Short Communication

3'-Hydroxy T-2 and 3'-Hydroxy HT-2 Toxins: New Metabolites of T-2 Toxin, a Trichothecene Mycotoxin, in Animals

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T-2 toxin, 4 β ,15-diacetoxy-8 α -(3-methylbutyryloxy)-3 α -hydroxy-12,13-epoxytrichothec-9-ene (**I**), is one of the most important trichothecene mycotoxins occurring naturally in various agricultural products, and is possibly involved in sublethal and lethal toxicoses of man and farm animals.¹⁾

The toxin, when orally administered to rodents, chickens and a lactating cow, is rapidly metabolized into unidentified compounds and eliminated into the excreta without its specific accumulation in any organ.^{2~4)} In this paper, we wish to describe the structural confirmation of major metabolites, named TC-1 and TC-3,⁴⁾ in a lactating cow.

A urine sample (500 ml) was collected from a lactating Jersey cow 12 hr after the oral administration of **I** (180 mg), and introduced successively into an Amberlite XAD-2 column (2 $\times$ 45 cm, eluted with 90% methanol in water), a Florisil column (1.5 $\times$ 17 cm, eluted with chloroform-methanol, 9:1), and a Sep-Pak C₁₈ cartridge (Waters Associates Inc., Mass.) according to the procedure described previously.³⁾ The major metabolites, TC-3 and TC-1 were eluted from the cartridge with 50% and 70% methanol in water, respectively. Each individual eluate was applied onto a silica gel TLC plate (20 $\times$ 20 cm, 0.25 mm gel thickness) and developed in chloroform-methanol (9:1).

Zones corresponding to TC-1 (between **I** and neosolaniol, **VIII**) and TC-3 (between HT-2 toxin, **II** and 4-deacetylneosolaniol, **IX**) were scraped off and eluted with acetone. Each eluate was further purified by rechromatography on the silica gel TLC plate using a solvent system of chloroform-acetone (3:2). Both TC-1 and TC-3 thus purified gave blue color spots on the TLC plate by treating with 4-(*p*-nitrobenzyl)pyridine,⁵⁾ suggesting the presence of the intact 12,13-epoxy ring in both metabolites. The *R_f* values of TC-1 and TC-3 were 0.51 and 0.26 (chloroform-methanol, 9:1), and 0.35 and 0.10 (chloroform-acetone, 3:2), respectively.

Both metabolites afforded T-2 tetraol (**X**) by hydrolysis with ammonium hydroxide in methanol, indicating that they contain the intact trichothecene nucleus in the molecule. By GC-MS, after derivatization to trimethylsilyl (TMS) ethers with TRI-SIL'TBT' reagent (Pierce Chemical Co., Ill.), their molecular ions were *m/z* 626 for TMS-TC-1 and at *m/z* 656 for TMS-TC-3. Diagnostic fragments at *m/z* 436 for TMS-TC-1 and at *m/z* 466 for TMS-TC-3 indicated the ions of 7,9-diene structures, which were also observed in the TMS ethers of **I** and **II**, respectively, and another diagnostic ion at *m/z* 83 (CH₂=C(CH₃)CH₂CO $\cdot$ or (CH₃)₂C=CHCO $\cdot$) was found in both metabolites instead of *m/z* 85 ((CH₃)₂CHCH₂CO $\cdot$) which was detected in **I** and **II**. These results indicate that a hydroxy group was located at either the C-3' or C-4' position of the C-8 substituent.

Both TC-1 and TC-3 were quantitatively derivatized to TMS ethers within 5 min with the TBT reagent, whereas with TRI-SIL'BT' reagent (Pierce Chemical Co., Ill.) it took more than 60 min for the etherification. Based upon the apparent difference in the reactivity among the metabolites, it is reasonable to conclude that a hydroxy group on the side chain must be situated at C-3' rather than at C-4'. Consequently, 3'-hydroxy T-2 toxin, 4 β ,15-diacetoxy-3 α -hydroxy-8 α -(3-hydroxy-3-methylbutyryloxy)-12,13-epoxytrichothec-9-ene (**IV**) and 3'-hydroxy HT-2 toxin, 15-

acetoxy-3 α ,4 β -dihydroxy-8 α -(3-hydroxy-3-methylbutyryloxy)-12,13-epoxytrichothec-9-ene (**V**) are proposed for the chemical structures of the new metabolites, TC-1 and TC-3, respectively, which correspond to the ω -1 oxidation products of **I** and **II**, respectively.

Compounds **IV** and **V** were synthesized as follows: a) T-2 tetraacetate (**VI**) prepared from **X** was selectively hydrolyzed to 3-acetylneosolaniol (**VII**) with swine liver homogenates in a Tris-HCl buffer (pH 7.5)⁶; b) the product was esterified with 3-hydroxy-3-methylbutyrylic acid in the presence of 4-dimethylaminopyridine and dicyclocarbodiimide⁷ to afford 3-acetyl-3'-hydroxy T-2 toxin (**III**); c) finally, the ester was partially hydrolyzed at the C-3 and C-4 ester-linkages with ammonium hydroxide to afford **IV** and **V**. The spectroscopic properties of these compounds were as follows; compound **IV**, IR $\nu_{\max}^{\text{KBr}}$ cm^{-1} : 3470 (–OH), 1730 (–COO–), 1370 (CH_3COO –), and 1245 (CH_3COO –), PMR (in CDCl_3 , 90 MHz) δ : 0.78 (3H, C-14, s), 1.27 (6H, C-4' and C-5', s), 1.72 (3H, C-16, s), 2.00 and 2.09 (each 3H, two acetyl, s), 2.3 to 2.5 (2H, C-7), 2.41 (2H, C-2', s), 2.74 and 3.00 (each 1H, C-13, d, $J=4$ Hz), 3.61 (1H, C-2, d, $J=5$ Hz), 3.97 and 4.23 (each 1H, C-15, d, $J=12.5$ Hz), 4.09 (1H, C-3, q, $J=3$ and 5 Hz), 4.27 (1H, C-11, d, $J=6$ Hz), 5.27 (1H, C-4, d, $J=3$ Hz), 5.28 (1H, C-8, d, $J=5.5$ Hz), and 5.74 (1H, C-10, d, $J=6$ Hz), and compound **V**, IR $\nu_{\max}^{\text{KBr}}$ cm^{-1} : 3440 (–OH), 1720 (–COO–), 1370 (CH_3COO –), and 1240 (CH_3COO –), PMR (in CDCl_3 , 90 MHz) δ : 0.75 (3H, C-14, s), 1.27 (6H, C-4' and C-5', s), 1.72 (3H, C-16, s), 2.01 (3H, acetyl, s), 2.39 (2H, C-2', s), 2.2 to 2.4 (2H, C-7), 2.71 and 2.97 (each 1H, C-13, d, $J=4$ Hz), 3.53 (1H, C-2, d, $J=4.5$ Hz), 3.88 and 4.26 (each 1H, C-15, d, $J=12$ Hz), 4.09 (1H, C-11, d, $J=6$ Hz), 4.29 (1H, C-4, d, $J=3$ Hz), 5.27 (1H, C-8, d, $J=5$ Hz), and 5.70 (1H, C-10, d, $J=6$ Hz). A singlet resonance due to *gem*-dimethyl protons of the C-8 substituent in compounds **IV** and **V** was observed at δ 1.27 instead of a doublet resonance at δ 0.96 ($J=6$ Hz) in **I** and **II**. The synthetic compounds were identical with the urinary metabolites by co-chromatographies

on TLC and GLC. Furthermore, mass spectra of the TMS-ethers of **IV** and **V** were also identical with those of the ethers of TC-1 and TC-3, respectively.⁸

The LD₅₀ values of **IV** and **V** in intraperitoneally administered male mice of ddys strain were 4.63 ± 0.63 and 22.8 ± 2.0 mg/kg, respectively. The former was slightly higher than that of **I** (5.31 ± 0.46 mg/kg), whereas the latter was approximately 30% in comparison with that of **II** (6.50 ± 0.51 mg/kg). The histological findings of mice given **IV** were necrotic epithelia in the intestinal mucosa and atrophic or hypoplastic changes in the thymus, spleen and bone marrow.

The metabolites **IV** and **V** were found in the plasma and milk in addition to the bovine excreta,⁴ and may correspond to unidentified metabolites found in the excreta of chicks and rats.^{2,3} This suggests that many animals are capable of metabolizing T-2 toxin into **IV** and **V**, which are diagnostic indices for estimating intake of the toxin.

TABLE. CHEMICAL STRUCTURES OF T-2 TOXIN AND ITS DERIVATIVES

	R ¹	R ²	R ³	R ⁴
I	H	Ac	Ac	–COCH ₂ CH(CH ₃) ₂
II	H	H	Ac	
III	Ac	Ac	Ac	
IV	H	Ac	Ac	–COCH ₂ C(OH)(CH ₃) ₂
V	H	H	Ac	
VI	Ac	Ac	Ac	Ac
VII	H	Ac	Ac	Ac
VIII	H	Ac	Ac	H
IX	H	H	Ac	H
X	H	H	H	H

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