Structure of the Cyclic Tetrapeptide Tentoxin. Crystal and Molecular Structure of the Dihydro Derivative

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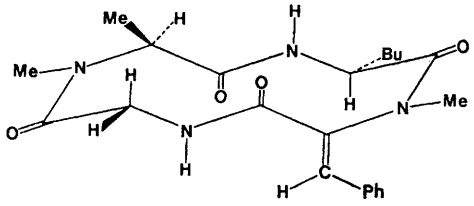
Summary The dihydro reduction product of tentoxin (I), a phytotoxic tetrapeptide from *Alternaria tenuis* Nees., is shown by *X*-ray crystallography and the known absolute configurations of its amino acid constituents to be cyclo-(*N*-methyl-L-alanyl-L-leucyl-*N*-methyl-D-phenylalanyl-glycyl); tentoxin is its dehydrophenylalanyl analogue.

TENTOXIN, a phytotoxic metabolite of *Alternaria tenuis* Nees.,[1] is a cyclic tetrapeptide containing one unit each of glycine, L-leucine, L-*N*-methylalanine, and *N*-methyldehydrophenylalanine.[2,3] Primarily on the basis of ^{1}H n.m.r. and m.s. data we recently proposed the full structure to be cyclo-(*N*-methyl-L-alanyl-L-leucyl-*N*-methyl-*trans*-dehydro-

phenylalanyl-glycyl) (I),[3] in contrast to a report which suggested an alternative amino-acid sequence.[4] Single crystal *X*-ray structural analysis of the dihydro compound produced by hydrogenation at the dehydrophenylalanyl



(I)

double bond[3] has now reached a sufficient state of refinement to demonstrate conclusively that this derivative was accurately formulated as cyclo-(L-MeAla-L-Leu-D-MePhe-Gly).[3] Thus structure (I) for tentoxin is correct.

Dihydrotentoxin crystallizes from methanol in space group $P1$, $a = 9{\cdot}511(2)$, $b = 9{\cdot}614(2)$, $c = 6{\cdot}920(1)$ Å; $\alpha = 114{\cdot}42(1)$, $\beta = 86{\cdot}77(1)$, $\gamma = 93{\cdot}94(1)°$, $D_m = D_c = 1{\cdot}21$ g cm^{-3}, and $Z = 1$. Intensity data for 2030 independent reflections (Mo-K_α radiation, $2\theta \leqslant 50°$) were collected using a manual diffractometer. Of these, **1364** had $I > 3\sigma$; these were used for the structure determination and refinement. Despite the size of the molecule, the structure was readily solved using Patterson methods through an initial search for the anticipated conformation[3,5] of an *NN*-αα-tetramethylated 12-membered cyclotetrapeptide skeleton ($C_{12}N_4O_4$) in the unit cell. Subsequent refinement using full-matrix least-squares and Fourier techniques led to the structure shown in the Figure, the absolute configuration being based on the reported con-

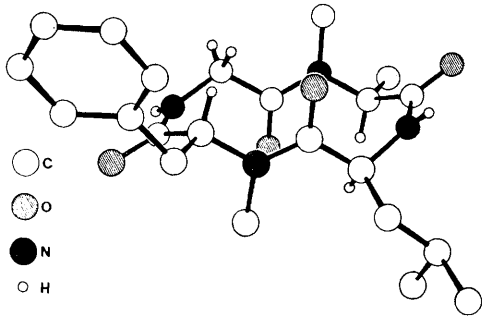


FIGURE. Crystal structure of dihydrotentoxin. Hydrogen atoms are shown only on the peptide ring.

figurations of the individual amino acids.[3] At the present state of refinement, using isotropic thermal parameters, the conventional *R* factor is 0·09. A difference map of this model has a maximum electron density of less than 0·4*e* Å^{-3}.

Not only the amino-acid sequence, but also the molecular conformation corresponds in all details to that deduced for dihydrotentoxin by ^{1}H n.m.r. spectroscopy.[3] This provides strong support for the validity of the analytical techniques used in interpreting the n.m.r. data and strengthens the suggestion that this conformation is very common among cyclotetrapeptides.[6] Hydrogenation of tentoxin could not alter the amino-acid sequence, and thus this result also confirms the amino-acid sequence (I) for the natural product itself.

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