TRICHOTHECENE METABOLISM STUDIES. 2. STRUCTURE OF
3α-(1"β-D-GLUCOPYRANOSIDURONYL)-8α-ISOVALERYLOXY-SCIRPEN-3,4β,
15-TRIOL 15-ACETATE PRODUCED FROM T-2 TOXIN IN VITRO

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Abstract

The preparation and structure determination of the title compound is described.

The epoxytrichothecene mycotoxins are a large family of fungal metabolites that exhibit a range of impressive biological activities.[3] They are potent inhibitors of protein synthesis in eukaryotes and many have been implicated in diseases of plants, animals, and humans.[3a] In spite of the toxicological significance of these compounds, however, relatively little is known about their metabolic fate in mammalian systems.[4,5] We recently described the in vitro production of glucuronide 2 from anguidine (1) and provided the first concrete evidence that
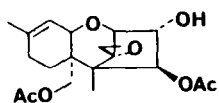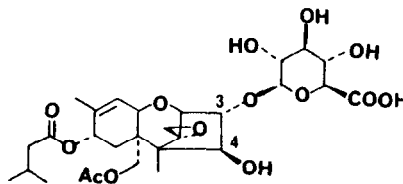

1
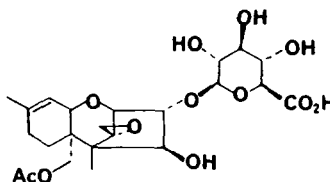
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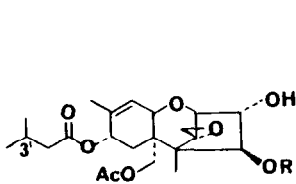
3, R=Ac
5, R=H

4

glucuronidation may be a significant pathway for the trichothecene metabolism in vivo.[4] Questions remained, however, about the generality of this conclusion. Consequently we have initiated a parallel series of experiments using T-2 toxin (3) and report herein the enzymatic production and structure determination of glucuronide 4 as the major in vitro T-2 conjugate.[6]

[^{3}H] T-2 toxin (4.7 nM, 0.026 μCi) [7] was incubated with uridine 5'-diphosphoglucuronic acid (UDPGA, 12 mM), β-naphthoflavone-induced hepatic microsomes from male Long-Evans rats (1.2 mg protein/mL),[8] $MgCl_2$ (10 mm), and phosphate buffer (10 mM, pH 7.7) at 37°C. HPLC analysis of the mixture after 2 h indicated the presence of a new product (49%) glucuronide 4 (R_t 15.5 min).[9,10] The same product was produced by incubation of T-2 toxin (150 μM) with [^{14}C] UDPGA (0.7 μM, >180 mCi/mmole) using the protocol outlined above. Scale-up of this procedure[11] using unlabelled T-2 toxin followed by HPLC purification[10] afforded pure 4[12] (10% isolated yield).

The FAB mass spectrum[13] of 4 is consistent with a 1:1 adduct of HT-2 toxin (5) and glucuronic acid [m/e 645 (MNa_2^+), 623 (MNa^+), 601 (MH^+), 389 ($M^+-C_6H_9O_7-H_2O$), 307 ($M^+-C_6H_9O_7-C_5H_9O_2$)], a conclusion supported by ^{1}H NMR data which showed a single acetyl resonance.[12] Comparison of the ^{1}H signals for H-4 (δ 4.58), H-3 (4.44) and H-15 (4.30 and 3.99) of 4 measured in MeOH-d_4 with those of authentic HT-2 toxin (5), (δ 4.38, H-4; 4.10, H-3; 4.27 and 4.00, H-15) suggested that 4 is a glucuronide derivative of 5.[14] The linkage between the trichothecene nucleus and glucuronic acid was determined by conversion to the peracetate methyl ester derivative 6.[15] This compound was identical to an authentic sample synthesized from T-2 toxin (3) and bromosugar 7 by a Koenigs-Knorr reaction (20% yield; 94% based on recovered T-2 toxin).[16] Thus, the structure of this metabolite is correctly described by formula 4.
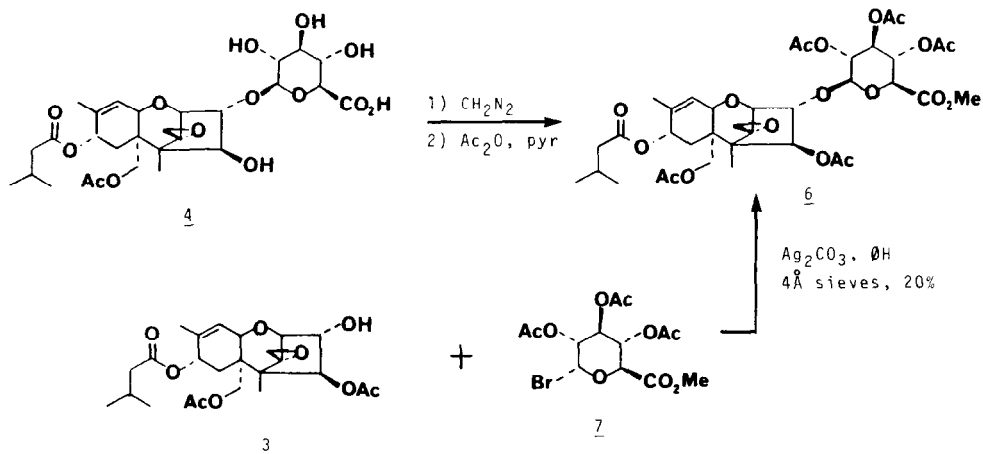
This experiment shows that T-2 toxin or its well-known hydrolysis product HT-2 (5) are viable substrates for microsomal glucuronyl transferase. Based on our previous experience with anguidine, it is likely that T-2 toxin is hydrolyzed to HT-2 before conjugation with UDP-glucuronic acid.[4] Nevertheless, it is now reasonable to speculate that glucuronic acid conjugates of T-2 metabolites[5a-c] will be produced in vivo. Structural studies of in vivo metabolites will be reported in due course.[17]

References

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6. The only metabolic transformations of 3 documented prior to our work had been deacylation reactions catalyzed by microsomal esterases,[5f-i] and the P_{450} mediated hydroxylation of the 3'-position.[5a,b]

7. We thank Dr. K. Hunter (Uniformed Services University of Health Sciences, Dept. of Defense, Bethesda, Maryland) for a generous sample of [³H] T-2 toxin.

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9. The microsomes were removed by centrifugation at the end of the incubation. The products were concentrated on a C18 Sep-Pak cartridge (Waters Assoc.) and then were separated by HPLC.

10. The μ-Bondapak C18 column (3.9 mm x 30 cm, Waters Assoc.) was used for all analyses and isolations (100% H_2O for 2 min, 0-45% MeOH linear ramp for 15 min., 45-60% MeOH linear ramp for 15 min., 1.5 mL/min).

11. UDPGA (12 mM), T-2 toxin (738 μM) and 1.22 mg/mL of microsomal protein (Sprague-Dawley rats) were incubated for 3.5 h.

12. Data for 4: ¹H NMR (MeOH-d₄, 250 MHz) δ 5.75 (d, 1 H, J=5.6 Hz, H-8), 5.31 (d, 1 H, J=5.0 Hz, H-10), 4.68 (d, 1 H, J=7.6 Hz, H-1"), 4.58 (d, 1 H, J=3.0 Hz, H-4), 4.44 (dd, 1 H, J=3.1, 4.8 Hz, H-3), 4.30 (d, 1 H, J=12.4 Hz), 4.23 (d, 1 H, J=5.8 Hz, H-11), 3.99 (d, 1 H, J=12.4 Hz, H-15B), 3.68 (d, 1 H, J=4.9 Hz, H-2), 3.5-3.2 (m, partially obscured by solvent peak, sugar), 2.96 (d, 1 H, J=4.1 Hz, H-13A), 2.77 (d, 1 H, J=4.0 Hz), 2.38 (dd, 1 H, J=5.6, 15 Hz, H-7A), 2.05 (s, 3 H, -OAc), 1.73 (s, 3 H, H-16), 1.00 (m, 6 H, H-4' isovaleryl), 0.82 (s, 3 H, H-14); FAB mass spectrum (glycerol dispersion) m/e 645

(MNa_2^+), 623 (MNa^+), 601 (MH^+), 389 $(M^+-C_6H_9O_7-H_2O)$, 307 $(M^+-C_6H_9O_7C_5H_9O_2)$.

13. The FAB mass spectral measurements were performed by Dr. C. Costello and S. Maleknia using the facility supported by NIH Research Grant #RR00317 from the Biotechnology Resources Branch, Division of Research Resources (Principal Investigator: Prof. K. Biemann).

14. The corresponding chemical shifts for H-4 (δ 5.70), H-3 (δ 4.23) and H-15 (4.32 and 4.10) of T-2 toxin (3) indicate that H-4 is not acylated in metabolite 4.

15. Data for 6: mp 82-84°C; $[\alpha]_D^{20}$ - 3.3° (c=0.61, $CHCl_3$); ^{1}H NMR ($CDCl_3$, 250 MHz) δ 5.88 (d, 1 H, J=2.7, H-4), 5.74 (d, 1 H, J=5.8 Hz, H-8), 5.25 (m, 3 H, H-8 and 2 sugar H's), 5.10 (m, 1 H, sugar), 4.79 (d, 1 H, J=7.5 Hz, H-1"), 4.32 (dd, 1 H, J=2.6, 5.0 Hz, H-3), 4.26 (d, 1 H, J=12.7 Hz, H-15A), 4.16 (d, 1 H, J=5.9 Hz, H-11), 4.07 (d, 1 H, J=12.6 Hz, H-15B), 3.97 (d, 1 H, J=9.7 Hz, H-5"), 3.73 (d, 1 H, J=5.0 Hz, H-2), 3.71 (s, 3 H, $-CO_2Me$), 3.04 (d, 1 H, J=3.8 Hz, H-13A), 2.77 (d, 1 H, J=3.9 Hz, H-13B), 2.36 (dd, 1 H, J=5.8, 15.2 Hz), 2.11-2.0 (5s, 15 H, -OAc), 1.74 (s, 3 H, H-16), 0.95 (m, 6 H, H-4'), 0.71 (s, 3 H, H-14); IR ($CHCl_3$) 2950, 1750 (br), 1430, 1365, 1210 (br), 1030 (br) cm^{-1}; FAB mass spectrum (DMSO/glycerol dispersion) m/e 783 (MH^+), 317 $(C_{13}H_{17}O_9^+$, sugar); EI mass spectrum m/e 767 (M^+-CH_3), 698 $(M^+-C_5H_8O)$, 680 $(M^+-C_5H_{10}O_2)$; high resolution mass spectrum, calcd for $C_{32}H_{40}O_{16}$ $(M^+-C_5H_{10}O_2)$ 680.2316; Found, 680.232 ± 0.001.

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