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Synthesis of a Naturally Occurring Inhibitor of Glutamine Synthetase, Tabtoxinine-β-lactam

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(±)-Tabtoxinine- β -lactam, a potent inhibitor of glutamine synthetase, has been synthesised by a route involving a nitroso Diels–Alder reaction.

Tabtoxin (1) is a dipeptide exotoxin produced by Pseudomonas tabaci, the organism responsible for Wildfire disease in tobacco plants.1 When hydrolysed by peptidases, in vivo, this exotoxin releases tabtoxinine- β -lactam (2), which inhibits the glutamine synthetase of the photorespiratory nitrogen cycle, causing chlorosis and death of the tobacco plant.² It seems likely that this inhibition is the result of tight binding of tabtoxinine- β -lactam (2) to the enzyme as an analogue of the postulated tetrahedral intermediate (3) involved in the enzymatic reaction.1a In 1983 we achieved a stereospecific synthesis of the dipeptide tabtoxin³ but found that this approach was not amenable to the synthesis of the actual toxin, tabtoxinine- β -lactam (2), nor was the hydrolysis of (1), under acidic^{1e} or enzymatic^{2b} conditions, a satisfactory source of (2). Consequently, we have developed a new route to the toxin (2) which is based on an improved Diels-Alder strategy (Scheme 1, X =CN) and offers direct access to (\pm) -(2), with the correct regioand stereo-chemistry.

Thus, 2-chloroacrylonitrile was treated with butadiene (sealed tube, 90-100 °C, 24 h) to give the cyclohexene (4) (85%),⁴ which on heating at reflux in pyridine gave the diene (5) \dagger (72%). Diene (5) was treated with benzyl nitrosoformate (generated in situ from benzyl N-hydroxycarbamate and tetraethylammonium periodate in CH₂Cl₂⁵) affording the adduct (6) as a single regioisomer $[73\%; \delta_{\rm H}(\rm CDCl_3) 4.89 (1H,$ m, allylic), 6.56 (1H, dd, J 8.5 and 2Hz, vinylic), and 6.70 (1H, dd, J 8.5 and 6.3 Hz, vinylic)]. The nitrile function of (6) was smoothly reduced with NaBH₃(OCOCF₃)⁶ to give the amine (7) (58%), identical to that synthesised previously by a more complex procedure,3 confirming the desired regiochemistry of the Diels-Alder step. After protection of the amine (7) with a t-butoxycarbonyl group [BOC-ON (2-t-butoxycarbonyloxyimino-2-phenylacetonitrile),⁷ CH₂Cl₂] to give (8) (83%; m.p. 115.5 °C), oxidative cleavage was accomplished by the method of Starks (KMnO₄, $Bu_4N^+HSO_4^-$, $C_6H_6-H_2O$, 25 °C)⁸ to give the diacid (9) [98%; $\delta_{\rm H}$ (CD₃COCD₃) 3.4–3.8 (2H, ABX, CH₂NH) and 4.63 (1H, m, allylic)]. The difficult step of differentiation of the two carboxy groups of (9) was successfully achieved by decarboxylative esterification [benzyl chloroformate (1.5 equiv.), pyridine, CH₂Cl₂, 25 °C] to give the monoester (10) [57%; $\delta_{\rm H}$ (CDCl₃) 5.08 (2H, br, benzylic), 5.15 (2H, br, benzylic), and 7.0–7.5 (10H, m, 2 × Ph)] along with a small amount of the diester (11) [4.8%; $\delta_{\rm H}$ (CDCl₃), 5.05–5.38 (7H, m, 6 benzylic H and NH), and 7.25–7.45



Scheme 1. $X = CO_2Et$, CN.

 $[\]dagger$ ¹H N.m.r. (300 MHz), i.r., and mass spectra were entirely consistent with the assigned structures for all new compounds and satisfactory combustion analyses were obtained.



(15H, m, $3 \times Ph$)]. Removal of the primary amino protection of (10) with 98% formic acid (25 °C, 3 h)⁹ gave the penultimate precursor, the β -amino acid (12) (99%). The β -lactam closure was achieved by Ohno's procedure [Ph₃P-di-2-pyridyl disulphide-MeCN, 80 °C, 2 h]¹⁰ to yield the spiro β -lactam (13) [63%; v_{max.} (CHCl₃) 1780, 1740, and 1710 cm⁻¹; δ_{H} (CDCl₃) 3.33 (1H, d, J 5 Hz, β -lactam) and 3.56 (1H, d, J 5 Hz, β -lactam)]. Hydrogenation of (13) (10% Pd-C, EtOH) resulted in complete deprotection and concomitant reductive cleavage of the N-O bond to provide (±)-tabtoxinine- β lactam (2) [quantitative, v_{max.} (D₂O) 1736 cm⁻¹; δ_{H} (D₂O) 1.62–2.00 (4H, m, CH₂CH₂), 3.15 (1H, d, J 6 Hz, β -lactam), 3.28 (1H, d, J 6 Hz, β -lactam), and 3.60 (1H, m, CHNH₂)] which was identical (500 MHz n.m.r.) to the sample isolated from *P. tabaci* and was biologically active *in vitro* and *in vivo*.

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