. Kosuge, H. Zenda, A. Ochiai, N. Masaki, M. Noguchi, S. Kimura and H. Narita, *Tetrahedron Lett.*, **1972**, 2545.
5) K. Okada, S. Inoue, H. Tanino, K. Hashizume, H. Kakoi and M. Hotta, The 1984 International Chemical Congress of Pacific Basin Societies, Hawaii, January, 1985.
6) S. Kimura, S. Sugiyama and H. Narita, *Annu. Rep. Shizuoka Pub. Health*, **16**, 163 (1972).

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