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THE STRUCTURE OF TENTOXIN

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Tentoxin is a metabolite of Alternaria tenuis Auct. which produces severe chlorosis in the cotyledons of many dicotyledonous plant species (1-3). It is an optically active neutral substance showing mp 172-175° after crystallization from benzene, and the composition $C_{22}H_{30}N_4O_4$ can be deduced from elemental analysis (found: C, 64.91; H, 7.20; N, 12.70. $C_{22}H_{30}N_4O_4 \cdot \frac{1}{2}C_6H_6$ requires: C, 65.03; H, 7.32; N, 12.91), molecular weight determination (molecular ion m/e 414 (rel. int. 100); mol. wt. 441 by isothermal distillation in acetone), and other data described below. The presence of at least one conjugated system is indicated by ultraviolet absorption at 285 nm (ϵ 12,200; in water) and the infrared spectrum shows absorption from NH (3345 cm^{-1}) and carbonyl (1670 and 1630 cm^{-1}) groups.

Resonance from all 30 protons is clearly evident in the 100 MHz pmr spectrum, and spin-coupling relations among many of them have been established by double resonance techniques. Two sharp amide N-CH_3 singlets (τ 7.20 and 6.81) and two broad amide NH doublets (τ 1.79, J = 10 Hz and τ 2.73, J = 9 Hz) are visible, identifying the four nitrogen atoms. The τ 1.79 NH doublet is converted to a singlet by irradiation of a one-proton doublet of doublets at τ 4.81 (J = 10 and 15 Hz) and the same irradiation collapses a one-proton doublet at τ 6.43 (J = 15 Hz) to a singlet; this result identifies either $CONHCH_2$ (with diastereotopic CH_2 protons) or CONHCHCH (with a C-C dihedral angle near 0° or 180°)

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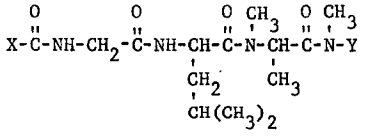
as the environment of one nitrogen. The τ 2.73 NH doublet becomes a singlet when an ill-defined one-proton multiplet at τ 5.82 is irradiated, and the same irradiation leads to a distinct alteration in the shape of an unresolved two-proton multiplet at τ 8.75; thus the other secondary nitrogen is $CONHCHCH_x$ (x = 1 or 2). A three-proton doublet (τ 8.46, J = 7 Hz) can be decoupled from a one-proton quartet (τ 5.60, J = 7 Hz), identifying the presence of a CH_3CH grouping, and two three-proton doublets (τ 9.40 and 9.50, each J = 6 Hz) are converted to singlets by irradiating at the same frequency under the unresolved 8.75 multiplet, suggesting the presence of a C-isopropyl group which contains diastereotopic methyls. A five-proton aromatic singlet (τ 2.59), a one-proton aromatic or deshielded olefinic singlet (τ 2.25), and a poorly-defined one-proton multiplet merged into the low-field side of the 8.46 methyl doublet round out the spectrum.

These strong indications of amide functionality led to examination of the acidic hydrolysis of tentoxin. Thin-layer chromatographic analysis of the hydrolysate shows five ninhydrin-positive spots. Two of these correspond to glycine and leucine, and a third has chromatographic behavior identical with methylamine hydrochloride. In view of the pmr data, a sample of dl-N-methylalanine was synthesized from methylamine and α-bromopropionic acid, and this proved to be chromatographically indistinguishable from a fourth product in the hydrolysate.

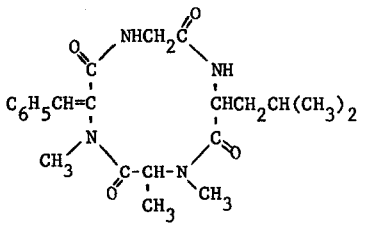


I

These data lead directly to the partial structure I for tentoxin (amino acid sequence not implied), in which X and Y are either joined (i.e. a cyclic tetrapeptide) or independent (a tripeptide with both end groups amidic). The protons shown in this structure readily account for all of the pmr signals except the τ 2.59 five-proton singlet and the τ 2.25 one-proton singlet, leaving these two pmr features and the 285 nm ultraviolet absorption to be derived from the distribution of C_8H_6 between X and Y. No reasonable distribution of the available C_8H_6 between independent end groups will accommodate

these spectroscopic properties, and tentoxin therefore must be cyclic. Two arrangements the C_8H_6 residue might give rise to the observed pmr and uv properties, $C_6H_5CH{=}C$ and $C_6H_5C{=}CH$, which leads to consideration of three structures for the fourth potential amino acid of the peptide: $C_6H_5CH{=}C(NHCH_3)CO_2H$, $C_6H_5C(NHCH_3){=}CHCO_2H$, and $C_6H_5C(CO_2H){=}CHNHCH_3$. Any of these would, of course, lead to methylamine as a hydrolysis product, together with an oxo acid or degradation product thereof.

Hydrogenation of tentoxin at atmospheric pressure over platinum in ethanol affords a mixture of dihydro, hexahydro, and octahydro products (molecular ions m/e 416, 420, 422). Acidic hydrolysis produces a mixture which upon thin-layer chromatography gives ninhydrin-positive spots corresponding to glycine, leucine, N-methylalanine, methylamine (presumably from incompletely hydrogenated by-products), and two additional substances. Through the sequence N-benzylation, Eschweiler-Clarke methylation, and hydrogenolytic debenzylation, phenylalanine was converted to its N-methyl derivative (4), hydrogenation of which over platinum afforded N-methyl-β-cyclohexylalanine. These two synthetic N-methyl-amino acids had chromatographic properties indistinguishable from those of the unidentified hydrolysis products. This allows selection of $C_6H_5CH{=}C(NHCH_3)CO_2H$ as the fourth potential amino acid in tentoxin, the uv absorption of which is in good agreement with that observed for α-acetamidocinnamic acid (λ_{max} 278 nm, ϵ = 8,300; in ethanol). Tentoxin is accordingly assigned structure II, with the sequence and configuration as yet undefined.



II

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REFERENCES

1) N. D. Fulton, K. Bollenbacher, and B. J. Moore, Phytopathology, 50, 575 (1960).

2) N. D. Fulton, K. Bollenbacher, and G. E. Templeton, Phytopathology, 55, 49 (1965).

3) G. E. Templeton, C. I. Grable, N. D. Fulton, and K. Bollenbacher, Phytopathology, 55, 1079 (1965); 57, 516 (1967).

4) P. Quitt, J. Hellerbach, and K. Bolger, Helv. Chim. Acta, 46, 327 (1963).