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Total Synthesis of the Cyclic Tetrapeptide, HC-Toxin

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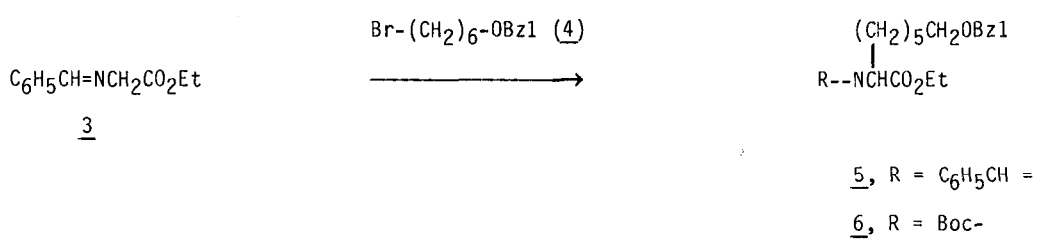
Summary: The amino acid sequence of the host-specific phytotoxin, HC-toxin, cyclo(L-Ala-D-Ala-L-Aoe-D-Pro) has been confirmed by total synthesis.

A host-specific toxin produced by the fungus, Helminthosporium (Cochliobolus) carbonum race 1, has been characterized as a cyclic tetrapeptide composed of the amino acids, proline, two alanines, and 2-amino-8-oxo-9,10-epoxydecanoic acid (Aoe).[1,2,3,4] The latter amino acid was first detected in the phytotoxin, Cyl-2.[5] Liesch et al.[2] proposed the sequence of amino acids in HC-toxin as cyclo(Ala-Ala-Pro-Aoe) while Walton et al.,[3] Gross et al.,[4a] and Pope et al.[4b] proposed the sequence, cyclo(Ala-Ala-Aoe-D-Pro) (1). By means of ^{1}H-NMR NOE studies, Kawai et al. determined that the configuration of Ala-2 must be D and also established the bis γ-turn conformation of the ring system in chloroform.[6] The configuration of the epoxide in 1 has not been established but is probably 9S based on the comparable biological activities of HC-toxin and chlamydocin (2).[7,22] We report herein the first synthesis of HC-toxin by a route that confirms the configurational sequence of amino acids and which can be utilized to prepare tritiated analogs.

SCHEME I



Alkylation of benzylidene glycine ethyl ester (3)[8] with 1-bromo-6-benzyloxyhexane (4)[9] gave the imine (5) which was hydrolyzed (1N HCl, 2 h, room temp.), and the resulting amine hydrochloride converted to racemic N-Boc-2-amino-8-benzyloxy-octanoic acid ethyl ester (Boc-Abo-

OEt;6) by reaction with di-tert-butyl-dicarbonate in 75% overall yield (Scheme I).[10,11] The linear tetrapeptide 7[11] was synthesized by stepwise elongation of the peptide in solution as developed for a homolog,[11] beginning with D-proline methyl ester hydrochloride (Scheme II). N,N'-Dicyclohexylcarbodiimide (DCC) and N-hydroxybenzotriazole (HOBt) were used together for all the coupling reactions[13] and the intermediates were isolated in good yield. Saponification (2N NaOH in dioxane:water, 2:1) gave tetrapeptide 7[11] in 97% yield. Cleavage of the Boc group (HCl:dioxane, 40 min) followed by cyclization with diphenylphosphoryl azide (DPPA)[14,15] (CH_2Cl_2, 48 h at -20°C, 48 h at 0°C, 5mM) gave cyclic tetrapeptide 8a[11] (21% yield) along with a small amount (4.2%) of the D-Abo epimer 8b. The configurations of the Abo peptides were assigned from their NMR data by comparison with model peptides of known configuration.[21,4]

SCHEME II

Boc-L-Ala-D-Ala-DL-Abo-D-Pro-OH (7)

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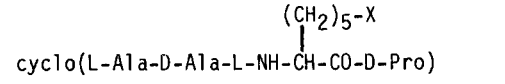
cyclo(L-Ala-D-Ala-X-Abo-D-Pro)



cyclo(L-Ala-D-Ala-L-NH-CH-CO-D-Pro)

8a, X = L

8b, X = D

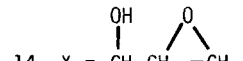
9, X = CH_2OH

10, X = CH=O

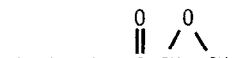
11, X = $CH_2OCH_2SCH_3$

12, X = CH(OH)-C≡CH

13, X = CH(OH)-CH=CH_2



14, X = CH-CH—CH_2



1a,b, X = C-CH—CH_2

The side chain of Abo in 8a was converted to Aoe by the series of reactions shown in Scheme II. Removal of the benzyl group by catalytic hydrogenolysis (Pd/C, t-butanol) gave alcohol 9[11] in quantitative yield. Oxidation of alcohol 9 (pyridine-SO_3, Me_2SO, Et_3N, 25°C, 5-10 min) gave the aldehyde 10 (71%) plus sulfide 11 (10% yield, δ = 2.13,s,3H, S-CH_3; 4.63,s,2H,-OCH_2-S; 3.5,m,CH_2-O) formed by a Pummerer rearrangement.[16] Reaction of aldehyde 10 with lithium acetylide (10 eq. mol of n-BuLi, CH CH gas, -78°C, THF, 4 h)[17] followed by careful quenching (sat. NH_4Cl) gave the acetylenic alcohol 12[11] in 64% yield. The triple bond in 12 was reduced using a large excess of Lindlar catalyst in methanol to give the allylic alcohol 13[11] (100%) which was converted to epoxy alcohol 14 (1.2 eq. mCPBA in CH_2Cl_2) and oxidized *in situ* to the epoxyketone 1a,b (mCPBA,1.5 eq., 2,2,6,6-tetramethyl piperidine hydrochloride, 0.02 eq.mol) in 72% overall yield.[11,19,20] The structure of 1a,b was confirmed by direct comparison of synthetic HC-toxin with an authentic sample by 270 ^{1}H-NMR, mass spectrometry, microanalysis, and

TLC. The NMR spectra were identical except for additional fine structure for the epoxy protons in synthetic HC-toxin caused by the presence of both epoxide epimers. The biological activity of synthetic HC-toxin was determined in the modified root growth assay[1] against susceptible and resistant maize hybrids.[3] In this assay, synthetic HC-toxin had an ED_{50} of 0.85 μg/ml (vs 0.5μ g/ml for natural HC-toxin) and, like natural HC-toxin, did not inhibit the resistant maize at 100-fold higher concentrations. The synthesis of radioactive HC-toxin and other analogs by this route is in progress.

Acknowledgements.

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in 48% yield: bp 121-124°C/0.25-0.3 mm Hg; 90 MHz NMR ($CDCl_3$) δ = 1.30-1.92(m,8H), 3.42(m,4H) 4.49(s, 2H), 7.31(s,5H).

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18. For example: Hilgetag, G. and Martini, A., "Weygand/Hilgetag Preparative Organic Chemistry," John Wiley and Sons, Inc., 1972, p40-45. We used Lindlar catalyst (Pd on $CaCO_3$ poisoned with Pb, 25 mgs) per 0.1 mmol of acetylenic alcohol 12 for 15-20 min. Rf values of 13 were almost identical with starting material 12.
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