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

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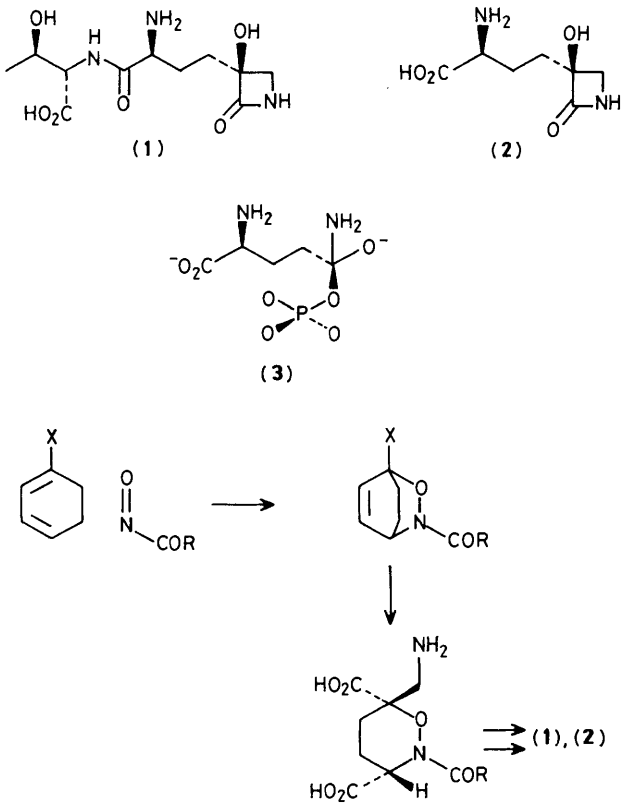
(±)-Tabtoxinine-β-lactam, a potent inhibitor of glutamine synthetase, has been synthesised by a route involving a nitroso Diels–Alder reaction.

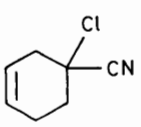
Tabtoxin (**1**) is a dipeptide exotoxin produced by *Pseudomonas tabaci*, the organism responsible for Wildfire disease in tobacco plants.[1] When hydrolysed by peptidases, *in vivo*, this exotoxin releases tabtoxinine-β-lactam (**2**), which inhibits the glutamine synthetase of the photorespiratory 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 tabtoxin[3] 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 (±)-(**2**), with the correct regio- and 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**)† (72%). Diene (**5**) was treated with benzyl nitrosoformate (generated *in situ* from benzyl *N*-hydroxycarbamate and tetraethylammonium periodate in CH_2Cl_2[5]) affording the adduct (**6**) as a single regioisomer [73%; $\delta_H(CDCl_3)$ 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 $NaBH_3(OCOCF_3)$[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] CH_2Cl_2] to give (**8**) (83%; m.p. 115.5 °C), oxidative cleavage was accomplished by the method of Starks ($KMnO_4$, $Bu_4N^+HSO_4^-$, C_6H_6–H_2O, 25 °C)[8] to give the diacid (**9**) [98%; $\delta_H(CD_3COCD_3)$ 3.4—3.8 (2H, ABX, CH_2NH) 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, CH_2Cl_2, 25 °C] to give the monoester (**10**) [57%; $\delta_H(CDCl_3)$ 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%; $\delta_H(CDCl_3)$, 5.05—5.38 (7H, m, 6 benzylic H and NH), and 7.25—7.45

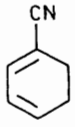


Scheme 1. X = CO_2Et, CN.

† ^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.



(4)



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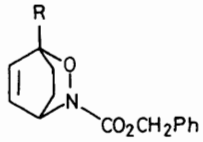
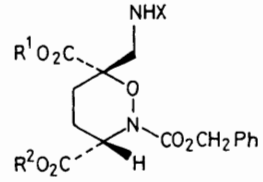
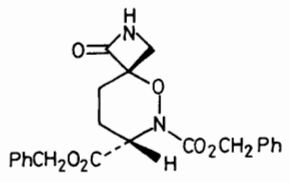


(6) R = CN
(7) R = CH_2NH_2
(8) R = $CH_2NHCO_2Bu^t$





(13)

(9) $R^1 = R^2 = H$, X = CO_2Bu^t
(10) $R^1 = H$, $R^2 = CH_2Ph$, X = CO_2Bu^t
(11) $R^1 = R^2 = CH_2Ph$, X = CO_2Bu^t
(12) $R^1 = H$, $R^2 = CH_2Ph$, X = H

(15H, m, 3 × 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 [Ph_3P–di-2-pyridyl disulphide–MeCN, 80 °C, 2 h][10] to yield the spiro β-lactam (**13**) [63%; $\nu_{max.}$($CHCl_3$) 1780, 1740, and 1710 cm^{-1}; δ_H($CDCl_3$) 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, $\nu_{max.}$ (D_2O) 1736 cm^{-1}; δ_H(D_2O) 1.62—2.00 (4H, m, CH_2CH_2), 3.15 (1H, d, *J* 6 Hz, β-lactam), 3.28 (1H, d, *J* 6 Hz, β-lactam), and 3.60 (1H, m, C*H*NH_2)] 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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