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Stereospecific Synthesis of Tabtoxin

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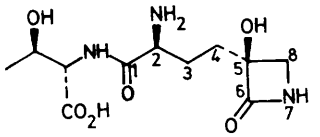
The exotoxin, Tabtoxin, from *Pseudomonas tabaci* (the organism responsible for Wildfire disease of tobacco plants) has been synthesised by a stereospecific route involving, as a key stereochemistry-defining step, the cycloaddition of an acylnitroso compound to a cyclohexadiene.

Wildfire disease is an infectious leafspot disease of tobacco plants first reported in 1917[1] and known to be caused by an exotoxin called Tabtoxin (**1**) produced by the infecting agent *Pseudomonas tabaci*. The structure[2] and stereochemistry[3] of (**1**) were revealed relatively recently, largely due to the instability ($t_{\frac{1}{2}}$, pH 7, 24 h at 25 °C) of the toxin, which undergoes a facile intramolecular transacylation to the stable, inactive, isotabtoxin (**2**). The toxin appears to exert its effect on the plant by inhibition of the photorespiratory nitrogen cycle *via* a specific blockade of glutamine synthetase.[4] We now report the first synthesis of this toxin, in which the crucial stereochemical relationship between C(2) and C(5) was achieved by simultaneous formation of C(2)–N and C(5)–O bonds *via* a Diels–Alder reaction of an acylnitroso compound with a suitable cyclohexadiene[5] (Scheme 1).



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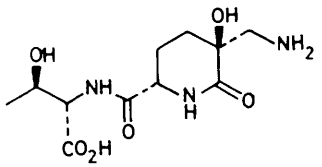
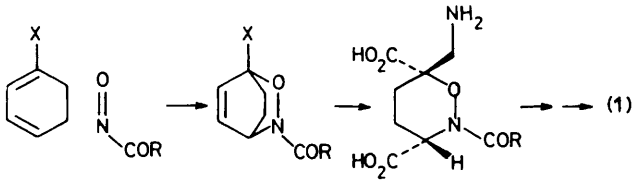
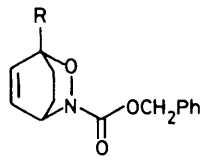


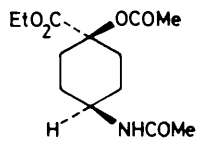
(2)



Scheme 1. *E.g.* R = OCH_2Ph; X = CO_2Et.

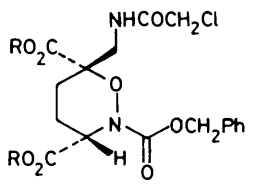
Thus ethyl cyclohexa-1,3-dienecarboxylate reacted with benzyl nitrosoformate (generated *in situ* from *N*-benzyloxycarbonyl hydroxylamine and $Et_4N^+IO_4^-$, CH_2Cl_2) to yield a single regioisomer (**3**) [93%, ^{1}H n.m.r., δ($CDCl_3$) 4.85 (1H, m, –C–H), 6.6 (2H, m, olefinic)].† The regiochemistry of this reaction was confirmed by hydrogenation (Pd/C, EtOH) and acetylation to (**4**) (m.p. 144—146 °C) in which the amide hydrogen showed splitting [^{1}H n.m.r., δ($CDCl_3$) 5.36 (d, *J* 5 Hz)] from a single methine hydrogen. Reduction with sodium borohydride gave the alcohol (**5**) (100%) which was oxidised [Moffat, dicyclohexylcarbodi-imide, Me_2SO; pyridine–trifluoroacetic acid (TFA), 68%] to the aldehyde (**6**), isolated in





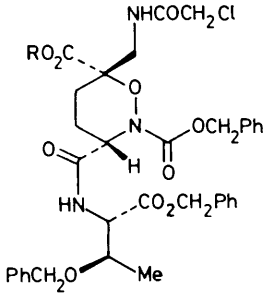
(**3**) R = –CO_2Et
(**5**) R = –CH_2OH
(**6**) R = –CHO
(**7**) R = –CH_2–NH–CH(4-MeO-C_6H_4)$_2$
(**8**) R = –CH_2NH_2
(**9**) R = –$CH_2NHCOCH_2Cl$

(**4**)

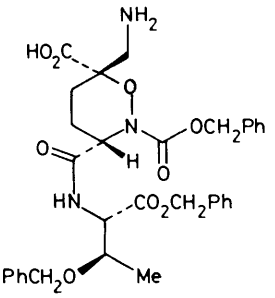


(**10**) R = H
(**11**) R = $COBu^t$

† All new compounds gave satisfactory analytical and spectral data.



(12) R = H
(13) R = $-CHPh_2$



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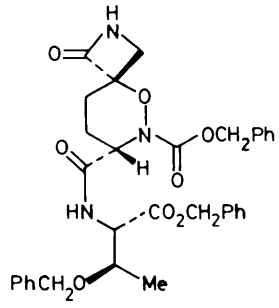

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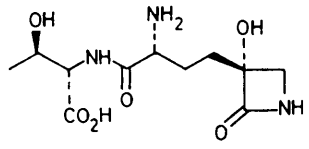

(16)

admixture with its hydrate. Since direct reductive amination was not possible, largely due to dialkylamine formation, the desired amine (**8**) was obtained indirectly by conversion of the aldehyde (**6**) into the protected amine (**7**), with 4,4′-dimethoxybenzhydramine and $NaBH_3CN$ (MeOH, HCl, pH 6, 3 Å molecular sieves, 59%), which was readily deprotected (TFA, anisole, 25 °C, 89%) to the amine (**8**) [^{1}H n.m.r., δ($CDCl_3$) 3.00 (2H, s, CH_2NH_2), 4.77—4.82 (1H, m, –CHN), 6.44—6.22 (2H, m, olefinic)] and then converted ($ClCH_2COCl$, CH_2Cl_2, Et_3N, 0 °C) into the chloroacetamide (**9**) (86%, m.p. 65—66 °C), in preparation for oxidative cleavage of the double bond. This step was achieved following a procedure of Starks ($KMnO_4$, H_2O, C_6H_6, $Bu_4N^+HSO_4^-$, 25 °C)[6] which provided the racemic diacid (**10**) {58%, m.p. 178—179 °C, ^{1}H n.m.r., δ(CD_3CN) 3.48 and 4.05 (2H, ABX, J_{AB} 14, J_{AX} 3, J_{BX} 8 Hz, $-CH_2NH-$), 3.92 (2H, s, $-CH_2Cl$), 4.73 [1H, dd, $-CH(CO_2H)N$]}.

Differentiation of the carboxy groups in (**10**) was achieved *via* the preparation of the dipivaloyl mixed anhydride (**11**) [MeCN, Et_3N (2 equiv.), 0 °C, Bu^tCOCl (2 equiv.), 30 min] which reacted *in situ* with *O*-benzyl-L-threonine benzyl ester[7] (0 °C, 1 h, 25 °C, 3 h) to give the product (**12**) resulting from selective attack at the less hindered of the two carbonyl groups, as a mixture of diastereoisomers,‡ which was then converted (Ph_2CN_2, CH_2Cl_2, 25 °C, 10 min) into the crystalline benzhydryl esters. One diastereoisomer crystallised from ethyl acetate [now known to be (**13**), m.p. 180—182 °C, 25% from (**10**)], and the other from diethyl ether [m.p. 98—100 °C, 28% from (**10**)]. Both isomers were carried through the rest of the synthesis. Thus the isomer (**13**) (m.p. 180—182 °C) was deprotected (TFA, 25 °C, 1 h) to the acid (**12**) [90%, m.p. 154—156 °C, ^{1}H n.m.r., δ(CD_3CN) 3.39 and 4.07 (2H, ABX, J_{AB} 14.5, J_{BX} 9.5, J_{AX} 2.8 Hz, CH_2NH), 3.92 (2H, s, $-CH_2Cl$)] and then further deprotected (thiourea, MeCN, EtOH, 40 °C, 48 h, 50%)[8] to the amino acid (**14**) which was directly cyclised (2-thiopyridinedisulphide, Ph_3P, MeCN, reflux, 6 h)[9] to the spirocyclic β-lactam (**15**) [30%, ν_{max} (neat) 1780, 1745, and 1680 cm^{-1}]. Hydrogenolysis (Pd/C, MeOH, 25 °C, 14 h) of (**15**) gave tabtoxin (**1**) [90%, ^{1}H n.m.r., δ(D_2O) 1.02 (3H, d, *J* 7 Hz, CH_3), 1.66—1.92 (4H, m, CH_2CH_2), 3.16 (1H, d, J_{AB} 6 Hz, H_A of CH_2N), 3.30 (1H, d, J_{AB} 6 Hz, H_B of CH_2N), 4.0—4.12 (3H, m, MeC–OH, CHOH, and $CHNH_2$)]. This material showed the same biological activity on the tobacco plant, the same glutamine synthetase and *E. coli* growth assay and had an identical ^{1}H n.m.r. spectrum (D_2O, 300 MHz) to the natural tabtoxin isolated from *P. tabaci*. The stereoisomer (**16**), obtained from the lower m.p. isomer of (**13**), showed virtually no activity in the biological tests.

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‡ The absolute stereochemistry of only one of the two diastereoisomers is depicted here.

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