Synthesis of a Toxic Octapeptide from the Larvae of Sawfly

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The synthetic octapeptide lophyrotomin (PhCO-D-Ala-D-Phe-L-Val-L-Ile-D-Asp-L-Asp-D-Glu-L-Gln), the toxic factor of the larvae of an Australian species of sawfly, *Lophyrotoma interrupta*, has been prepared by solid-phase synthesis and proved to be identical with the natural material.

Lophyrotomin is an *N*-blocked octapeptide, first isolated from the larvae of an Australian species of sawfly (*Lophyrotoma interrupta*), which has been recognized as a toxic factor responsible for cattle poisoning, by ingestion, in Queensland areas.[2] Its sequence was proposed to be the following: PhCO-D-Ala-D-Phe-L-Val-L-Ile-D-Asp-L-Asp-D-Glu-L-Gln.[1]

The presence of D amino acids and a blocked *N*-terminus allows resistence to enzymatic hydrolysis, suggesting an evolutionary role of this molecule as an effective feeding deterrent. We thus synthesized the peptide to unambigously prove its primary structure, also being in the process of having a sample of toxin for future studies on its biological activity. Stimulus to the synthesis, performed by the solid-phase method,[3] came also from the uncommon nature of the peptide structure due to the presence of a benzoylated *N*-terminus. A general procedure for the benzoylation of synthetic peptides was thus developed. Final comparison by HPLC and FAB-MS analyses of the natural and synthetic products provided the necessary evidence for the sequence originally proposed.[1] Byproduct structures were also determined.

Preparation of the protected peptide resin was carried out by using the Fmoc-based solid-phase peptide synthesis procedure (Fmoc = fluoren-9-ylmethoxycarbonyl).[4] The *C*-terminal glutamine was transesterified to the 4-hydroxymethylphenoxyacetic acid functionalized resin using the *p*-nitrophenyl ester derivative (2 × 3.6 equiv.).[5] These conditions allowed the minimization of the side chain dehydration reaction to nitrile, described for the carbodiimide method.[6] Aspartic acid and glutamic acid were protected as their *tert*-butyl ester derivatives. D and L amino acids, as required, were condensed in a stepwise manner as symmetric anhydrides (3.6 equiv.) using a manual synthesizer. *N*-Terminal benzoylation was acheived with benzoic acid (2 × 3.6 equiv.) in the presence of equimolar amounts of *N,N'*-diisopropylcarbodiimide and 1-hydroxybenzotriazole (HOBt) in DMF (Scheme 1). Pilot benzoylation experiments with benzoyl chloride (3.6 equiv.) and *N*-methylmorpholine in DMF yielded almost 40% of a byproduct with the undesired *N,N'*-dimethylformamidine group at the *N*-terminus.[7] The structure of this analogue was established by FAB-MS and specific colorimetric test.[8] Cleavage from the solid support and side chains deprotection was obtained with 12 mol dm^{-3} HCl, at 0 °C for 30 min. This permitted the rehydration of the nitrile functionality formed eventually.[6]

The pure peptide was obtained finally by purification of the crude material *via* semipreparative HPLC, using a Lichroprep RP18 column (10 × 250 mm) eluted with a H_2O–MeCN gradient, containing 0.1% TFA, flow rate 7 ml min^{-1}. The overall yield was 12.3% (from the introduction of Gln onto the resin).

Coinjection of synthetic and natural lophyrotomin gave a single peak in analytical HPLC, on a Bondapak C18 column (4.6 × 100 mm) using different eluting solvents and gradients (a typical one was 10% aqueous MeCN–90% aqueous MeCN, containing 0.1% TFA; flow rate 1 ml min^{-1}). The synthetic peptide showed the correct amino acid composition (Asp 2.07, Glu 2.10, Ala 0.96, Val 0.80, Ile 1.00 and Phe 1.00).[9] An abundant MH^+ peak at *m/z* 1040 was present in the positive-ion FAB-MS spectrum of our synthetic lophyrotomin. The spectrum showed identical fragmentation peaks with respect to the natural product. Among others, two kinds of sequence ions were particularly significant: the *N*-terminal b_n ions (*m/z* 535, 650, 765 and 894) and the *C*-terminal y_n ions (*m/z* 619, 718, 865 and 936).[10] As a result of the chelating ability of lophyrotomin toward sodium and potassium ions, the spectra were recorded only after accurate ion exchange chromatography.[1]

Structure elucidation of the artefacts was essential in order to minimize their formation. In the case of the *N*-terminal *N,N'*-dimethylformamidine analogue of lophyrotomin, the MH^+ peak was present in the spectrum at *m/z* 991; the mass difference with respect to the protonated natural product, the presence of y_n ions, equally shifted by 49 mass units, and the b_n ions at the same mass values of lophyrotomin, allow the determination of the correct structure of this byproduct. Similarly, the 'nitrile' product showed a mass shift to lower values of 18 mass units, both in the MH^+ ion and in the b_n series, the *C*-terminal y_n ions remaining unshifted.

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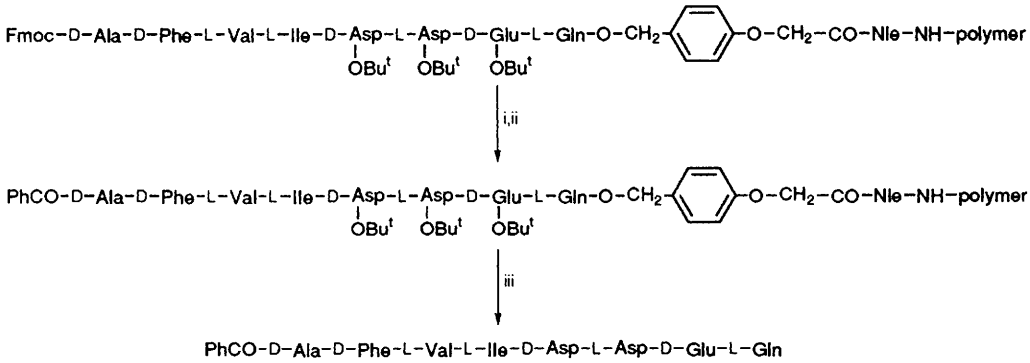

Scheme 1 *Reagents and conditions*: i, 20% piperidine–DMF; ii, benzoic acid, HOBt, *N,N'*-diisopropylcarbodiimide in DMF; iii, 12 mol dm^{-3} HCl, 30 min at 0 °C

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