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TOTAL SYNTHESIS OF CLAVAMINE, INSECTICIDALLY ACTIVE COMPOUND ISOLATED FROM VENOM OF JORO SPIDER (*NEPHILA CLAVATA*)

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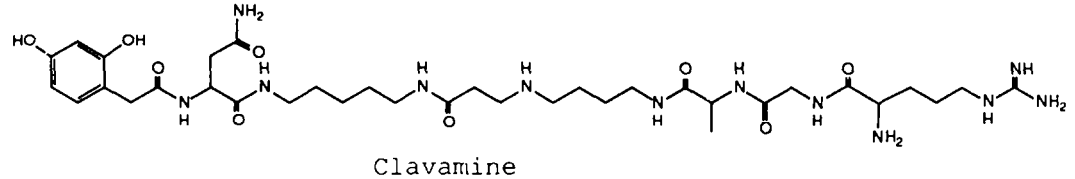
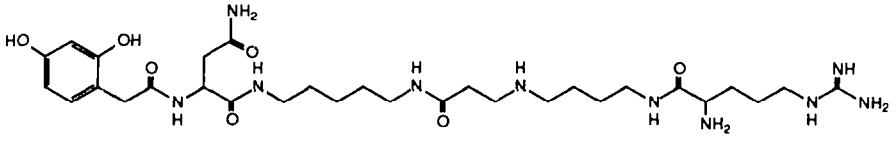
Clavamine, a new insecticidal toxin isolated from the venom of Joro spider (*Nephila clavata*) was totally synthesized in order to confirm the proposed structure. In clavamine, a fragment of glycylalanine is inserted in the structure of NSTX-3, a New Guinean spider toxin. The synthesis determined the chemical structure of clavamine unambiguously.

From the venom of Joro spider (*Nephila clavata*), a novel toxin called clavamine with insecticidal activity against wrigglers, larvae of the mosquito (*Culex pipens molestus*) was isolated by Yoshioka *et al.* The toxin did not show an irreversible suppression of excitatory postsynaptic potential (EPSP), which is characteristic to spider toxins, NSTX and JSTX[1], at the lobster neuromuscular junction[2]. The finding of this new toxin added an



Clavamine



NSTX-3

Fig.1 The structures of clavamine and NSTX-3.

example to biologically interesting neurotoxin group found either in spider or wasp, e.g. NSTX[2], JSTX[2], argiopin[3], argiotoxin[4], philantotoxin[5], etc.

The structure of clavamine was deduced as shown in Fig.1 by NMR, FAB-MS, and Edman degradation recently[6]. The constituent components include 2,4-dihydroxyphenylacetic acid, cadaverine(Cad), and putreanine (Pua), besides four normal amino acids, *i.e.* Asp, Gly, Ala, and Arg. Clavamine also contains 2,4-dihydroxyphenylacetyl-L-asparaginyl-1,5-pentanediamine which is the structure common to JSTX-3, NSTX-3, and argiopin. In order to establish the proposed structure of clavamine synthetically, total chemical synthesis was attempted and performed in this study.

The protected derivative, 2,4-dibenzyloxyphenylacetyl-Asn→Cad←Pua(Z)-H (**2**), used as the intermediate in total synthesis of NSTX-3[7], took an important role as the key synthon for the synthesis of clavamine too as shown in Fig. 2.

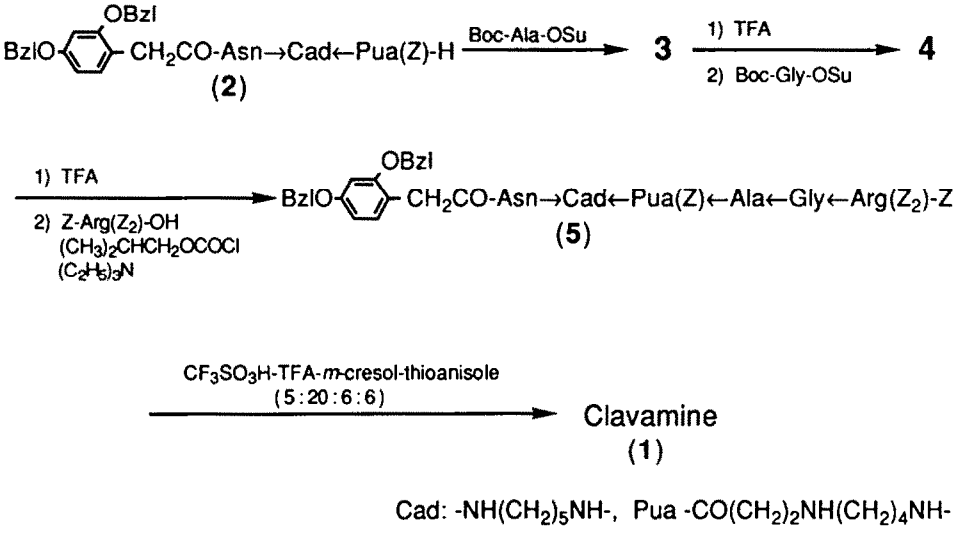


Fig. 2 Synthetic scheme of clavamine.

To the compound **2** as a starting material, three amino acids Ala, Gly, and Arg were successively introduced by stepwise elongation. Protected clavamine thus obtained was deprotected by CF_3SO_3H-TFA-*m*-cresol-thioanisole. Purification was carried out by ion-exchange column chromatography (CM-sephadex) and reversed phase HPLC. Synthetic clavamine was identical with natural sample in all respects such as retention time of HPLC, ^1H and ^{13}C-NMR, and insecticidal activity against the larvae of the mosquito (*Culex pipens molestus*).

This synthetic study thus established the chemical structure of the new insecticidal active compound isolated from the venom of Joro spider. Moreover, the synthesis can now supply the natural toxin which is only available in minor amount from the natural sources in sufficient amount as powerful tool in the investigation of insecticidal action.

Experimental

2,4-Dibenzyloxyphenylacetyl-Asn→Cad←Pua(Z)←Ala-Boc (**3**) To a solution of 2,4-dibenzyloxyphenylacetyl-Asn→Cad←Pua(Z)-H·CH_3COOH[7] (**2**) (500 mg, 566 μmol) in DMF (50 ml) were added triethylamine (68.5 mg, 677 μmol) and Boc-Ala-OSu (324 mg, 1.13 mmol). The reaction mixture was allowed to react at room temperature for 6 h, and then concentrated *in vacuo*. The residue was triturated with water and ethyl acetate to give solid. Yield 500 mg (88.8 %). The solid was reprecipitated for the elemental analysis. mp 148–150°C. Found: C, 64.51; H, 7.18; N, 9.72 %. Calcd for $C_{54}H_{71}N_7O_{11}\cdot 0.5H_2O$: C, 64.65; H,7.23; N, 9.77 %. $[\alpha]_D^{15}$ -2.6°(c 1.00, DMF).

2,4-Dibenzyloxyphenylacetyl-Asn→Cad←Pua(Z)←Ala←Gly-Boc (**4**) 2,4-Dibenzyloxyphenylacetyl-Asn→Cad←Pua(Z)←Ala-Boc (3) (380 mg, 382 μmol) was dissolved in TFA (10.9 ml) and stirred for 1 h at room temperature. The reaction mixture was concentrated *in vacuo* and the residue was washed with hexane and ether. To the solution of the solid thus obtained in DMF (49 ml) were added triethylamine (42.3 mg, 418 μmol) and Boc-Gly-OSu (70.4 mg, 760 μmol). Work up of this reaction were carried out by the same manner as that of **3**. Yield 302 mg (75.1 %). mp 146-147°C. Found: C, 63.16; H, 7.08; N, 10.46 %. Calcd for $C_{56}H_{74}N_8O_{12}\cdot H_2O$: C, 62.90; H,7.16; N, 10.47. $[\alpha]_D^{15}$ -2.7°(c 1.04, DMF).

2,4-Dibenzyloxyphenylacetyl-Asn→Cad←Pua(Z)←Ala←Gly←Arg(Z_2)-Z (**5**) 2,4-Dibenzyloxyphenylacetyl-Asn→Cad←Pua(Z)←Ala←Gly-Boc (**4**) (100 mg, 95 μmol) was treated with TFA by the same manner as that of **3** to give 2,4-dibenzyloxyphenylacetyl-Asn→Cad←Pua(Z)←Ala←Gly-H·TFA. To a solution of Z-Arg(Z_2)-OH (71.3 mg, 124 μmol) in THF (3.3 ml) were added isobutyl chloroformate (24 μl,186 μmol) and triethylamine (12.2 μg, 121 μmol) at -18°C and stirred for 10 min. The reaction mixture was added to the solution of 2,4-dibenzyloxyphenylacetyl-Asn→Cad←Pua(Z)←Ala←Gly-H·TFA obtained above and triethylamine (9.4 mg, 94 μmol) in DMF (3.3 ml) at -18°C for 2 h, and then stirred at room temperature overnight. After the reaction mixture was concentrated *in vacuo*, the residue was washed with water and ethyl acetate. The solid thus obtained was reprecipitated from DMF-ethanol. Yield, 101

mg(70.5 %). mp 163-165°C. Found: C, 63.81; H, 6.59; N, 10.70 %. Calcd for $C_{81}H_{96}N_{12}O_{17}\cdot H_2O$: C, 63.68; H, 6.47; N, 11.00. $[\alpha]_D^{15}$ +1.1°(c 0.83, DMF).

Clavamine (1) 2,4-Dibenzyloxyphenylacetyl-Asn→Cad←Pua(Z)←Ala←Gly←-Arg(Z_2)-Z (**5**) (40.0 mg, 26.5 μmol) was dissolved in CF_3SO_3H-TFA-*m*-cresol-thioanisole (5:20:6:6) (760 μl). The solution was stirred for 60 min. at room temperature. To the reaction mixture ether was added to give a precipitate. The solution of the solid in water was washed with ether and purified by the ion-exchange column chromatography (CM-sephadex, 18 x 300 mm, eluant 0.9 M ammonium acetate buffer, pH 6.90). Fractions containing clavamine were lyophilized and purified by HPLC (Cosmosil $5C_{18}$, 16.7 x 250 mm, eluant CH_3CN - 0.1% CH_3COOH, 0 - 30 % linear gradient (15 min), flow rate 5.0 ml/min). Yield 9.4 mg (36.4 %), $[\alpha]_D^{15}$ -12.6°(c 0.94, H_2O). Amino Acid Analysis (6M HCl, 110°C, 48 h): Asp, 0.91(1); Gly, 1.04(1); Ala, 1.00(1); Pua, 0.97(1); Arg, 1.07(1). HPLC retention time: 14.61 min (Cosmosil $5C_{18}$, 4 x 250 mm, CH_3CN - 0.1 % TFA (0 - 30 %, 15 min), flow rate 1.0 ml/min, detection: 220 nm). For the comparison with natural clavamine NMR was measured as a TFA salt. ^1H NMR (500 MHz, D_2O): 1.18(m,2H), 1.36(d,3H), 1.44(m,4H), 1.56(m,2H), 1.67(m,4H), 1.93(m,2H), 2,63(t,2H), 2.73(ddd,2H), 3.05(t,2H), 3.10(m,4H), 3.21(m,4H), 3.26(t,2H), 3.52(dd,2H), 4.01(d,2H), 4.06(t,1H), 4.24(q,1H), 4.60(m,1H), 6.44(m,2H), 7.08(d,1H).

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