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SYNTHESIS OF SPIDER TOXIN (JSTX-3) AND ITS ANALOGS

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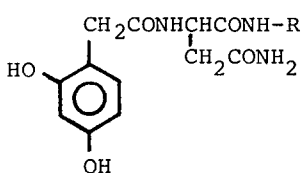
Abstract: One of the active principles isolated from spider venom, JSTX-3, and its analogs of the polyamine part were synthesized.

A toxin derived from a spider venom has been established to block specifically the glutamate receptor.[1,2] Though a specific blocker of the glutamate transmitter is considered to be an essential tool in the area of neurobiological research, very few blockers with a high degree of specificity has been reported. Recently, the active principles (JSTX's) were isolated from Nephila clavata (Joro spider) and their structures were proposed.[3] The spider toxin is composed of several structurally similar toxic principles, and three isolated principles (named as JSTX-2, 3 and 4) showed similar blocking actions on the lobster neuromuscular synapse. The active principles (JSTX-2, 3 and 4) are deduced to possess a 2,4-dihydroxyphenylacetyl-asparaginylcadaverino-moiety as a common structure, which suggests that this moiety would be the minimum requirement for the biological activity. Therefore, we scheduled to synthesize compound 1 as one of the proposed structures, and compound 3 as the common part for JSTX's. To investigate the structure-activity relationships and the role of the polyamine part, we also synthesized compound 2 (one methylene unit deleted from 1) and 4 (possessing a polyamine part longer than that of 3, shorter than that of 1 or 2).



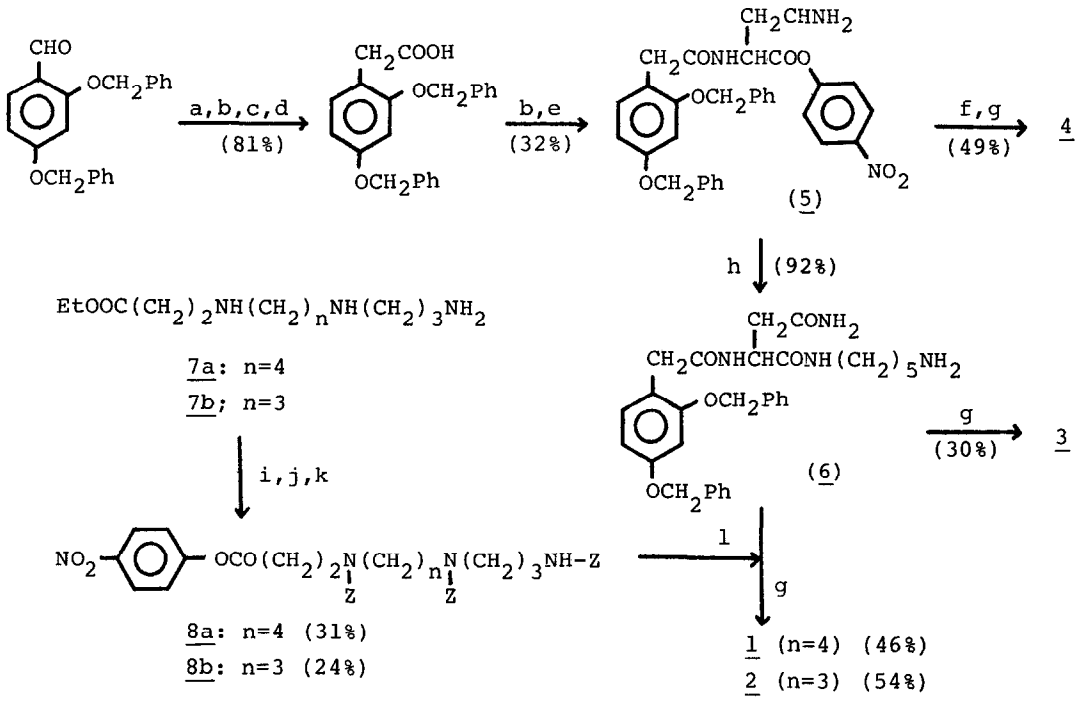
1: R=$(CH_2)_5NHCO(CH_2)_2NH(CH_2)_4NH(CH_2)_3NH_2$

2: R=$(CH_2)_5NHCO(CH_2)_2NH(CH_2)_3NH(CH_2)_3NH_2$

3: R=$(CH_2)_5NH_2$

4: R=$(CH_2)_3NH(CH_2)_4NH(CH_2)_3NH_2$

The brief synthetic scheme was shown below. Resorcylaldehyde was protected with benzylchloride (y: 75%, mp. 89-90°C) and then reduced with $NaBH_4$ in MeOH at room temperature to give 2,4-dihydroxybenzyl alcohol (y: 99%, mp. 84-85°C), which was treated with $SOCl_2$ in dry benzene under reflux for 20 min. Evaporation of the reaction mixture gave crude 2,4-dibenzyloxy-benzyl chloride, which was reacted with NaCN without purification to give 2,4-



a: $NaBH_4$, MeOH; b: $SOCl_2$, benzene, reflux; c: NaCN, DMSO; d: KOH, EtOH, reflux; e: L-Asn-ONp, TEA, DMF; f: spermine, DMF; g: 10% Pd-C, H_2, AcOH; h: cadaverine, DMF; i: Z-Cl, $NaHCO_3$, ether-H_2O; j: KOH, EtOH, r.t.; k: p-nitrophenol, DCC, DMF; l: 1-hydroxybenzotriazole, DMF

dibenzyloxyphenylacetonitrile (y: 85%, pale yellow oil). The product was hydrolyzed with NaOH in EtOH containing 30%(v/v) of H_2O (reflux, overnight) to give 2,4-dibenzyloxyphenylacetic acid (y: 95%, mp. 139°C). The carboxylic acid was converted to acid chloride with $SOCl_2$ in dry benzene at room temperature for 20 min, and then coupled with L-asparagine-p-nitrophenol ester (L-Asn-ONp, derived from L-Z(OMe)-Asn-ONp by treatment with CF_3COOH in the presence of p-cresol) in dry DMF containing triethylamine (TEA) to give compound 5. The compound 5 was purified by silica gel column chromatography (eluted with AcOEt), and the structure was confirmed by ^{1}H-NMR. The yield was 21% from resorcylaldehyde.

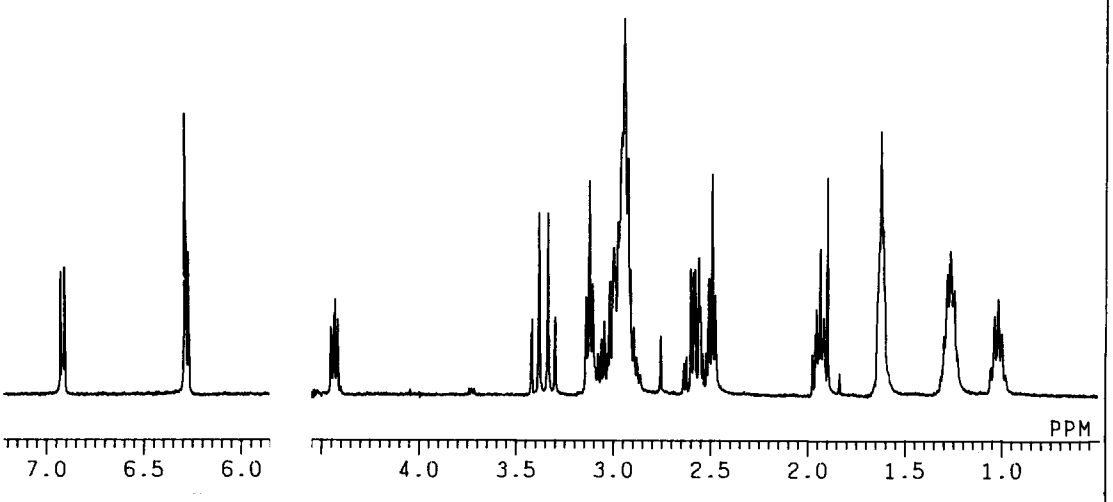
Reaction of 5 with spermine in dry DMF at room temperature for 10 sec. and deprotection by catalytic hydrogenation (10% Pd-C, H_2 gas in AcOH for 3 hr) gave compound 4. 4 was purified by HPLC (ODS, 3% CH_3CN in 0.02% HClaq.) and obtained as HCl salt in the yield of 49%. The structure of 4 was confirmed by 400 MHz ^{1}H-NMR in D_2O.[4] Reaction of 5 with cadaverine in dry DMF at room temperature for 10 sec. gave compound 6 in the yield of 92%. Deprotection of 6 by catalytic hydrogenation gave compound 3, which was purified with HPLC (ODS, 7% CH_3CN in 0.02% HClaq.) and obtained as HCl salt in the yield of 30%. The data of 400 MHz ^{1}H-NMR was shown in the note.[5]

The polyamine parts of the compounds 1 and 2 (compounds 7a and 7b) were prepared as described by Yamamoto and Maruoka.[6] The amino groups of the compound 7 were protected using carbobenzyloxy chloride (Z-Cl) by Schotten-Baummann method (y: 30-40%), and then the ethoxy group was hydrolyzed (quantitative yields). Resulting carboxylic acid was condensed with p-nitrophenol in dry DMF in the presence of DCC to give activated ester 8 (y: 60-70%).

Coupling 8a and 8b with 6 in dry DMF in the presence of catalytic amounts of 1-hydroxybenzotriazole at room temperature for 1-2 hr, and deprotection by catalytic hydrogenation (10% Pd-C, H_2 gas in CH_3COOH at room temperature for 2 hr) gave compounds 1 and 2, respectively. Compounds 1 and 2 were purified by HPLC (ODS, 5% CH_3CN in 0.02% HClaq.) and obtained as HCl salts. The yields were 46% for 1 and 53% for 2. The structures of 1 and 2 were confirmed by 400 MHz ^{1}H-NMR in D_2O.[7,8] The 400 MHz ^{1}H-NMR spectra of 1 was shown in the figure below (data were described in the note[7]).

Comparison of ^{1}H-NMR spectra, the retention times on HPLC, UV spectra, biological activties, and diastereomeric derivatization of 1 with those of natural JSTX-3 revealed that the structure of these two compounds is strictly identical including the optical center.

All of the synthesized compounds showed the suppressing effect on excitatory postsynaptic potentials. The potencies decreased in the order of $1 \geq 2 > 4 > 3$ with the ID_{50} in the range of 10^{-8}-10^{-10} mg/ml (details will be published elsewhere). The results suggest that 2,4-dihydroxyphenylacetyl-asparaginylcadaverino-moiety is an essential structure for the glutamate



400 MHz ^{1}H-NMR spectrum of compound 1 in D_2O (10 mg/ml). ^{1}H-NMR was measured using JEOL JMN GX-400 under autoreference conditions (the chemical shift of DHO was adjusted to 4.65 ppm). Data were shown in the note.[7]

receptor inhibiting effect, and the long polyamine moiety causes increasing affinity toward the glutamate receptor of these compounds.

In conclusion, one of the active principles derived from spider toxin, JSTX-3 and its analogs of polyamine part were synthesized (compounds 1-4). These compounds possess glutamate receptor inhibiting activity. They may serve as powerful tools in the study of elucidating the function and the structure of the glutamate receptor as well as in the development of clinically useful agents.

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2) T.Abe, N.Kawai and A.Miwa, J.Physiol., 339, 243 (1983).
3) Y.Aramaki, T.Yasuhara, T.Higashijima, M.Yoshioka, A.Miwa, N.Kawai and T.Nakajima, Proc.Japan Acad., 62(B), 359 (1986).
4) 400 MHz ^{1}H-NMR was measured using JEOL JMN GX-400 in D_2O under auto-reference conditions (DHO = 4.65 ppm). ^{1}H-NMR of compound 4: ppm: 1.56 (quin., 4H), 1.69(quin., 2H), 1.92(quin., 2H), 2.59(dd, 7Hz, 17Hz, 1H), 2.62 (dd, 7Hz, 17Hz, 1H), 2.76(t, 2H), 2.77(t, 2H), 2.92(t, 2H), 2.95(t, 2H), 2.98(t, 2H), 3.11(ddt, 1H), 3.18(ddt, 1H), 3.35(d, 15Hz, 1H), 3.41(d, 15Hz, 1H), 4.42(t, 7Hz, 1H), 6.30(s-like, 2H), 6.92(m, 1H).
5) 400 MHz ^{1}H-NMR of compound 3: ppm: 1.09(quin., 2H), 1.31(quin., 2H), 1.42 (quin., 2H), 2.55(dd, 6.4Hz, 13Hz, 1H), 2.60(dd, 6.4Hz, 13Hz, 1H), 2.72 (dt-like, 2H), 3.00(ddt, 1H), 3.06(ddt, 1H), 3.32(d, 15Hz, 1H), 3.39(d, 15Hz, 1H), 4.43(dd, 1H), 6.30(s-like, 2H), 6.94(m, 1H).
6) H.Yamamoto and K.Maruoka, J.Am.Chem.Soc., 103, 6133 (1981).
7) 400 MHz ^{1}H-NMR of compound 1: ppm: 1.02(quin., 2H), 1.27(quin., 4H), 1.63 (br, 4H), 1.94(quin., 2H), 2.49(t, 7Hz, 1H), 2.57(dd, 4Hz, 12Hz, 1H), 2.60 (dd, 7Hz, 12Hz, 1H), 2.92(ddt, 1H), 2.93(t, 8H), 2.98(t-like, 2H), 3.04 (ddt, 1H), 3.12(t, 7Hz, 1H), 3.32(d, 15Hz, 1H), 3.41(d, 15Hz, 1H), 4.44 (dd, 4Hz, 7Hz, 1H), 6.30(s-like, 2H), 6.92(m, 1H).
8) 400 MHz ^{1}H-NMR of compound 2: ppm: 1.01(quin., 2H), 1.27(quin., 2H), 1.28 (quin., 2H), 1.94(quin., 2H), 1.96(quin., 2H), 2.50(t, 7Hz, 1H), 2.58(dd, 6Hz, 18Hz, 1H), 2.61(dd, 5Hz, 18Hz, 1H), 2.92(ddt, 1H), 2.93(t, 4H), 3.00 (t-like, 6H), 3.07(ddt, 1H), 3.14(t, 7Hz, 1H), 3.31(d, 15Hz, 1H), 3.40(d, 15Hz, 1H), 4.44(dd, 5Hz, 6Hz, 1H), 6.30(s-like, 2H), 6.92(m, 1H).

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