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Reaction Products (Isomers) of Two Metabolic Derivatives of T-2 Toxin (TC-1 and TC-3) When Reacted with Trifluoroacetic Acid Anhydride

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T-2 metabolites TC-1 and TC-3 (3′-hydroxy-T-2 toxin and 3′-hydroxy-HT-2 toxin) when reacted with trifluoroacetic acid anhydride each forms two isomers. The isomers resolve on SE-54 (DB-5) capillary columns and are unsaturated in either the C-3′ or C-2′ position. This characteristic reaction serves as an aid in locating other unknown metabolites of T-2 that contain a hydroxyl group on the C-3′ position.

T-2 toxin [4β,15-diacetoxy-8-[(3-methylbutyryl)oxy]-3-hydroxy-12,13-epoxytrichothec-9-ene] is a potent mycotoxin produced by some isolates of various species of *Fusarium* that can cause hemorrhaging, abortion, dermal necrosis, emesis, leukopenia, immunodepression, hindquarter paresis, and inhibition of protein synthesis in various animals and biological systems (Mirocha, 1983). When intubated into dairy cows, various metabolic derivatives were discovered, some of which were called TC-1 and TC-3 (Yoshizawa et al., 1981). The latter were identified as the C-3′ hydroxylation products of T-2 and HT-2, respectively (Yoshizawa et al., 1982). Their structures are shown in Figure 1. When reacted with silylating reagents such as TBT (Pierce Chemical Co.), one major trimethylsilyl ether derivative is formed of each metabolite that is resolvable by capillary gas chromatography. When TC-1 and TC-3 are reacted with trifluoroacetic acid anhydride, two isomers of each derivative are formed and are the subject of this paper. Trifluoroacetyl (TFA) derivatives were chosen because their detection sensitivity is much greater than that of Me_3Si derivatives in chemical ionization mass spectroscopy.

EXPERIMENTAL SECTION

Derivative Formation (Trimethylsilyl Ethers) of T-2, HT-2, TC-1, and TC-3 Toxins. Standards of T-2 and HT-2 toxin were obtained from *Fusarium* cultures that were extracted and purified in our laboratory. TC-1 and TC-3 were supplied by T.Y. and were obtained from a synthetic preparation. The purity of the toxins approximated 98%. Stock solutions (20 μL each) of 100 ng/μL were pipetted into a $^1/_2$-dram vial with a Teflon-lined screw cap. After the solvent was evaporated, 10 μL of the silylating reagent (Tri-Sil/TBT, Pierce Co., Rockford, IL) and 10 μL of chloroform were added. The samples were left at room temperature for 5 min to equilibrate, and then 1 μL was injected for analysis.

Derivative Formation (Trifluoroacetate) of T-2, HT-2, TC-1, and TC-3. Standards were transferred to $^1/_2$-dram vials as described in the silylation procedure above. The solvent was evaporated, and 200 μL of trifluoroacetic acid anhydride (Pierce Chemical Co., Rockford, IL) was added. The samples were heated at 60 °C for 20 min. The TFAA was then evaporated under nitrogen, and chloroform was added as the carrier solvent. One microliter was injected into the gas chromatograph.

Gas Chromatographic and Mass Spectral Conditions. A 30-m, 0.25-i.d. fused silica capillary column coated with dimethyl silicone gum (SE-54) was used. The samples were introduced by splitless injection with a delay time of 0.5 min. Flow rates were 60 mL/min, inlet, and 1.5 mL/min, exit. The injection port temperature was 250 °C. The GC oven was programmed from 80 to 300 °C at 30 °C/min.

The mass spectrometer was tuned with perfluorotributylamine with methane as the reagent gas. The source temperature was set at 150 °C, and pressure was measured at 0.6 torr for methane and 0.2 torr for ammonia. The electron energy was set at 200 eV.

RESULTS AND DISCUSSION

TC-1 (III), when reacted with a Me_3Si-donating reagent, will produce the predominant species consisting of the reaction product of two sylilated hydroxyl groups, one at C-3 and the other at C-3′, resulting in a molecular ion of 626. On the other hand, the TFA derivative of TC-1 forms two predominant isomeric species resolvable by capillary gas chromatography, neither of which represents reaction with two hydroxyls but rather only one of the pair. The two isomers formed have a molecular ion of 560. The molecular ion of the TFA derivative of the parent compound (T-2 toxin) is 562, 2 mass units more than that of

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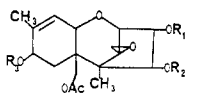
Table I. Physical, Chemical, and Chromatographic Properties of T-2, HT-2, TC-1, and TC-3

property	T-2	HT-2	isomer TC-1 one	isomer TC-1 two	isomer TC-3 one	isomer TC-3 two
empirical formula	$C_{24}H_{34}O_9$	$C_{22}H_{32}O_8$	$C_{24}H_{34}O_{10}$		$C_{22}H_{30}O_9$	
molecular weight	466	424	482		440	
M_r of TFA	562	616	560	560	614	614
M_r of Me_3Si	538	568	626		656	
retention time (GC, TFA),[a] min	12.9	10.9	13.2	13.8	11.2	11.5
R_f value (TLC)[b]	0.66	0.43	0.56		0.34	
reaction—*p*-anisaldehyde	purple	purple	purple		purple	
reaction—H_2SO_4	gray-brown	gray-brown	gray-brown		gray-brown	
reaction—Katos' reagent	blue	blue	blue		blue	

[a] GC conditions: Durabond-5 column (J & W Scientific, Inc., de Palma, CA), 30 M; flow 60 mL/min; temperature program 80–300 °C at 30 °C/min. [b] TLC conditions chloroform–MeOH, 90:1.



		R_1	R_2	R_3	
I	T-2 TFA	OC_2F_3	OAc	$(CH_3)_2CHCH_2CO$	
II	HT-2 TFA	OC_2F_3	OC_2F_3	$(CH_3)_2CHCH_2CI$	
III	TC-1	OH	OAc	$(CH_3)_2COHCH_2CO$	
IV	TC-1 TFA	OC_2F_3	OAc	$H_2C{=}CH_3CH_2CO$	ISOMER I
				$(CH_3)_2C{=}CHCO$	ISOMER II
V	TC-1 TMS	OTMS	OAc	$(CH_3)_2C(OTMS)CH_2CO$	
VI	TC-3	OH	OH	$(CH_3)_2COHCH_2CO$	
VII	TC-3 TFA	OC_2F_3	OC_2F_3	$H_2C{=}CH_3CH_2CO$	ISOMER I
				$(CH_3)_2C{=}CHCO$	ISOMER II
VIII	TC-3 TMS	OTMS	OTMS	$(CH_3)_2C(OTHS)CH_2CO$	

Figure 1. Structures of T-2 derivatives.

the corresponding TC-1. This can only be explained if the C-3′ hydroxyl is acylated and immediately deacylated, resulting in a net dehydration reaction. Protons for the dehydration are available from either the methylene carbon at C-4′ or the methyl carbon at C-2′. The resulting unsaturation results in two positional isomers as shown in Figure 2.

The above reaction can also be confirmed with the positive chemical ionization spectra (CH_4 plasma) of T-2 toxin and TC-1. Under these conditions the base peak (M^+ 401) remains the same but the molecular ion ($M^+ + 1$) for T-2 is 563 and $M^+ + 1$ for TC-1 is 561. The positive CI spectra become the basis for establishment of the molecular ion as both contain the methane dimer ($M^+ + C_2H_5$) and trimer ($M^+ + C_3H_5$), which helps locate the molecular ion. The positive CI mass spectra of these compounds are shown in Figure 3. The TC-1 and T-2 mass spectra are compared to show that the dehydration occurs in the isovaleroxy group on C-8. In the case of T-2, the isovaleroxy group cleavage represents the loss of 102 mass units from the $M^+ + 1$ forming ion 461. In the case of TC-1, ion 461 is formed by a loss of 100 mass units. Thus, the isovaleroxy group undergoes a change that results in a loss of 2 mass units and net unsaturation supposedly through dehydration.

In summary, TC-1 reacts with a sylilating reagent (TBT, Pierce Chemical Co.) to form the C-3 and C-3′ di(trimethylsilyl) reaction product. Conversely, the reaction with trifluoroacetic acid anhydride forms a C-3 monotrifluoroacetate derivative consisting of two isomers with a

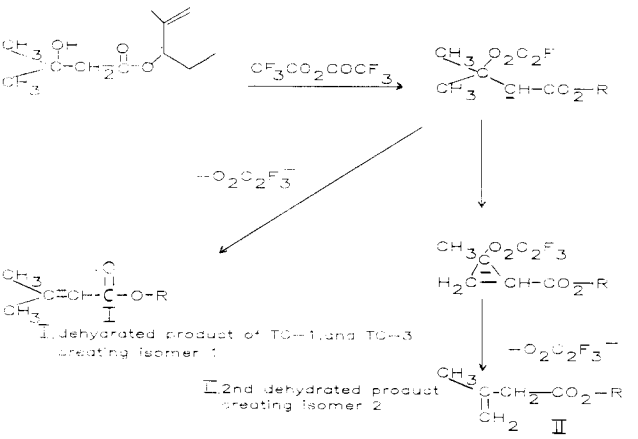


Figure 2. Dehydration with trifluoroacetic acid anhydride of the C-3 hydroxyl of the isovaleroyl group at C-8 by proton acquisition from the C-4′ methyl group or C-2′ methylene resulting in two isomers resolvable by capillary gas chromatography.

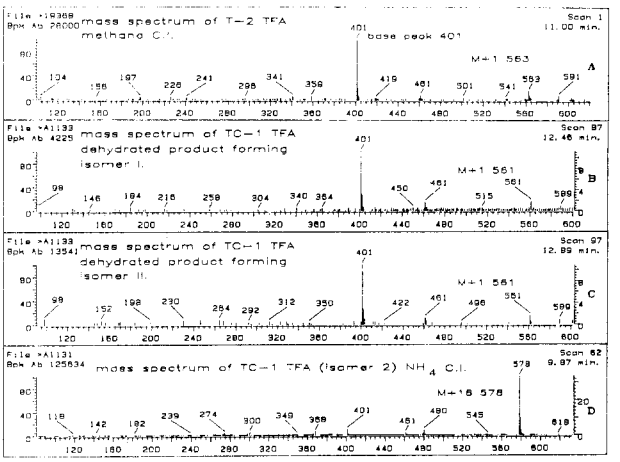


Figure 3. Mass spectra of monotrifluoroacetate derivatives of (A) T-2 toxin (positive chemical ionization in methane), (B) TC-1, isomer 1 (positive chemical ionization in methane), (C) TC-1, isomer two (positive chemical ionization in methane), and (D) TC-1, isomer one, positive chemical ionization in ammonia. Note the same base peak value in all positive CI methane spectra (M^+ 401) and a 2 mass unit difference between T-2 and TC-1 isomers.

molecular ion of 560. The isomers are formed by a random dehydration of the hydroxyl at C-3′ due to deprotonation at C-2′ (methylene group) or C-4′ (methyl group).

Similar to TC-1 is the C-3′ hydroxylation product of HT-2 (II) known as TC-3 (IV). It has reactive hydroxyls on C-3, C-4, and C-3′, and when reacted with a silylating reagent (TBT), all three hydroxyls are silylated, resulting in an M^+ of 656. When reacted with TFA anhydride, two isomers are found after resolution by capillary gas chro-

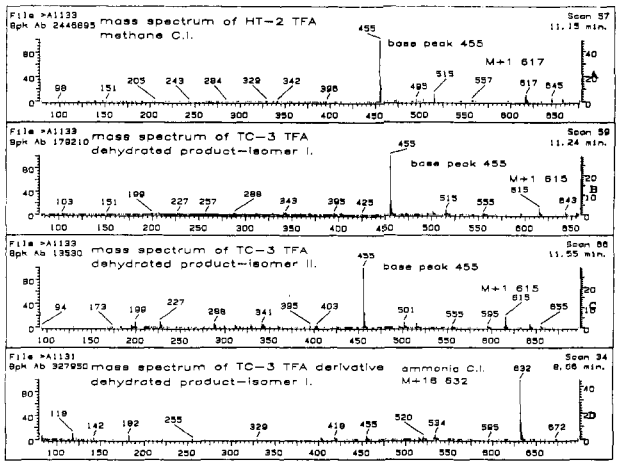


Figure 4. Mass spectra of di-TFA derivatives of (A) HT-2 toxin (+CI CH_4), (B) TC-3 isomer 1 (+CI CH_4), (C) TC-3 isomer 2 (+CI CH_4), and (D) TC-3 isomer 1 (+CI NH_3). Note the same base peak (M^+ 455) in all spectra (CH_4 CI) and a two proton difference in $M^+ + 1$ of T-2 and TC-3 isomers. The +CI in ammonia helps establish the molecular ion.

matography, one with a retention time of 11.2 min and the other 11.5 min (Table I). The mass spectrum of HT-2 at 70 eV shows a molecular ion of 616 in electron impact and 617 in positive chemical ionization (Figure 4) with a base peak of 455 in the latter. The TFA derivatives of TC-3 (both isomers) also show a base peak of 455 and a molecular ion at M^+ 615. Both isomers are dehydration products analogous to those found in TC-1 explained above. The identity of the molecular ion of TC-3 is further confirmed by the $M^+ + 18$ (632) shown in Figure 4 when detected by chemical ionization in NH_3.

Thus, the reactivity of TC-1 and TC-3 with trifluoroacetic acid anhydride forms isomeric dehydration products that simplifies procedures for the detection of C-3′ hydroxylated products of T-2 metabolism. This characteristic reaction has assisted us in detecting another T-2 derivative (TC-6) that produces isomers similar to the TFA reaction products of TC-1 and TC-3.

Registry No. III, 84474-35-1; VI, 78368-54-4; TC-1 (isomer I), 91860-58-1; TC-1 (isomer II), 91860-59-2; TC-3 (isomer I), 91860-60-5; TC-3 (isomer II), 91860-61-6.

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Metribuzin Metabolism in Soybeans. Characterization of the Intraspecific Differential Tolerance

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Differential tolerance of soybean [*Glycine max* (L.) Merr.] to [^{14}C]metribuzin was not due to absorption or translocation differences but due to the rate of metabolism. After 106-h treatment by subirrigation, the radioactivity in the susceptible cultivar "Semmes" was primarily in leaf interveinal tissue as unmetabolized metribuzin while in the tolerant cultivar "Coker 338" the majority of the radioactivity was in the more mature shoot tissue as polar metabolites and was restricted primarily to the vascular tissue. Differential tolerance was attributed to at least the following factors: (a) the restriction of metribuzin to the vascular tissue in tolerant "Coker 338" with movement mostly into the interveinal tissue in "Semmes", (b) the higher metribuzin concentration in "Semmes" leaves (9 μg/g of dry weight) than in "Coker 338" leaves (3 μg/g of dry weight), and (c) the higher rate of polar product (metribuzin conjugate) formation in "Coker 338".

Metribuzin, 4-amino-6-*tert* butyl-3-(methylthio)-*as*-triazin-5(4*H*)-one, is an asymmetrical triazine herbicide used on soybeans. However, cultivar tolerance to metribuzin varies dramatically (Andersen, 1976). Intraspecific differential tolerance is apparently due to differential rates of metabolism (Mangeot et al., 1979; Smith and Wilkinson, 1974). Metribuzin is metabolized to polar and nonpolar metabolites and incorporated into the insoluble residue (Mangeot, et al., 1979; Smith and Wilkinson, 1974). Smith and Wilkinson (1974) reported that tolerance resulted from metribuzin detoxification through polar conjugate formation. Mangeot et al. (1979) indicated that formation of the 6-*tert*-butyl-3-(methylthio)-*as*-triazin-5(4*H*)-one (DA) nonpolar metabolite, numerous unidentified aqueous metabolites, and incorporation into the insoluble fraction all contributed to cultivar tolerance to metribuzin. In tomato, the polar metabolites have been identified as the β-D-(*N*-glucoside) and malonyl β-D-(*N*-glucoside) conjugates of metribuzin (Frear et al., 1983).

The objective of this study was to characterize, more definitively, the cause(s) of soybean intraspecific differ-

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