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SYNTHESIS OF THE CYCLIC TETRAPEPTIDE TENTOXIN.
EFFECT OF AN N-METHYLDEHYDROPHENYLALANYL RESIDUE ON CONFORMATION OF LINEAR TETRAPEPTIDES

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Tentoxin, a cyclic tetrapeptide produced by the phytopathogenic fungus, Alternaria tenuis, causes chlorosis when applied to germinating seed.[1] Recent studies have proposed that tentoxin is cyclo(N-methyl-L-alanyl-L-leucyl-N-methyl-Z-dehydrophenylalanyl-glycyl) (8).[2] We report here the first synthesis of the cyclic tetrapeptide 8, which confirms the proposed structure, and present n.m.r. and c.d. evidence which suggests that the conformation of the linear tetrapeptide 5a is similar to cyclic peptide 8.

N-t-Butoxycarbonyl-N-methyl-L-alanyl-L-leucyl-3-benzylthio-D,L-phenylalanyl-glycyl-O-resin (1) was prepared by solid phase synthesis from N-t-butoxycarbonyl-glycyl-O-resin (0.6 mmol Gly/g, 1% divinyl benzene) according to the general procedure of Merrifield.[3] N-t-Butoxycarbonyl-3-benzylthio-D,L-phenylalanine[5] was prepared from 3-benzylthio-D,L-phenylalanine[4] and used as the mixture of diastereomers. Methanolysis of the resin 1 gave the diastereomeric sulfides 2a,b[5] in an overall yield of 64%. Treatment of sulfides 2a,b with sodium periodate in aqueous methanol for 24 h gave the sulfoxides 3a,b (yield 94%)[5] which were converted to the dehydrophenylalanyl peptides 4a,b (60% yield)[5] by heating in refluxing xylene under an atmosphere of nitrogen for 30 h.[6] The geometric isomers 4a (R_f, EtOAc = 0.62) and 4b (R_f, EtOAc = 0.81) were separated by column chromatography on silica gel. Peptide 4a (λ_{max} 276, ϵ18,400) was assigned the Z-configuration and peptide 4b (λ_{max} 282, ϵ9080) assigned the E-configuration on the basis of the greater intensity of absorbance observed for the trans-isomer of cinnamic acids.[7] These assignments are consistent with the observations that the chemical shifts (Table) of the leucyl and alanyl protons in 4a (Z) are shielded with respect to the same protons in 4b (E) while the glycyl protons are shielded in 4b.

Peptide 4a was transformed to the N-methyl-Z-dehydrophenylalanyl peptide 5a (89% yield)[5] by reaction with methyl iodide and potassium carbonate in dimethylformamide at room temperature for 72 h. This methylation reaction was found to proceed stereospecifically on model compounds. For

example, the methylesters of Z- and E- N-t-butoxycarbonyl-3-methyldehydroalanine were converted to the Z- and E- N-methyl derivatives respectively in high yield without isomerization of the double bond.[8] No methylated products (either the E- or Z- isomers) were detected when the E-dehydrophenylalanyl isomer 4b was subjected to the same methylation procedure. Peptide 4b was recovered in high yield.

The conformation of peptide 5a ($N-CH_3$-ΔPhe) is substantially different from that of peptide 4a (NH-ΔPhe). The chemical shifts (Table) of the α- and δ-protons of leucine, and the methyl and vinyl protons of N-methyl-Z-dehydrophenylalanine in 5a are nearly the same as those determined for tentoxin, 8. The leucine resonances in both noncyclic precursor 5a and natural cyclic peptide 8 are shifted upfield from what one finds in other linear dehydropeptides (4a,b; 9) which contain the dehydrophenylalanyl unit suggesting that the orientation of the leucyl isobutyl group with respect to the phenyl ring in both 5a and 8 is similar. In contrast, peptide 4a which contains three secondary amide bonds should be in an all trans amide bond conformation similar to 10a. The chemical shift of the leucyl isobutyl group in 10a should be normal (about 0.91) since the isobutyl group is too far from the phenyl ring to be shielded by it. The circular dichroism spectra of 5a (277 nm, [θ] + 6640) and 8 (285 nm, [θ] 3593) showed comparable positive elipticities in the 260-320 nm region, indicating similar stereochemical and electronic environments about the α,β-dehydro chromophore. Unmethylated peptide 4a showed a strong negative elipticity in the same region (275 nm, [θ]-12,212). The close similarity between the n.m.r. and c.d. spectra of 5a and 8 suggests that the preferred conformation of 5a resembles structure 11 (and not 10b) in that the amide bond between the N-methyldehydrophenylalanyl and leucyl residues is predominently the cis conformer.

The protected peptide acid (6, R = H),[5] obtained from ester 5a by saponification (93% yield), was converted to the 2,4,5-trichlorophenyl ester (7. R = $C_6H_2Cl_3$) by reaction with dicyclohexylcarbodiimide in pyridine. After removal of the N-t-butoxycarbonyl group by treatment of 7 with 1 N hydrochloric acid in acetic acid, the resulting peptide was cyclized by reaction for 24 h in refluxing pyridine (0.18 mmolar) containing one equivalent of diisopropylethylamine. The cyclic tetrapeptide 8 was purified by chromatography and isolated in a yield of 18% based on the protected acid 6. The n.m.r., i.r., u.v. mass spectral data and the chromatographic behavior of synthetic 8 were identical with those of natural tentoxin. The biological potencies of synthetic and natural tentoxin on germinating lettuce seedlings were indistinguishable. These data confirm the sequence reported for tentoxin.[2]

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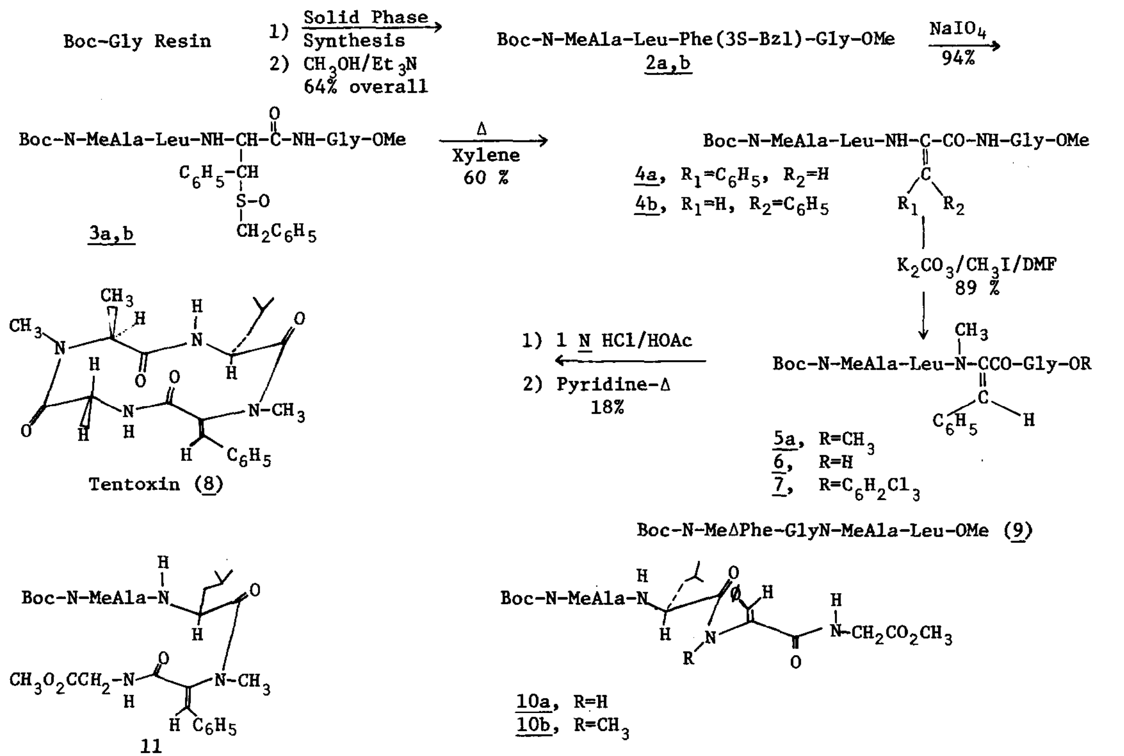

Table++

Compound	Leu		Ala			Gly		Me Phe	
	δH	α,H	β-H	$N-CH_3$	α-H	α-H	CO_2CH_3	$N-CH_3$	β-H
4a	0.91	4.4	1.27	2.77	4.50	4.05 (d, J=6)	3.71	---	---
4b	0.92	4.56	1.36	2.79	4.50	3.91 (d, J=6)	3.64	---	---
5a	0.54 0.60	4.18	1.26	2.70	4.66	4.14 (d, J=6)	3.71	3.21	7.74
8	0.53 0.63	4.18	1.53	2.80	4.37	5.21 (d,d; J=10,9) 3.57 (d, J=15)	---	3.18	7.75
9	0.93	4.6	---	2.96	---	4.16 4.22	3.71	3.05	7.35

++The spectra were obtained with a Bruker-HX90E spectrometer in deuteriochloroform. The chemical shifts are expressed in ppm relative to internal TMS.

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