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NOTE

THE SYNTHESIS OF DEUTERIUM LABELED T-2 TOXIN

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SUMMARY

We report the synthesis of isotopically pure trideuteriated T-2 toxin, a useful internal standard for mycotoxin analysis. Peracetylation of HT-2 toxin with hexadeuteriated acetic anhydride, followed by selective hydrolysis of the C-3 acetate afforded the desired compound in good yield.

Key words: T-2 toxin, deuterium, mycotoxin, mass spectrometry, trichothecene.

The determination of trichothecene mycotoxins has been a subject of considerable interest in recent years(1) in view of the fact that ingestion of contaminated foodstuffs by humans or farm animals is known to cause a wide variety of toxicoses often leading to death(2). The methods used in trichothecene analysis include TLC, GC, GC-MS, LC, and LC-MS(3). In our analysis of trichothecene mycotoxins and their metabolites in biological fluids by combined HPLC-MS, we required a deuterium labeled standard for quantitation. To our surprise we found that simple deuteriated trichothecenes are not readily available for use as internal standards(4). The use of deuteriated trimethylsilyl (TMS) derivatives of T-2 and other related trichothecenes or reference standards for analysis by GC/MS has been recently reported(5). However, trimethylsilyl ethers are susceptible to hydrolysis and, in a mixture of TMS-d_0 and TMS-d_9 derivatives of an analyte, cross silylation which may lead to erroneous results is always a possibility.

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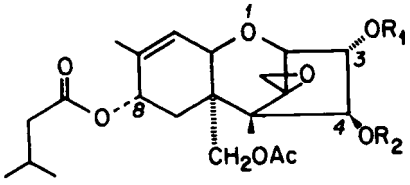
In our approach the trichothecene of interest, T-2 toxin, was labeled with deuteriated acetate. Investigations into the chemistry of trichothecenes have shown that the ester groups located in different areas of the molecule exhibit different hydrolysis rates(6). Thus several trichothecenes may be made available by carefully monitored hydrolysis of a per-esterified precursor.



	R_1	R_2
1	H	H
2	$COCD_3$	$COCD_3$
3	H	$COCD_3$

For the synthesis of the title compound 3, we envisioned a two-step sequence involving peracetylation of HT-2 toxin 1 with deuterium labeled acetic anhydride followed by selective hydrolysis of the C-3 acetate. Acetylation of HT-2 toxin with acetic anhydride-d_6 in pyridine yielded quantitatively the peracetate 2 as evidenced by TLC, the incorporation of six deuterium atoms in the mass spectrum and the presence of only one acetate methyl and the shift of the C-3 and C-4 protons from 4.26 and 4.42 ppm to 5.21 and 5.95 ppm respectively in the ^1H NMR spectrum. A portion of peracetate 2 was hydrolyzed in 1M ammonium hydroxide in methanol-water (4:1). The hydrolysis of peracetate 2 was carefully monitored by TLC at half hour intervals. After 90 min, TLC revealed that the peracetate 2 was no longer present, and the major component was T-2 toxin 3 along with smaller amounts of HT-2 toxin. The ratio of T-2 toxin to HT-2 toxin in the crude reaction mixture was 4:1 as determined by respective signal heights of the H-2 and H-13 protons in the region 2.5-4.0 ppm of the ^1H NMR spectrum. The d_3-T-2 toxin 3, purified by prep TLC, had a mass spectrum (chemical ionization, NH_3) in which the $(M+NH_4)^+$ ion (base) peak was clearly shifted by 3 atomic mass units from m/z 484 to m/z 487 compared to its unlabeled counterpart. The $(M+NH_4)^+$ adduct ion will be monitored when using 3 as an internal standard. The isotopic purity for compounds 2 and 3 was determined to be greater than 98%.

EXPERIMENTAL

All chemicals were used as obtained from the manufacturer except where noted otherwise. Melting points were obtained on a Thomas Hoover melting point apparatus and are uncorrected. ^{1}H NMR spectra were recorded in deuteriochloroform using a Varian 300MHz spectrometer with tetramethylsilane as the internal reference. Chemical ionization (NH_3) and electron impact mass spectra, respectively mentioned in the discussion and experimental sections, were obtained with a Finnigan Model 4000 spectrometer.

Hexadeuterioacetyl T-2 toxin, 2

HT-2 toxin (Myco Labs, 50 mg) was dissolved in freshly distilled pyridine (1 ml) and hexadeuterioacetic anhydride (1 ml) and allowed to react at room temperature for 24 hrs. Water was then added and the mixture was extracted with ethyl acetate. The organic layer was washed with 1N HCl, water and then dried over sodium sulfate. A pale yellow oil was obtained, 60 mg. ^{1}H NMR:δ 0.77(s, 3H, H-14), 0.98(d, 3H, J = 6Hz, H-4'), 0.99(d, 3H, J = 6Hz, H-4'), 1.77(brs, 3H, H-16), 1.92(brd, 1H, J = 15Hz, H-7α), 2.05-2.20(m, 3H, H-2' and H-3'), 2.10(s, 3H, OAc), 2.39(dd, 1H, J = 5, 15Hz, H-7β), 2.85(d, 1H, J = 4Hz, H-13a), 3.10(d, 1H, J = 4Hz, H-13b), 3.89(d, 1H, J = 5Hz, H-2), 4.14(d, 1H, J = 12Hz, H-15a), 4.24(brd, 1H, J = 6Hz, H-11), 4.38(d, 1H, J = 12Hz, H-15b), 5.21(dd, 1H, J = 5, 4Hz, H-3), 5.32(brd, 1H, J = 5Hz, H-8), 5.78(brd, 1H, J = 6Hz, H-10), 5.95(d, 1H, J = 4Hz, H-4); EI-MS m/z (rel. intensity) 430(8,M^+-C_4H_8CO), 412(37,M^+-C_4H_9COOH), 370(5), 353(5), 352(6), 326(6), 275(28), 227(44), 180(43), 121(47), 105(52), 85(44), 57(62,$C_4H_9^+$), 46(100,CD_3CO^+).

Trideuterio T-2 toxin, 3

Hexadeuterioacetyl T-2 toxin, 2, was dissolved in a solution (4 ml) of 1M ammonia in methanol-water (4:1). Aliquots of the reaction mixture were analyzed by TLC at 30 min intervals. The TLC plates (Analtech Uniplates silica gel GF) were developed in hexane: ethyl acetate (1:5) and visualized

using the method suggested by Takitani, et al.(7) After 90 min of reaction time the reaction was quenched with 1N HCl and the mixture was extracted with ethyl acetate, washed with water, and dried over sodium sulfate. The T-2 toxin residue was purified by TLC using hexane: ethyl acetate (1:5). The zone corresponding to R_f 0.44 to 0.63 was scraped, and the silica was extracted with ethyl acetate, filtered, and the filtrate was dried to afford the product (34 mg as needles from hexane/ethyl acetate, 62% isolated yield) mp 144-146°. ^{1}H NMR δ 0.83 (s, 3H, H-14), 0.99 (d, 3H, J = 7Hz, H-4'), 1.00 (d, 3H, J = 7Hz, H-4'), 1.78(brs, 3H, H-16), 1.94(brd, 1H, J = 16 Hz, H-7α), 2.00-2.20(m, 3H, H-2' and H-3'), 2.07(s, 3H, OAc), 2.43(dd, 1H, J = 16, 6 Hz, H-7β), 2.83(d, 1H, J = 4Hz, H-13a), 3.09(d, 1H, J = 4Hz, H-13b), 3.73 (d, 1H, J = 5Hz, H-2), 4.09(d, 1H, J = 14Hz, H-15a), 4.19(dd, 1H, J = 5, 4Hz, H-3), 4.32(d, 1H, J = 14Hz, H-15b), 4.38(brd, 1H, J = 5Hz, H-11), 5.32 (brd, 1H, J = 5Hz, H-8), 5.35(d, 1H, J = 4Hz, H-4), 5.84(brd, 1H, J = 5Hz, H-10); EI-MS m/z 385(1.1, M^+-C_4H_8CO), 367(3.3, M^+-C_4H_9COOH), 325(0.5), 308(2), 307(2), 281(4), 261(2), 205(6), 180(16), 121(51), 105(26), 85(33), 57(69, $C_4H_9^+$), 46(100, CD_3CO^+).

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