Structural Characterization of Three New AAL Toxins Produced by *Alternaria alternata* f. sp. *lycopersici*

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Three new pairs of biologically active regioisomeric toxins (AAL toxins TC, TD, and TE) were isolated from liquid cultures of *Alternaria alternata* f. sp. *lycopersici*, purified using standard chromatographic procedures, and their structures elucidated following interpretation of spectra obtained from NMR and mass spectrometry experiments. Each of the toxin congeners is structurally similar to the AAL toxin TA, which was characterized earlier from culture filtrates of this fungus. AAL toxin TC resembles TA but differs in its lack of hydroxyl groups at C_4 and C_5. AAL toxin TE is the N-acetylated form of TC. Spectroscopic data are reported to confirm that TB is similar to TA but lacks a hydroxyl group at C_5, as suggested earlier. AAL toxin TD is the N-acetylated form of TB. All five pairs of regioisomers arise as products of fungal biosynthesis and do not appear to be generated during isolation. All regioisomeric pairs induce the genotype-specific necrosis characteristic of AAL toxin TA in tomato bioassays but differ markedly in relative toxicity.

INTRODUCTION

Alternaria alternata f. sp. *lycopersici* is a fungal pathogen which causes the alternaria stem canker disease of tomato (Gilchrist and Grogan, 1976). During disease development and in liquid culture, the pathogen secretes host-specific toxins (AAL toxins) which, in purified form, elicit cellular necrosis patterns characteristic of the disease (Siler and Gilchrist, 1983). The ability of the pathogen to infect leaves, stems, and green fruit of tomato is limited to genotypes that are homozygous for the recessive allele (*asc/asc*) of the Asc gene (Clouse and Gilchrist, 1986). The sensitivity of tomato tissues to the toxins also is regulated by the *Asc* gene, with the *asc/asc* genotype exhibiting the most sensitivity to the toxins. Thus, the toxins appear to function as chemical determinants of the stem canker disease (Clouse and Gilchrist, 1986).

The AAL toxins originally were detected as two ninhydrin-positive compounds isolated from cell-free culture filtrates of the fungus by thin-layer chromatography (TLC) (Bottini et al., 1981). Both purified compounds reproduced the necrotic symptoms of the disease, were genotype-specific in the induction of necrosis, and were toxic at equal molar concentrations. Each of the fractions consisted of a mixture of two structural isomers of tricarballylic acid esters at either C_{13} or C_{14} of an amino polyol backbone (designated AAL toxins TA_1,TA_2, TB_1, and TB_2) (Bottini and Gilchrist, 1981; Bottini et al., 1981). The structures of the TA isomers were completely elucidated (Figure 1), while the TB isomers were suggested as having the same isomeric pattern as TA but lacking the C_5 hydroxyl group (Figure 1). However, spectroscopic evidence for confirmation of the structure of the TB isomers has not been reported.

Until recently, the synthesis of the AAL toxins was considered to be unique to the genus *Alternaria* and the molecules were thought to be acutely toxic only to tomato cells. However, it was reported in 1988 that molecules structurally related to the AAL toxins were secreted by *Fusarium moniliforme* (Bezuidenhout et al.,1988). Given the trivial name, fumonisins, widespread interest in these molecules followed reports that animal (Marasas et al., 1988a; Gelderblom et al., 1988) and human diseases (Marasas et al., 1988b; Sydenham et al., 1990) associated with consumption of maize colonized by *F. moniliforme* were linked to fumonisins present in the grain. The predominant form of fumonisin, FB_1, also induced genotype-specific cell death in tomato leaf cells like the AAL toxins (Gilchrist et al., 1992). FB_1 and the AAL toxin TA inhibit cell proliferation in both rat liver and dog kidney cells (Mirocha et al., 1992). Inhibition of ceramide synthase in animal cells by both fumonisins and AAL toxins (Merrill et al., 1993) suggests a specific molecular target for the toxins in animals. The toxicological properties and structural similarities of AAL toxins and fumonisins have raised concern about the presence of both in the food chain (Mirocha et al. 1992; Pittet et al., 1992; Sydenham et al., 1992; Thiel et al., 1992) with an attendant threat to human and animal health (Gelderblom et al., 1992). The isolation and structural characterization of all related isomers of these toxins produced by *A. alternata* f. sp. *lycopersici* and *F. moniliforme* are necessary to critically evaluate biological activity, assess potential health risks, and address possible economic consequences of food contamination by this family of mycotoxins.

In this paper, we report the structural characterization of three new pairs of biologically active regioisomeric AAL toxins which were extracted from liquid cultures of *A. alternata* f. sp. *lycopersici*. The structure proposed by Bottini et al. (1981) for AAL toxin TB also was confirmed following interpretation of positive ion fast atom bombardment (FAB) mass spectrometry and NMR spectroscopy.

MATERIALS AND METHODS

Chemicals. Amberlite XAD-2 and *p*-anisaldehyde were purchased from Sigma Chemical Co. (St. Louis, MO). Silica gel used for flash chromatography (40-μm average particle diameter) was obtained from J. T. Baker Inc. (Phillipsburg, NJ). Strong anion-exchange columns (SAX) were purchased from Varian (Harbor City, CA). All organic solvents used were from Fisher Scientific, ACS or Optima grade. Methyl-d_3 alcohol-*d* (99.8%

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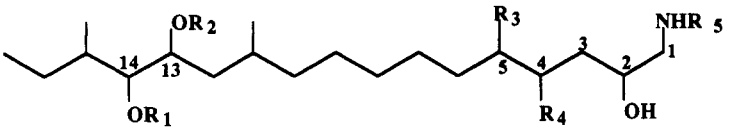

TOXIN	R1	R2	R3	R4	R5
TA1	H	CO-CH_2-CH(COOH)-CH_2-COOH	OH	OH	H
TA2	CO-CH_2-CH(COOH)-CH_2-COOH	H	OH	OH	H
TB1	H	CO-CH_2-CH(COOH)-CH_2-COOH	H	OH	H
TB2	CO-CH_2-CH(COOH)-CH_2-COOH	H	H	OH	H
TC1	H	CO-CH_2-CH(COOH)-CH_2-COOH	H	H	H
TC2	CO-CH_2-CH(COOH)-CH_2-COOH	H	H	H	H
TD1	H	CO-CH_2-CH(COOH)-CH_2-COOH	H	OH	C(=O)CH_3
TD2	CO-CH_2-CH(COOH)-CH_2-COOH	H	H	OH	C(=O)CH_3
TE1	H	CO-CH_2-CH(COOH)-CH_2-COOH	H	H	C(=O)CH_3
TE2	CO-CH_2-CH(COOH)-CH_2-COOH	H	H	H	C(=O)CH_3

Figure 1. AAL toxins.

D) was purchased from Aldrich Chemical Co. (Milwaukee, WI). All other reagents were of analytical grade. Silica TLC plates were purchased from Analtech (Newark, DE).

Fungal Cultures. The isolate of *A. alternata* f. sp. *lycopersici* (AS27-12) was originally isolated from a field-infected tomato plant, maintained in the laboratory on V-8 agar, and tested for both pathogenicity and toxin production before being used in these experiments. Both traits have remained stable in culture for more than 10 years in this isolate and AS27-3, which has been used routinely in our laboratory, gave the same results as reported herein. Liquid cultures were grown and processed as described previously (Clouse et al., 1985). Cultures that were frozen after harvesting were thawed, refiltered, and processed as described below. The presence of each of the toxin forms was confirmed in culture filtrates of isolate AS27-3 produced from 1985 through 1992 and other cultures routinely isolated from plants over this period.

Extraction and Purification. XAD-2 (50 g/L) was added to culture filtrates and stirred for 4–6 h, the adsorbed XAD beads were washed with water, and the toxins were eluted with 100% methanol. The extract was evaporated to dryness under vacuum at 50 °C, dissolved in ethyl acetate/acetic acid/hexane/water (6:2:2:1), and fractionated on a silica gel column (2.0 × 22 cm; 30 g) using the same solvent system as the mobile phase by flash chromatography. Collection of the effluent was initiated when the front, indicated by migration of a yellow nonphytotoxic component, reached the bottom of the column. Twenty fractions (5 mL) were then collected, at which point the mobile phase was changed to ethyl acetate/acetic acid/water (6:3:1), and additional fractions were collected at a flow rate of 7 mL min^{-1}. Fractions were evaluated for individual isomers by separation on analytical TLC using a solvent system consisting of ethyl acetate/acetic acid/water (6:3:1). The separated toxins were detected by spraying the plate with *p*-anisaldehyde [50 μL in 10 mL of methanol/acetic acid/sulfuric acid (8:1.0:0.5)]. Fractions containing a single component with the same R_f value were combined and evaporated to dryness. Fractions that revealed two or more *p*-anisaldehyde positive areas on the TLC plate were separated again using a second silica gel column (11 mm × 22 cm; 8.5 g) and the same solvent system as indicated previously. Passage through two columns was sufficient to resolve all of the *p*-anisaldehyde positive material.

Final purification prior to characterization was performed on a SAX column (3 mL) previously washed successively with 3 volumes of 0.1 N NaOH, sufficient water to pH 7.0, and then equilibrated with 100% methanol, pH 7.7. The pooled fractions containing the separated *p*-anisaldehyde positive compounds were dissolved in 100% methanol (pH 7.7), applied to the SAX column, washed with 3 mL of 100% methanol, and eluted with 1% acetic acid in methanol. The acidic effluent was evaporated at 50 °C under vacuum and rechecked for purity on analytical TLC prior to structural analysis.

Tomato Leaflet Bioassays. Tomato leaflet bioassays were conducted as described previously (Clouse and Gilchrist, 1986). Each bioassay was repeated three times with duplicate leaflets of the sensitive (*asc/asc*) and the resistant (*Asc/Asc*) isoline at concentrations ranging from 10 to 4000 ng mL^{-1} unless indicated otherwise. Five different preparations were assayed and characterized for TA and TB, four for TC, and two each for TD and TE. The purity of each separated regioisomer was confirmed by analytical TLC.

Spectroscopic Data. NMR spectra were obtained at 500.136 (^1H) or 125.758 MHz (^{13}C) on a GE Ω500 instrument, at room temperature, using a 5-mm probe. Chemical shifts are reported relative to the solvent (CD_3OD) peak, 3.30 ppm for ^1H and 49.00 ppm for ^{13}C. Correlated spectroscopy (COSY) experiments (Bax and Freeman, 1981) were performed with 128 experiments of 32 or 64 scans each and size 512. Positive ion FAB mass spectra were obtained using a VG ZAB-HS-2F double-focusing mass spectrometer with BE geometry (VG Analytical, Ltd., Wythenshawe, U.K.) from a matrix of 3-nitrobenzyl alcohol. Ionization was conducted using xenon atoms with a measured current of 1 mA and a beam energy of 8 keV. Daughter spectra were obtained using helium for collisional activation–dissociation/mass-analyzed ion kinetic energy spectrometry (CAD/MIKE) at a flow that gave 50% attenuation of the parent beam.

RESULTS AND DISCUSSION

Chromatographic evidence indicates that *A. alternata* f. sp. *lycopersici* produces at least five congeners of the AAL toxins, three of which had not been reported previously. In the original paper by Bottini and Gilchrist (1981), the reagent used to detect the toxins on the TLC plates (TA and TB) was ninhydrin, which reacts only with primary amines. Subsequent use of *p*-anisaldehyde, which reacts also with hydroxyl groups, permitted detection of two additional biologically active compounds with different R_fs, designated TD and TE, which constitute up to 40% of the total AAL toxins produced under the conditions used in these experiments (Table 1). The fact that the latter two species were ninhydrin negative indicated that the amino group was either absent or blocked in each case. The fifth AAL toxin form was detected when chromatographic separations were made of culture filtrates that had been highly concentrated before chromatography. This additional component was ninhydrin positive but occurred at less than 5% of the concentration of TA and TB

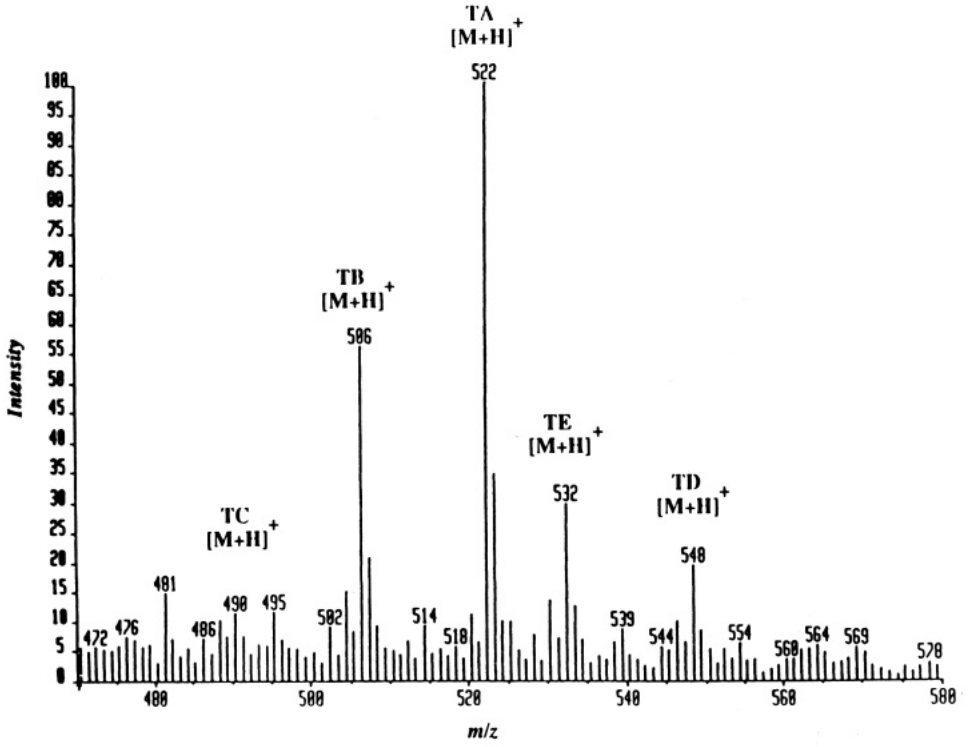

Figure 2. Positive ion FAB spectrum of AAL toxins isolated from culture filtrate using conditions described in the text. Protonated molecules are observed for the five toxins TA, TB, TC, TD, and TE.

(calculated by comparing the $[M + H]^+$ peaks of a FAB spectrum). The amount of TA + TB + TC ranged from 15 to 75 mg L^{-1} of culture filtrate, calculated using the trinitrobenzenesulfonic acid (TNBS) method for free amino groups (Habeeb, 1966). The designations proposed for all of the congeners reported herein are based on the order of the R_f values on analytical TLC (Table 1; Figure 1).

All five congeners were toxic to leaf tissue of the *asc/asc* isoline of tomato in the standard detached leaflet bioassay (Clouse and Gilchrist, 1985). In each case the genotype-specific difference, characteristic of TA, was observed, although significant differences in specific activity existed among the congeners (Table 1). The lower biological activity of the TD and TE, compared to TA, is in agreement with the previous report that blockage of the primary amine by acetylation or maleylation results in a reduction in absolute toxicity (Siler and Gilchrist, 1983; Gilchrist et al., 1992). In the present experiments, the relative toxicity of TD and TE was more than 100 times lower than that of the nonacetylated TA form. The activity of AAL toxin TC was lower than that of TA but was consistently higher than that of either of the ninhydrin negative congeners TD and TE.

Spectroscopic evidence for the presence of five congeners of the AAL toxins was obtained from positive FAB mass spectra of fractions isolated from filtrates of fungal cultures which consistently exhibited ions at m/z 522, 506, 490, 548, and 532, corresponding to $[M + H]^+$ ions for AAL toxins TA, TB, TC, TD, and TE (Figure 2), as determined from spectra of the individual toxins. The signal at m/z 522 (TA) is consistent with the structure proposed by Bottini and Gilchrist in 1981, and the mass of protonated TB (m/z 506) agrees with their suggestion that TB contained one fewer hydroxyl group than TA. The signal at m/z 490 indicates the presence of another congener (TC) in the series with two fewer hydroxyl groups than TA.

Signals corresponding to toxins TD and TE have molecular weights consistent with N-acetylated forms of TB and TC, which is consistent with their failure to give a positive reaction with ninhydrin. Interestingly, we did

Table 1. Toxicity of AAL Toxin Congeners to Detached Leaflets of *Lycopersicum esculentum*[a]

Rf[c]	TLC[d]	AAL-toxin congeners	Phytotoxic concentration of AAL-toxin congeners (ng ml^{-1})[b] Asc/Asc isoline	asc/asc isoline
0.80		TE	>4000	4000
0.73		TD	>4000	4000
0.61		TC	4000	300
0.48		TB	1000	10
0.37		TA	1000	10

[a] Detached leaflets of *L. esculentum* F_9 lines near-isogenic for the *Asc* gene were assayed for sensitivity to purified AAL-toxin congeners as described in the materials and methods section.

[b] Concentration of AAL-toxin congeners in ng ml^{-1} required to induce interveinal necrosis in 25% of the area of individual leaflets of the indicated isolines. Values represent the mean of four leaflets where the variation in necrosis among leaflets was less than 10% of mean at each concentration reported.

[c] Rf of the individual congeners TE to TA separated and visualized on analytical silica thin-layer chromatography as described in the materials and methods section.

[d] Figure represents a typical TLC separation conducted as indicated in the materials and methods section.

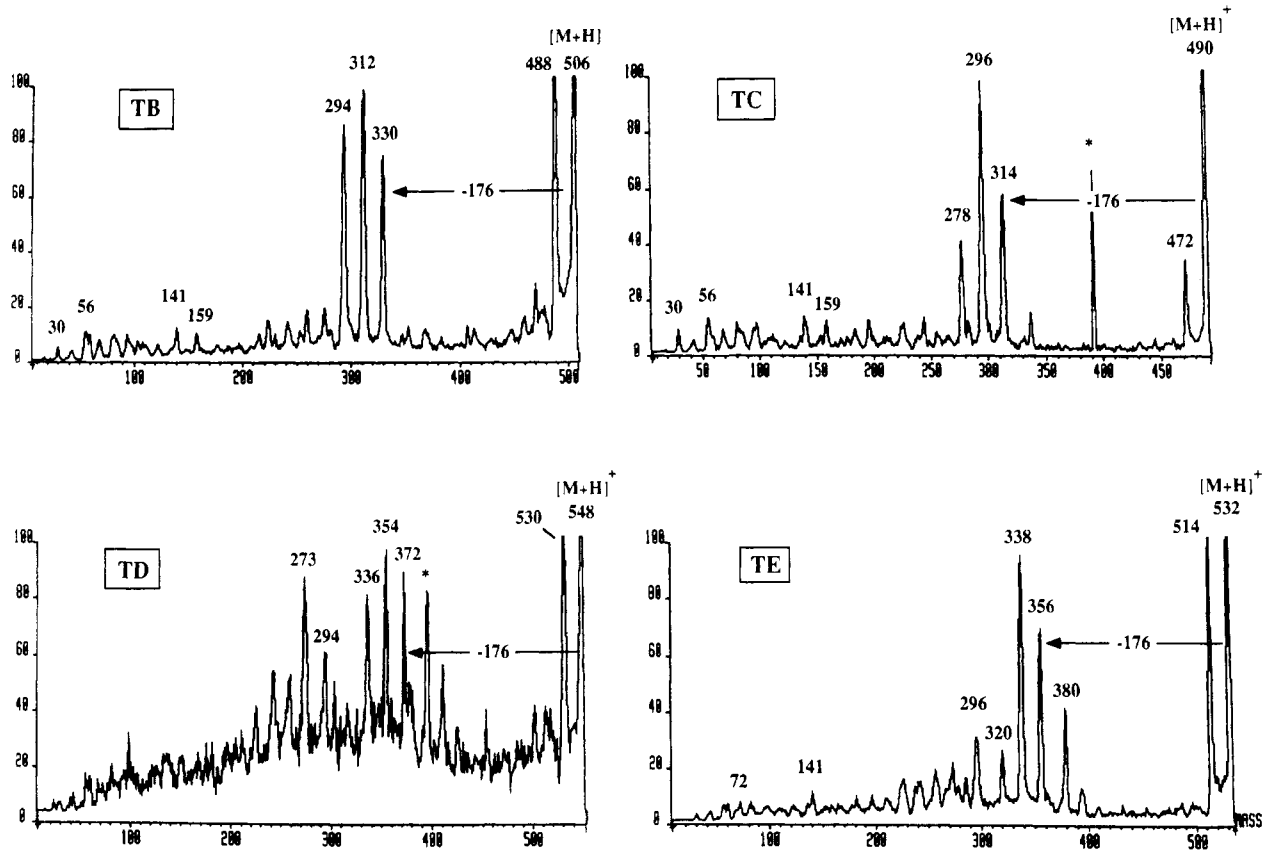

Figure 3. Daughter CAD/MIKE spectra for AAL toxins TB (B), TC (C), TD (D), TE (E). Spectra were obtained using conditions described in the text. Fragment ions attributed to the FAB matrix are labeled with an asterisk (*).

Table 2. ^{1}H NMR[a] Chemical Shifts for the AAL Toxins

	TA_1/TA_2[b]	TB_1/TB_2	TC_1/TC_2	TD_1/TD_2	TE_1/TE_2
H_1	3.05	3.03	3.01	3.25[c]	3.26[d]
$H_{1'}$	2.82	2.82	2.75	3.12	3.06
H_2	4.01	4.02	3.77	3.82[e]	3.60[f]
H_3	1.72	1.57	1.70	1.48	1.36
$H_{3'}$	1.51	1.44	1.46	1.45	1.33
H_4	3.67	3.79		3.76	
H_5	3.44	1.53		1.39	
H_6	1.36				

	TA_1	TA_2	TB_1	TB_2	TC_1	TC_2	TD_1	TD_2	TE_1	TE_2
H_{12}	1.68	1.31	1.63	1.31	1.67	1.31	1.60	1.31	1.60	1.31
H_{13}[g]	5.12	3.75	5.11	3.76	5.02	3.74	5.07	3.78	5.11	3.77
H_{14}	3.39[h]	4.75[i]	3.39[h]	4.77[i]	3.39[h]	4.75[i]	3.44[h]	4.77[i]	3.36[h]	4.78[i]
H_{15}	1.34	1.77	1.37	1.74	1.37	1.75	1.36	1.74	1.36	1.74

[a] Spectra obtained in a GE Ω500 instrument at 500.136 MHz. Chemical shifts are reported relative to the CD_3OD peak at 3.30 ppm. [b] (TA_1/TA_2...TE_1/TE_2) represent pairs of isomers that have the same chemical shifts for H1 to H6. [c] dd (J = 5.0, 7.0 Hz). [d] dd (J = 4.0, 4.5 Hz). [e] dd (J = 4.8, 5.2 Hz). [f] dd (J = 2.9, 3.0 Hz). [g] m (J = 2.2–2.8 Hz). [h] dd (J = 3.5, 3.7 Hz). [i] t (J = 5.7 Hz).

not find any evidence for an acetylated form of TA or any indication of fumonisin FB_1 produced by *A. alternata* f. sp. *lycopersici* as reported recently by Chen et al. (1992). Culture filtrates from this fungus, spiked with FB_1, gave distinct reproducible signals above background at m/z 722, where the added FB_1 concentration was less than 1% of the concentration of TA in a 27-day-old culture filtrate. However, no evidence of FB_1 was found in culture filtrates of isolates AS27-12 and AS27-3 which were produced and stored over the period from 1985 to 1992 as well as fresh cultures produced in 1993 using both static and continuous flow FAB mass spectrometry to attempt to detect the presence of FB_1.

FAB mass spectra of the *p*-anisaldehyde reactive compounds exhibit abundant signals corresponding to the expected protonated molecules related to TA, but this information alone is not sufficient to confirm that the signals arise from the AAL toxins. Daughter ion MS/MS spectra, generated for the $[M + H]^+$ ions corresponding to each of the five toxins, yielded an abundant fragment corresponding to $[M + H - 176]^+$ (Figure 3), which corresponds to the loss of a tricarballylic acid group. Furthermore, the daughter spectra also contained evidence of the number of hydroxyl groups which were indicated by the presence of a succession of fragments attributed to $[M + H - 176 - nH_2O]^+$, where n is an integer that ranges from 1 to the number of hydroxyl groups (Figure 3). The presence of the *N*-acetyl group in TD and TE was suggested by peaks in the daughter spectra at m/z 294 and 296, respectively (losses of tricarballylic acid, nH_2O, and an additional 42 daltons due to loss of ketene). Further evidence of the presence of the *N*-acetyl group came from daughter spectra generated for peaks at m/z 370 and 356 obtained via *in situ* hydrolysis of TD and TE on the FAB probe using 1% trifluoroacetic acid. In both cases, a characteristic fragment was observed at m/z 60, corresponding to $[CH_3CONH_2 + H]^+$ (data not shown).

The NMR data (^{1}H, COSY experiment, ^{13}C) of TA agreed with the results of Bottini et al. (1981) and form the basis for the assignments for TB, TC, TD, and TE. The ^{1}H NMR spectra of each toxin showed two characteristic pairs of peaks corresponding to the protons H_{13} and H_{14}. One pair corresponds to the isomer that has tricarballylic acid esterified to C_{13} (TA_1, TB_1, etc.), and the other pair corresponds to the isomer with esterification at C_{14} (TA_2, TB_2, etc.). The coupling network of these protons is shown clearly by the COSY spectra (Figure 4

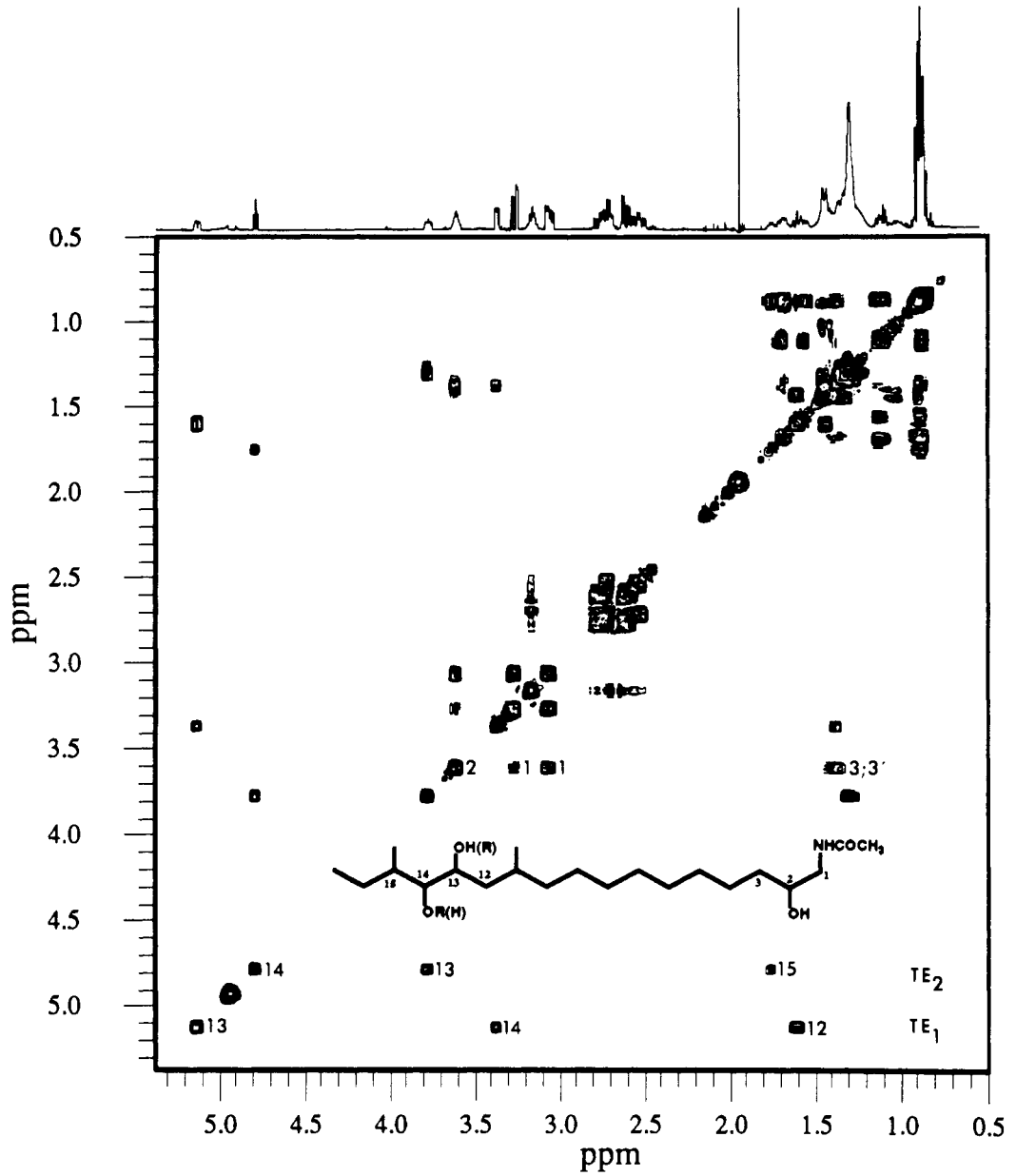

Figure 4. COSY spectra of TE showing the coupling patterns of the hydrogens at C_{12}, C_{13}, C_{14}, and C_{15} of isomers TE_1 and TE_2 and the coupling of the hydrogens at C_1, C_2, and C_3 in both isomers. The diagonal represents a contoured one-dimensional spectrum. The enumerated off-diagonal resonances represent the proton–proton coupling of the hydrogens at C_{13} and C_{14} of isomers TE_2 and TE_1 and the coupling of the hydrogens at C_2 in both isomers.

for TE). At this region, the ^1H chemical shifts of each isomer vary slightly among the toxins (Table 2).

The positions of the OH groups were assigned on the basis of the COSY experiment. A downfield multiplet was shown by TD proton H_2, indicating coupling with two downfield protons (H_1 and $H_{1'}$) and with two upfield protons (H_3 and $H_{3'}$). The hydrogens at C_1 (H_1 and $H_{1'}$) were assigned easily because they do not couple to any peak in the upfield region. Another downfield peak (H_4) couples with the protons on the C_3 position (H_3 and $H_{3'}$), showing that the second hydroxyl group is at C_4. The structure predicted by Bottini et al. (1981) for TB with the hydroxyl groups at C_2 and C_4 was confirmed by the current COSY spectrum for TB, which showed the same coupling pattern observed for TD. Toxins TC and TE exhibited COSY spectra with the same coupling pattern; each has one hydroxyl group at the C_2 position. A downfield proton (H_2) couples with two downfield protons (H_1 and $H_{1'}$) and with two upfield protons at the methylene region (H_3 and $H_{3'}$). The chemical shifts of the hydrogens for TA–TE are not affected by the position of the tricarballylic ester linkage at either C_{13} or C_{14} in the respective isomers (Table 2).

Toxins TD and TE showed peaks at 1.95 ppm, corresponding to the methyl protons of the *N*-acetyl group. Three sets of multiplets appear in the spectra of all five regioisomers, between 2 and 3.2 ppm, each of which showed coupling in the COSY experiment and correspond to the hydrogens of the saturated carbons of the tricarballylic acid group. The chemical shifts of these multiplets will vary with pH as expected with an increase in net charge associated with increasing pH. When assayed at pH 6.0, the chemical shifts were assigned as 3.08, 2.68, and 2.29 ppm, with only slight variation in the specific values for each toxin.

The ^{13}C spectra of TB showed two peaks in the carboxylic acid region, at 178.61 and 178.56 ppm, with relative intensities of 2:1, corresponding to the three carbonyl carbons of the tricarballylic acid. Bottini et al. (1981) found four peaks in this region for TA, two for each isomer. As

Table 3. ^{13}C NMR Shifts[a] of the Carbons Attached to the OH, Tricarballylic Acid (R), and Nitrogen of TA,[b] TB, TD, and TE

	TA_1/TA_2[c]	TB_1/TB_2	TD_1/TD_2	TE_1/TE_2
C_1	45.28	46.50	46.13	46.71
C_2	64.97	66.27	68.36	69.49
C_4	70.46	68.58	69.49	
C_5	74.70			

	TA_1	TA_2	TB_1	TB_2	TD_1	TD_2	TE_1	TE_2
C_{13}	74.46	69.16	74.62	69.75	69.56	68.94	74.89	71.33
C_{14}	76.30	81.72	77.30	82.12	77.49	82.24	77.58	82.30

[a] Spectra obtained in a GE Ω500 instrument at 125 MHz. Chemical shifts are reported relative to the CD_3OD peak at 49.00 ppm. [b] From Bottini et al. (1981). [c] (TA_1/TA_2...TE_1/TE_2) represent pairs of isomers that have the same chemical shifts for C_1, C_2, C_4, and C_5.

in the ^1H NMR spectra, the chemical shifts of these carbons also change with the pH (Plattner et al., 1992) and might account for the different number of peaks found in this work, when overlapping should have occurred. In contrast, TD and TE each showed four peaks in the carboxylic acid region and one additional peak at 173.64 ppm, which corresponds to the carbonyl group of the *N*-acetyl moiety. In the region between 65 and 85 ppm, the ^{13}C spectra of TD showed six peaks. Four peaks correspond to C_{13} and C_{14} (two for each isomer), and two are assigned as C_2 and C_4, the ones bound to the OH groups. TE showed five peaks, which indicates that it possesses one fewer hydroxyl group than TD (Table 3). The ^{13}C and ^1H chemical shifts assigned for TB, TC, TD, and TE indicate the four toxins share a common structural basis, directly related to TA.

Fumonisins also have been reported to occur as acetylated forms, FA_1 and FA_2 (Cawood et al., 1991), in cultures of *F. moniliforme*, but questions have been raised as to whether they are produced by the fungus or if their appearance is the result of exposure to acetic acid during isolation (Plattner et al., 1992) or when separated by TLC using solvent systems containing acetic acid. Our evidence indicates that the acetylated congeners TD and TE are present in the culture filtrate as products of fungal metabolism, rather than isolation artifacts, for three reasons. First, the acetylated AAL toxins are resolved in FAB/MS spectra of XAD and C_{18} fractionated preparations of the toxins that were not exposured to acetic acid prior to characterization. Second, rechromatography of purified TA, TB, and TC by TLC with the solvent systems containing acetic acid did not lead to the appearance of detectable additional TD or TE. Third, we have not detected an acetylated form of TA either in the culture filtrates or in preparations that had been exposed to acetic acid, as would be expected if acetylation was a random chemical event occurring after secretion by the fungus or during purification.

The subtle structural complexity of the AAL toxins and fumonisins is intriguing in terms of (a) the biosynthesis of the congeners, (b) the possible structure–function relationships to fungal ecology, and (c) the specific toxicity of the various forms against both animal and plant cells. Viewed as a set, the specific activities required to induce necrosis in tomato leaflets differed as much as 1000-fold among the AAL toxin congeners. This raises the question of whether TD and TE have the same mode of action as TA, TB, and TC in inducing necrosis in plant cells. The answer appears to reside with the fact that the differential sensitivity of the *Asc* isolines of tomato to the toxins is conserved against all five toxins, regardless of the relative toxicity of the individual toxins. We take this to confirm that the site of action is regulated in each case by the product of the *Asc* gene and that the same toxic mechanism in tomato is used by all AAL toxins, including TD and TE. Explanations for the differences in activity are unknown but may involve either transport into the cell or differences in binding kinetics at the target site.

The biological activity of TC, TD, and TE in animal systems is unknown but clearly needs to be assessed. It will be interesting to determine if the suggested target site in animal cells, ceramide synthase (Merrill et al., 1993), is differentially sensitive to the various toxin forms. Since one of the unique attributes of the AAL toxins and fumonisins is that they are biologically active against both plant and animal cells, it seems essential that the activity of each of the forms of the toxins produced by both *Alternaria* and *Fusarium* be evaluated for toxicity in both systems. Furthermore, it will be necessary to determine whether the acetylated and nonacetylated toxins interact, either positively or negatively, in animal systems before conclusions regarding potential impacts of the toxin congeners on animal health are reached. In a similar context, the potential presence of multiple forms of these sphingosine-like toxins in foodstuffs requires that assays for these mycotoxins be sufficiently sensitive to detect and distinguish the respective forms.

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