Tetrahedron Letters Vol. 21, pp 1537 - 1540
0040-4039/80/0415-1537$02.00/0

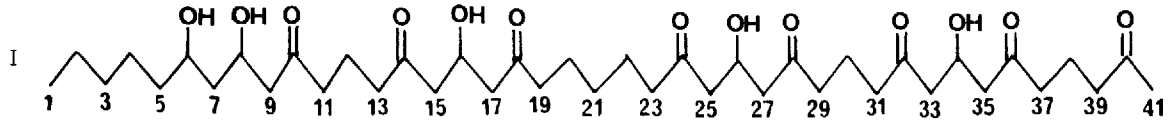
STRUCTURE OF THE HOST-SPECIFIC PATHOTOXINS PRODUCED BY *Helminthosporium maydis*, race T

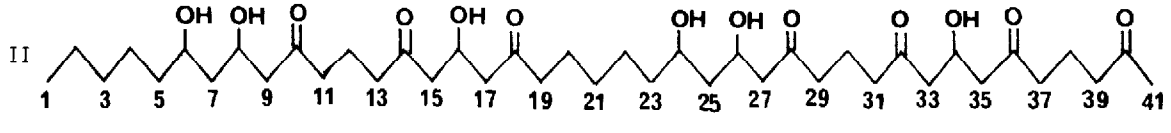
Y. Kono, S. Takeuchi and A. Kawarada
Lab. of Phytochemistry, The Institute of Physical and Chemical Research, Wako-shi, Saitama 351, Japan

J.M. Daly and H.W. Knoche
Lab. of Agricultural Biochemistry, University of Nebraska, Lincoln, Nebraska 68583, USA

Abstract: Polyketo-polyalcohol structures of the host-specific pathotoxins (Band 1- and Band 2-toxins) from *Helminthosporium maydis*, race T were elucidated by NMR analysis and chemical degradations.

The phytopathogenic fungus *Helminthosporium maydis*, race T, produces a complex of chemically analogous host-specific pathotoxins that have specific blighting effect on leaves of sensitive corn. We have earlier isolated four toxins from this origin, examined their physicochemical properties and proposed a tentative polyketo-polyalcohol structure for one of them, Band 1-toxin, which is the principal component of the toxin complex[1]. However, the precise positions of the oxygen groups on the long-chain carbon skeleton have not been established. In the present report we wish to present a complete structural assignments for the Band 1-toxin and the Band 2-toxin, which is a reduced analogue of the former. The unambiguous assignment of oxygen groups are given in structures I and II below.





Band 1-toxin is a colorless powder of mp. 132-133°C. Its molecular formula has been determined to be $C_{41}H_{68}O_{13}$ by high resolution MS of its penta-TMS derivative[1] and also by the high-resolution field-desorption MS of its sodium complex $C_{41}H_{68}O_{13}Na$ (m/e 791.4436, calc. 791.4556). λ_{max}^{MeOH} = 280 nm (ε = 154), $[\alpha]_D^{25°}$ = + 12.0 (c= 0.12, in MeOH). Its pentaacetate analyzed as $C_{51}H_{78}O_{18}$. The other, Band 2-toxin, is a colorless powder of mp. 127-128°C, with a molecular formula of $C_{41}H_{70}O_{13}$, as determined from the high-resolution MS of its hexa-TMS derivative[1], as well as by the low-resolution field-desorption MS of its sodium complex

$C_{41}H_{70}O_{13}Na$ (m/e 793). λ_{max}^{MeOH} = 280 nm (ε= 154), $[\alpha]_D^{25°}$ = + 17.5 (c= 0.16, in MeOH). Its hexaacetate analyzed as $C_{53}H_{82}O_{19}$. The results of ^{1}H-NMR with ^{1}H decoupling in the previous work[1] has suggested that the two toxins have many common partial structures but in different proportions in respective molecules: the three quintets (δ: 5.48, 5.22 and 4.90 ppm) that appeared for both toxins corresponded to 3H:1H:1H in the Band 1-toxin and 2H:2H:2H in the other, obviuosly due to the replacement of carbonyl group by a hydroxyl group in Band 2-toxin. The ^{13}C-NMR studies of Band 1-toxin and Band 2-toxin using single-frequency ^{13}C-{^{1}H} technique were carried out (Fig. 1), which revealed that the one of the two carbonyl groups in Band 1-toxin which is five methylene groups apart is reduced to a hydroxyl group. Chemical shifts were calculated using available data[2,3].

Band 1-toxin

	43.5	23.6	28.9	23.6	43.5	50.6	64.4	50.6
—CO—	CH_2	CH_2	CH_2	CH_2	CH_2	CO— CH_2	CH(OH)	CH_2 —CO—
calc.	43.9	23.6	28.5	23.6	43.9	50.5	63.4	50.5

Band 2-toxin

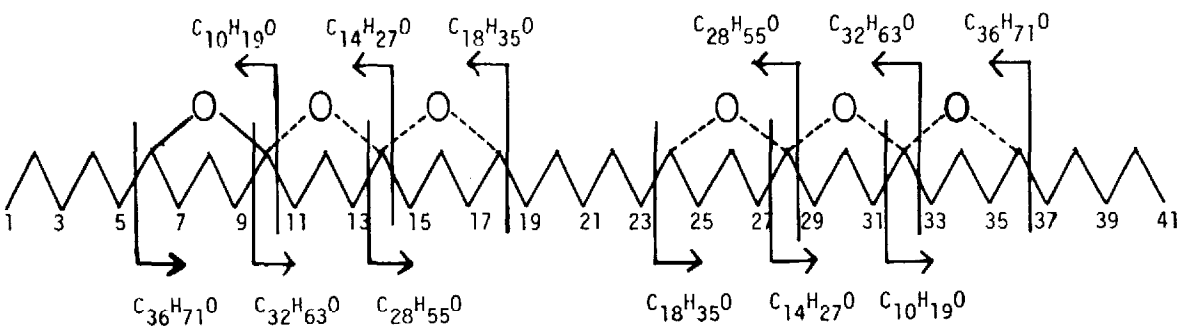
	43.5	23.9	29.6	25.8	38.7	70.7	44.7	68.0	50.6
—CO—	CH_2	CH_2	CH_2	CH_2	CH_2	CH(OH)	CH_2	CH(OH)	CH_2 —CO—
calc.	43.9	23.6	29.6	27.5	38.7	72.0	45.5	66.4	51.2

Fig. 1 ^{13}C-NMR assignment of the each characteristic parts of Band 1-toxin and Band 2-toxin

The presence of β-oxyoxo groups and absence of β-dioxo group in these toxins has been suggested from spectroscopic and chemical evidence[1]. Accordingly, each toxin (2-3 mg each) was oxidized with 0.2 ml of Jones' reagent (13 % CrO_3 in 4.3 N H_2SO_4) in 3 ml acetone at 40°C for 3 min, to convert hydroxyl groups into ketones; the formation of β-dioxo groups was expected. From mass and ^{1}H-NMR spectra, the products from both toxins were identical. The oxidized toxins were refluxed in 5 ml of 3 % potassium hydroxide in 80 % ethanol for 18 hrs to cause β-dioxo cleavage[4], and from each hydrolysate were isolated glutaric, pimelic and β-hydroxyoctanoic acids as main acidic products, which were identified through comparisons with authentic specimens by GC-MS. No detectable amount of similar acidic components were present in the hydrolysates of the toxin themselves. The likely presence of ζ-dioxo or ζ-oxyoxo and two δ-dioxo groups has been suggested previously[1], and the isolation of two dibasic acid from CrO_3 oxidation substantiated this supposition (ζ means five methylene groups apart). Examination of the results revealed that the number of the possible structures that are in accord with these data are restricted to three for Band 1- and five for Band 2-toxins.

What is left to be elucidated are the precise positions of ζ-dioxygenous and δ-dioxo groups, in order to establish the complete structures of the toxins.

Conversion of the Band 1- and 2-toxins to a hydrocarbon by removal of all oxygen groups was carried out according to Cope et al.[5], with some modifications. Each toxin (3-5 mg) was hydrogenated to a polyalcohol by catalytic hydrogenation with Adams platinum oxide (20 mg) in 10 ml of methanol-acetic acid (9:1) until keto groups disappeared. The resultant polyalcohols were converted to iodides in the following two-step treatment to avoid side reactions: in the milder first treatment the polyalcohols were refluxed for 24 hrs in a boiling mixture of red phosphorus (20 mg), hydriodic acid (3 ml) and n-heptane (2 ml). The iodides were dehalogenated with $LiAlH_4$ in 5 ml of tetrahydrofuran. The products were again halogenated as above but without n-heptane, and then were dehalogenated in the same manner as above. The products, from the two toxins, were separately hydrogenated with Adams platinum oxide as catalyst, and purified on silica gel columns with n-hexane to yield two fractions each. The first fraction of each gave an identical product that showed a molecular ion peak of m/e 576.6594 (n-$C_{41}H_{84}$, calc. 576.6573). The two second eluates of each were mixtures of products. Each of the mixtures seemed to consist of products carrying a single 4H-pyran ring on the carbon chain, according to the MS fragmentation patterns. The 4H-pyran ring must have come from cyclization of δ-dioxo or δ-oxyoxo groups during the above reductive treatments[6,7]. GC-MS of the two mixtures gave identical parent ion peaks of $C_{41}H_{82}O$ (m/e 590.6428, calc. 590.6488) and accompanying peaks by fragmentation. With high resolution GC-MS three pairs of prominent peaks which can be interpreted as the result of cleavage of the molecule on each side of 4H-pyran ring were observed. The MS data which gave evidence for the ultimate structures of the toxins are summarized in Fig. 2. The absence of peaks corresponding to $C_{22}H_{43}O$ and $C_{24}H_{47}O$ in the above



$C_{10}H_{19}O$	Obs.	155.1436	Calc.	155.1436	$C_{36}H_{71}O$	Obs.	519.5555	Calc. 519.5504
$C_{14}H_{27}O$	Obs.	211.2100	Calc.	211.2062	$C_{32}H_{63}O$	Obs.	463.4826	Calc. 463.4876
$C_{18}H_{35}O$	Obs.	267.2694	Calc.	267.2688	$C_{28}H_{55}O$	Obs.	407.4253	Calc. 407.4252

Fig. 2 HR-GC-MS of 4H-pyran compounds

mass spectrum indicated that no oxygen groups had been present between the C18 to C24 positions in the original toxins.

The high-resolution mass spectrometry of underivatized toxins in an EI mode provided fragment patterns which gave supporting evidence for the presumed structures of the toxins (Table 1). The presence of the fragments $C_{33}H_{48}O_5$ and $C_{25}H_{36}O_5$ in the dehydrated fragments of Band 2-toxin is explained by the presence of an additional hydroxyl group situated at C24 in the Band 2-toxin, which also supports the supposition of the positions of ζ-oxygen group on C18 to C24 in both toxins.

Table 1. Fragments in HR-MS of Band 1- and Band 2-toxins

Fragments	Obs.	Calc.	For
$C_8H_{13}O_3$	157.0879	157.0864	Band 1- and Band 2-toxins
$C_{10}H_{19}O_3$	187.1330	187.1333	Band 1- and Band 2-toxins
$C_{15}H_{24}O_2$	236.1775	236.1775	Band 1- and Band 2-toxins
$C_{16}H_{24}O_3$	264.1716	264.1724	Band 1- and Band 2-toxins
$C_{15}H_{22}O_4$	266.1520	266.1518	Band 1- and Band 2-toxins
$C_{25}H_{36}O_5$	416.2557	416.2562	Band 2-toxin
$C_{33}H_{48}O_5$	524.3504	524.3502	Band 2-toxin

The authors wish to present the structures I and II for Band 1- and Band 2-race T toxins respectively, based on the evidence described above. Both toxins show nearly identical biological activities[8,9].

Acknowledgment

We thank Dr.P. Lyon and Dr.M. Gross, University of Nebraska, Dr.K. Sasaki, Dr.S. Tanji and Dr.U. Miyazaki, Kakenkagaku Ltd., JEOL Ltd. and Hitachi Ltd. for high-resolution mass spectra Dr.J. Uzawa, Institute of physical and chemical Research, for NMR, and Dr.I. Nojima, JEOL Ltd for high-resolution field-desorption MS.

References

1. Y. Kono and J.M. Daly, Bioorganic Chemistry 8, 391 (1979).
2. J.B. Stothers, "Carbon-13 NMR Spectroscopy", Academic Press, New York, 1972.
3. L.F. Johnson and W.C. Jankowski, "Carbon-13 NMR spectra", Wiley-Interscience, New York, 1972.
4. R.B. Meyer and C.H. Hauser, J. Org. Chem., 25, 158 (1960)
5. A.C. Cope, R.K. Bly, E.P. Burrow, O.J. Ceder, E. Ciganek, B.T. Gillis, R.F. Porter and H.E. Johnson, J. Amer. Chem. Soc., 84, 2170 (1962).
6. M. Ikeda, M. Suzuki and C. Djerassi, Tetrahedron letters, 3745 (1967).
7. O. Ceder, J.M. Waisvisz, M.G. Van der Hoeven and R. Ryhage, Acta Chem. Scand., 18, 83 (1964)
8. G. Payne, H.W. Knoche, Y. Kono and J.M. Daly, Physiol. Plant Path., in press
9. G. Payne, Y. Kono and J.M. Daly, Plant Physiol., in press

(Received in Japan 25 January 1980)