CHEMICAL INTERCONVERSION OF T-2 AND HT-2 TOXINS AND RELATED COMPOUNDS

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SUMMARY

Mild alkaline hydrolysis of 3α-hydroxy-4β,15-diacetoxy-8α-isovaleroxy-12,13-epoxytrichothec-9-ene (T-2 toxin) gave the 3α,4β-diol (HT-2 toxin) and the 3α,4β,15-triol as well as the 3α,4β,8α,15-tetraol. Partial acetylation of the diol gave mainly the 4β-hydroxy-3α-acetate, isomeric with T-2 toxin.

T-2 (I) and HT-2 (IV) toxins were isolated in this laboratory from cultures of Fusarium tricinctum, and characterized as derivatives of a trichothecen-3,4,8,15-tetraol.[1,2] T-2 toxin is readily prepared in gram quantities by fermentation,[3] but HT-2 toxin is produced in smaller amounts and is rather difficult to isolate from the culture. We have developed a procedure for the chemical interconversion of I and IV and have also obtained two new compounds of this family, a structural isomer of T-2 toxin (II) and a triol (V). The structures assigned these compounds are given in Figure 1.

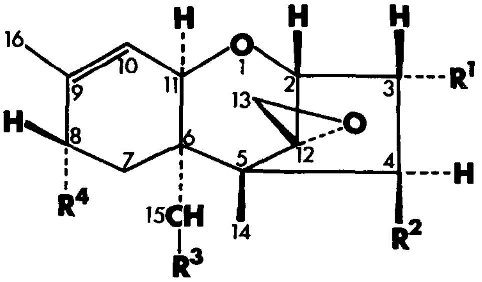
EXPERIMENTAL

T-2 toxin

T-2 toxin was produced by F. tricinctum NRRL 3299 grown at 8°C on corn[4] and isolated according to Bamburg *et al.*[1]

Chromatography solvents

Two solvent systems were used for both thin-layer chromatography (TLC) and column chromatography: A, ethyl



	R^1	R^2	R^3	R^4
(I)	OH	OAc	OAc	$OOCCH_2CHMe_2$
(II)	OAc	OH	OAc	$OOCCH_2CHMe_2$
(III)	OAc	OAc	OAc	$OOCCH_2CHMe_2$
(IV)	OH	OH	OAc	$OOCCH_2CHMe_2$
(V)	OH	OH	OH	$OOCCH_2CHMe_2$
(VI)	OH	OH	OH	OH

Fig. 1. Structures of T-2 toxin and related compounds.

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acetate:acetone:methanol (50:50:1 v/v); B, toluene-ethyl acetate (1:3 v/v).

Alkaline hydrolysis of T-2 toxin

T-2 toxin (500 mg) was dissolved in a 1.0 N solution of ammonia in methanol:water (4:1 v/v) (25 ml), pH 11.6, and allowed to react at room temperature for 48 hr. Two µl of the reaction mixture, monitored by TLC followed by charring with H_2SO_4, revealed that the T-2 toxin was converted almost entirely into 3 products having R_f values in solvent system A of 0.27, 0.42 and 0.53 (Fig. 2A). Two of these corresponded in R_f value to VI and IV, respectively. The third product was shown by NMR and mass spectroscopy to be compound V.

The reaction mixture was evaporated *in vacuo* to an oil which was chromatographed on a 100 g silica gel column developed with solvent system A. Appropriate effluent fractions, monitored by TLC, when combined and evaporated in vacuo, yielded colorless oils weighing: IV, 196 mg; V, 71 mg; VI, 98 mg and unreacted I, 28 mg.

Compound V was redissolved in solvent system A and slowly evaporated at room temperature for 2 weeks. White hexagonal prisms were obtained, m.p. 164-165°C.

Acetylation of HT-2 toxin

A mixture of HT-2 toxin (50 mg), pyridine (2.5 ml) and acetic anhydride (50 µl) was heated at 100° in a stoppered bottle for 2 hr. TLC of 2 µl indicated about 85% of the toxin had been converted to 3 products with R_f values in solvent

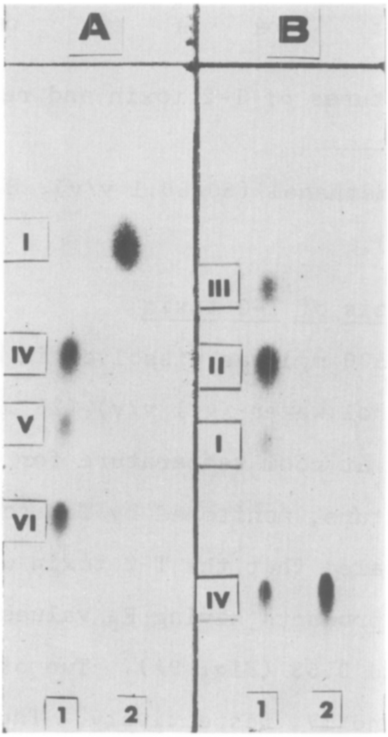

Fig. 2. Thin layer chromatograms. Plate A, sample 1: T-2 toxin hydrolysis mixture developed with solvent system A. Plate B, sample 1: HT-2 toxin acetylation mixture developed with solvent system B. Sample 2: T-2 and HT-2 standards on plates A and B, respectively. For numbering of components see Fig. 1.

system B of 0.15, 0.40, 0.51 and 0.64 (Fig. 2B). The first, second and fourth spots corresponded to authentic samples of unreacted IV, I and III, respectively. The third spot was subsequently shown by NMR and mass spectroscopy to be compound II.

Ten ml of ethanol was added to the reaction mixture and the solution evaporated to an oil _in vacuo_. The oil was applied to a preparative silica gel H plate (20 x 20 cm) and developed in solvent system B. The edge of the plate was sprayed with conc. H_2SO_4, charred over a hot plate to locate the bands, and the uncharred portion of the bands was removed and eluted with methanol. Evaporation of the eluates gave clear oils. The yields were I, 2.7 mg; II, 19.9 mg; III, 11.4 mg and unreacted IV, 7.7 mg.

Compound II was dissolved in warm Skellysolve B and crystallized on cooling; m.p. 149-152°C.

DISCUSSION

Hydrolysis of T-2 toxin

In methanolic ammonia solution T-2 toxin was converted to HT-2 toxin in 43% yield. The product showed the correct molecular ion at m/e 424 by mass spectrometry and produced IR and NMR spectra identical to those previously reported by Bamburg.[5] Preferential mild alkaline hydrolysis of the secondary 4β-acetoxy versus the primary 15-acetoxy function of diacetoxyscirpenol (like I except R^4 = H) has been reported[6,7] and has been attributed[8] to participation by the neighboring 3α-hydroxyl group.

By use of 0.65 N NH_4OH or by substituting $Ca(OH)_2$ for NH_4OH, it was possible to convert T-2 exclusively to HT-2 toxin, albeit in low yields. However, by using 0.01 N KOH in 90% methanol the major product after 3 hr reaction at room temperature was the completely hydrolyzed product, T-2 tetraol, VI. In a preliminary experiment on the hydrolysis of III with NH_4OH, the major product was I.

The minor product from the hydrolysis of T-2 toxin was shown to be the previously unreported triol, V, by the mass spectrum (molecular ion at m/e 382), NMR spectrum, which lacked the singlet at δ 1.99 ($COC\underline{H}_3$) but retained the 6-proton doublet at δ 0.97 (J ∿ 6.5 Hz) corresponding to the terminal Me groups of the isovaleric acid residue, and by conversion to the 3,4,15-triacetate, III, identical to that from T-2 and HT-2 toxins.

<u>Acetylation of HT-2 toxin</u>

HT-2 toxin was quantitatively converted to III by excess acetic anhydride.[2] When HT-2 toxin was treated with a limited amount of Ac_2O (0.53 molar ratio in the present work) the major product was II and only a very small amount of I was formed. Thus the acetylation favors the 3α-hydroxyl group.

As compound II was obtained by acetylation of IV and was isomeric with I (molecular ion at m/e 466) the structure indicated in Fig. 1 was highly probable. The signals attributable to the 3β- and 4α-protons in the NMR spectrum of II appeared at δ 4.9 and ca. δ 4.5, respectively, as compared to ca. δ 4.1 and δ 5.47 for these protons in the spectrum of T-2 toxin. These shifts are consistent with the presence of an acetoxy group at C-3 and an unesterified hydroxyl at C-4 in compound II.

The structure was further confirmed by the fragmentation pattern shown by II in the mass spectrometer. Trichothecenes bearing free hydroxyl substituents at C-3 and C-4 (T-2 tetraol, HT-2 toxin, scirpene triol) undergo a characteristic cleavage[5,9] by which these carbons are lost as the fragment -CHOHCHO, m/e 59, whereas those having a free hydroxyl at C-4 only (trichodermol, trichothecolone) lose the analogous fragment $-CH_2CHO$, m/e 43. Acetylation of the hydroxyl at C-4 (but not C-3) almost completely suppresses this type of cleavage. Compound II, therefore, was expected to lose -CH(OAc)CHO, m/e 101, and appropriate peaks, for example at 365 (M^+-101) and 263 (M^+-101-isovaleric acid) were prominent in the mass spectrum. Similarly, the mass spectrum of V showed an intense peak at 323 (M^+ -59), further confirming the $3\alpha,4\beta$-diol structure of this compound.

Aside from differences discussed above, the IR, NMR, and mass spectral properties of II and V closely resemble those of T-2 toxin and related trichothecenes. Biological properties, to be reported elsewhere, were also generally similar except that II showed unusually low activity in the rat skin test.

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