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STRUCTURE OF THE BIOLOGICAL ACTIVE CYCLOPEPTIDES PRODUCED BY ALTERNARIA MALI ROBERTS

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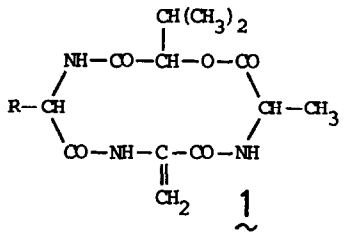
Previously we proposed the planar structure 1 for alternariolide, a toxin isolated from the culture broth of Alternaria mali Roberts and responsible for necrotic brown spots of apple, on the basis of spectroscopic evidence.[1] We describe here chemical studies, which establish the stereochemistry of the toxin and confirm the structure of the new component amino acid 2 (named as alternamic acid), as well as isolation of the second toxin from the same source.



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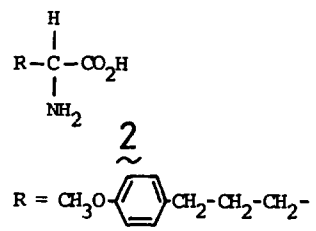

2

Acid hydrolysis (6N HCl / 16 hr / 115°C) of 1 afforded α-hydroxyisovaleric acid (identified as methyl ester by glc and nmr) as an ether soluble product, together with a volatile product pyruvic acid (identified as 2,4-DNPH), ammonia, alanine (identified by means of an amino acid analyser) and the new amino acid (2). The hydroxy acid exhibited in the ord spectrum a positive plain curve and accordingly the L configuration was concluded for this acid.[2] On treatment of the hydrolysate with L-amino acid oxidase chromatogram peaks due to alanine and alternamic acid disappeared, while in the case with D-amino acid oxidase the presence of the two amino acids were clearly indicated. Therefore each configuration of the two amino acids was also L.[3]

The structure of alternamic acid (2) was confirmed by the conventional Strecker synthesis from δ-p-methoxyphenylbutyraldehyde[4], obtained by reduction of the corresponding acid chloride[5] with $LiAlH(t\text{-}OBu)_3$. The HCl salt, mp 194-196°C, of the d,l acid 2 showed nmr peaks (D_2O, 60 MHz)

at δ 1.8 (4H,m), 2.60 (2H,t,J=6 Hz), 3.72 (3H,s), 4.0 (1H,m), 6.82 and 7.12 (4H,A_2B_2q, J=8 Hz). These spectral data and the retention time observed by means of an amino acid analyser were identical with those of the optical active amino acid, obtained from the hydrolysate described above. Consequently the structure of alternariolide is cyclo-[L-2-amino-5-(p-methoxyphenyl)-pentanoyl-dehydroalanyl-L-alanyl-L-α-hydroxyisovaleryl] (1).

A compound $C_{22}H_{30}N_4O_4$, mp 171-173°C, M^+ 414, $[\alpha]_D^{20}$ -244°(c=0.67), which exhibited similar, but less potent activity compared with 1, was isolated from the mother liquor of 1. The spectroscopic data[6] suggested the new toxin to be tentoxin[7], cyclo-(L-leucyl-N-methyl-trans-dehydrophenylalanyl-glycyl-N-methyl-L-alanyl) and in fact, the identity was confirmed by comparison of spectral data of the two compounds.

We are very grateful to Prof. W. L. Meyer, University of Arkansas, for sending us the nmr spectrum of tentoxin.

REFERENCES AND FOOTNOTES

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2. L-α-Hydroxyisovaleric acid prepared from L-valine[8] showed a positive plain curve and, on the other hand, D-acid did the mirror image.

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4. Oil; reasonable ir, nmr, mass spectral and analytical data have been obtained.

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6. Uv (λ_{max}^{EtOH}) 282 nm(ε=9300), ir ($\nu_{max}^{CHCl_3}$) 3350, 1670, 1630 and 1510 cm^{-1}; nmr ($CDCl_3$,60 MHz) δ 0.50, 0.62(each 3H,d,J=6 Hz), 1.25(2H,m), 1.52(3H,d,J=7 Hz), 1.7(1H,m), 2.81(3H,s), 3.18 (3H,s), 3.59(1H,d,J=15 Hz), 4.25(2H,m), 5.19(1H,q,J=15 Hz), 7.25(1H,m), 7.40(5H,s), 7.74 (1H,s) and 8.05(1H,d,J=10 Hz).

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