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CHARACTERIZATION OF ALTERNARIOLIDE, A HOST-SPECIFIC TOXIN PRODUCED BY <u>ALTERNARIA MALI ROBERTS</u>

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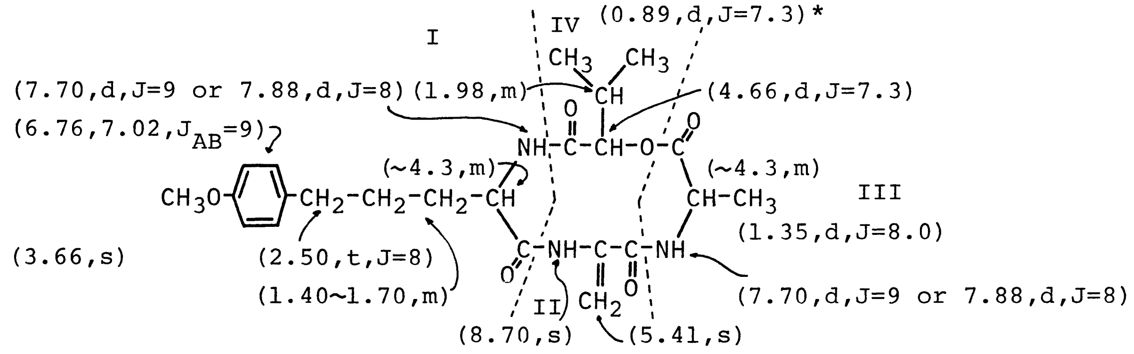
Alternariolide, a host-specific toxin responsible for Alternaria blotch of apple, has been characterized as a depsipeptide of the planar formula 1 on the basis of spectroscopic evidence.

Alternaria blotch caused by a pathogenic strain of <u>Alternaria mali Roberts</u> is one of the destructive diseases of apple. Some varieties of apple, such as Indo and Delicious, are highly susceptible to the disease.[1)] We recently reported isolation of a host-specific[2)] toxin produced by the fungus which caused necrotic brown spots characteristic for the disease on leaves and fruits of the plant.[3)] The present paper describes structural studies[4)] of the toxin, for which we suggest the name alternariolide.

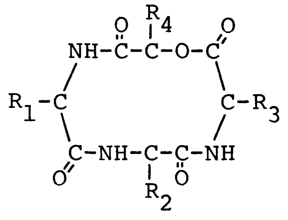
Alternariolide 1, mp 220-222°C, $[\alpha]_D^{18}$ -87.2° (c=1.66, $CHCl_3$), showed the M^+ peak at m/e 445.2194 corresponding to $C_{23}H_{31}N_3O_6$ (445.2210).[5)] The IR spectrum (KBr) suggested the presence of secondary amide groups (3325, 3300, 3280, 1660, 1630, 1535, 1515 cm^{-1}) as well as ester (1745 cm^{-1}), phenyl (3060, 1610, 1495, 820 cm^{-1}), methoxy (2840, 1250, 1050 cm^{-1}) and exomethylene (990, 900 cm^{-1}) groups. The NMR spectrum



*Chemical shifts and J values are given in δ and Hz units respectively.

1

(220 MHz, DMSO-d_6, 83°C$^{6)}$) exhibited peaks shown in formula 1. By addition of deuterium oxide, three 1H signals at δ 7.70 (d), 7.88 (d), and 8.70 (s) disappeared and therefore the two doublets were assigned to CH-N$\underline{H}$-CO protons and the singlet was ascribed to the amide proton of ■-N$\underline{H}$-CO type (■ denotes a carbon atom bearing no hydrogen atom). The resonance lines due to the two methines bearing the acylamino groups appeared as a 2H multiplet at δ 4.3 and a 1H doublet at δ 4.66 (J=7.3 Hz) indicated the presence of a methine linked to an acyloxy group. The 2H singlet peak at δ 5.37 is attributed to an exomethylene group.$^{7)}$ All above spectroscopic data, coupled with the elemental composition, are reasonably interpreted in terms of a tripeptide lactone structure 2$^{8)}$, in which one of R_1, R_2 and R_3 is the exomethylene group. Decoupling experiments at 60 MHz readily revealed the presence of



2

2-hydroxyisovaleric acid (H-IV-OH, see formula 1) and alanine (H-III-OH) and the structure of remaining amino acid, designated as alternamic acid, was deduced to be 2-amino-5-(p-methoxyphenyl)-pentanoic acid (H-I-OH) as follows. Since the NMR spectrum exhibits an aromatic methoxyl peak (δ 3.66) and a typical p-phenylene A_2B_2 quartet (δ_A 6.76, δ_B 7.02, J_{AB} = 9 Hz), the acid contains p-methoxyphenyl group. Extensive decoupling experiments (60 MHz) further indicated the presence of an eight-spin system conformable to the Ar-C$\underline{H}_2$-C$\underline{H}_2$-C$\underline{H}_2$-C$\underline{H}$(N$\underline{H}$-COR)-CO moiety (the assignment is given in formula 1). The four structural units of alternariolide have thus been clarified. The UV spectrum [λ_{max}^{EtOH} nm (ε); 224 (11,000), 275 (1,120), 284 (890)] is also consistent with the presence of α-acylaminoacrylamide and p-methoxyphenyl chromophores.

The high resolution mass spectrum provided not only supporting evidence for the structure of the above four components (Table 1), but also clue for the sequence of these units (Table 2). There are abundant peaks indicating the presence of connections II——III and moreover, the sequence I←—IV is demonstrated by the peaks No.90 and 88. The presence of the alanyl ester moiety (i.e. the linkage III—→IV) is shown by the fragment M - III - H_2 (No.99), since otherwise elimination

Table 1. Component analysis by exact MS.

Peak No.	Int. (%, rel.)	m/e (obs.)	Elements	Interpretation 9)
21	3	68.0122	C_3H_2NO	II - H*
24	6	70.0301	C_3H_4NO	III - H or IV + H
30	14	83.0496	C_5H_7O	IV - OH
31	2	84.0608	C_5H_8O	IV - O
33	3	97.0158	$C_4H_3NO_2$	$\mathrm{II}^{\bullet}$ + CO
73	4	175.0986	$C_{11}H_{13}NO$	I - CO - H_2
74	4	176.1085	$C_{11}H_{14}NO$	I - CO - H
75	9	178.1241	$C_{11}H_{16}NO$	I - CO + H
76	13	188.0835	$C_{12}H_{12}O_2$	I - NH_3
77	3	189.0921	$C_{12}H_{13}O_2$	I - NH_2
81	4	205.1105	$C_{12}H_{15}NO_2$	I

* The symbols (-) and (+) denote minus and plus signs respectively.

Table 2. Sequence analysis by exact MS.

Peak No.	Int. (%, rel.)	m/e (obs.)	Elements	Interpretation 9,10)
39	5	111.0559	$C_5H_7N_2O$	II + III - CO - H*
40	11	112.0626	$C_5H_8N_2O$	II + III - CO
41	8	113.0709	$C_5H_9N_2O$	II + III - CO + H
54	4	139.0512	$C_6H_7N_2O_2$	II + III - H
55	27	140.0579	$C_6H_8N_2O_2$	II + III
57	9	141.0651	$C_6H_9N_2O_2$	II + III + H
70	17	168.0537	$C_7H_8N_2O_3$	II + III + CO
71	2	169.0577	$C_7H_9N_2O_3$	II + III + CO - H
87	4	257.1046	$C_{15}H_{15}NO_3$	I + II - NH_3
88	3	272.1278	$C_{16}H_{18}NO_3$	I$\leftarrow$IV - H_2O - CH_3
90	7	287.1521	$C_{17}H_{21}NO_3$	I$\leftarrow$IV - H_2O
93	2	305.1612	$C_{17}H_{23}NO_4$	I + IV
99	8	356.1741	$C_{20}H_{24}N_2O_4$	M - III - H_2O

* see Table 1.

of alanine is highly improbable. The alternative possibility of dehydroalanyl ester structure is excluded by the IR band at 1745 cm^{-1}. Consequently, the planar formula <u>1</u> offers the most satisfactory explanation of all the spectral observations of alternariolide. Chemical and stereochemical studies of the toxin are now in progress and will be reported later.

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References and notes

1) K. Sawamura, Bull. Hort. Res. Station (Morioka), Series C, No.4, 43 (1966).

2) R.K.S. Wood, A. Granti, and A. Ballio Eds., Phytotoxins in Plant Diseases, Academic Press, New York, p. 251 (1972).

3) T. Okuno, Y. Ishita, S. Nakayama, K. Sawai, T. Fujita, and K. Sawamura, Ann. Phytopath. Soc. Japan, in press. Isolation of the same toxin has recently been announced also by T. Ueno, Y. Hayashi, T. Nakajima, H. Fukami, M. Nishimura, and A. Sekiguchi at the West Japan Local Meeting of the Agricultural Chemical Society of Japan on Jan. 10, 1974 (Okinawa).

4) The content of the present paper was read at the Annual Meeting of the Agricultural Chemical Society of Japan on Apr. 4, 1974 (Tokyo).

5) The molecular formula was supported by combution analysis.

6) At rt, line broadening was observed.

7) This signal was observed as two broad singlets at δ 5.23 and 5.36 at rt and the other ones scarcely changed.

8) For the NMR spectrum of the related depsipeptide valinomycin, see M. Ohnishi and D.W. Urry, Biochem. Biophys. Res. Comm., <u>36</u>, 194 (1969).

9) Here, for example, the fragment III does not necessarily mean $[NH-CH(CH_3)-CO]^+$. Other isomeric fragments such as $[OC-NH-CH(CH_3)]^+$ are of course also possible. However, these possibilities do not alter essentially the discussions in the text.

10) The arrow (→) means the direction of linkage, while the (+) sign does merely a linkage with unknown direction. For example, since the loss of H_2O from the fragment I→IV is highly improbable, the peak No.90 is interpreted as I←IV - H_2O.

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