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THE STRUCTURES OF TOXINS FROM TWO STRAINS OF *FUSARIUM TRICINCTUM**

J. R. BAMBURG, N. V. RIGGS† and F. M. STRONG
Department of Biochemistry, University of Wisconsin, Madison, Wisconsin, U.S.A.

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Abstract—In the United States there have been serious outbreaks of toxicosis among farm animals that have ingested moldy corn. The most toxic fungus frequently isolated from moldy corn in Wisconsin is *Fusarium tricinctum* of which there are many toxic strains. Among these, strain B-24 was originally chosen for growth in pure culture, and isolation of a crystalline toxin from it has been described.[1] At this point a much more toxic strain, T-2, became available and a crystalline toxin related to that from strain B-24 was isolated from it by a similar procedure. The B-24 toxin has been proved, by comparison of IR and PMR spectra and by mixed m.p. determination, to be identical with diacetoxyscirpenol (Ia), the principal toxic metabolite of *Fusarium scirpi*.[2–4] Results described below show that the T-2 toxin is 8α-(3-methylbutyryloxy)4β,15-diacetoxyscirp-9-en-3α-ol (Ib).

Fusarium tricinctum, strain T-2, was grown at 8° on Gregory's medium[5] and the whole culture was blended and lyophilized. The toxin, herein referred to as toxin T-2, crystallized readily from purified ethyl acetate extracts and was obtained in 0·5% yield based on the dry weight of lyophilized culture.

Analysis and mol wt determination gave the molecular formula $C_{24}H_{34}O_9$. Only end absorption appeared in the UV spectrum. The IR spectrum in a potassium bromide pellet showed the presence of OH and ester CO groups and an olefinic linkage; this spectrum showed only minor differences from that of diacetoxyscirpenol (Ia). Quantitative saponification, however, led to the uptake of three equivalents of alkali and liberation of acetic and isovaleric acids. The neutral product Ic from the hydrolysis showed OH and olefinic absorption but no CO absorption in the IR. The PMR spectrum of the neutral product Ic integrated for 12 less protons than did the spectrum of the parent, which corresponds to the loss of one isovaleric and two acetic acid residues. The parent contained also one free OH group which was readily acetylated (PMR spectrum) but the product Id was not obtained crystalline. Attempts to prepare the benzoate and 3,5-dinitrobenzoate led to amorphous products which appeared to be the desired esters (IR spectrum) but were unsatisfactory as derivatives for characterization.

Toxin T-2 (Ib) took up one mole of hydrogen over a PtO_2 catalyst in ethanol. The dihydrotoxin (IIb) was devoid of absorption in the UV, and its IR spectrum showed the expected bands for OH and ester CO absorption but lacked those due to olefinic absorption in the parent. The formula for toxin T-2 may therefore be expanded to

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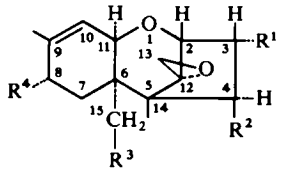
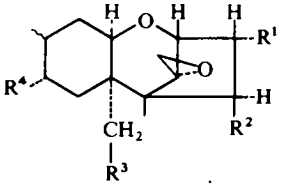
† Present address: Department of Organic Chemistry, University of New England, Armidale, N.S.W., Australia.



I



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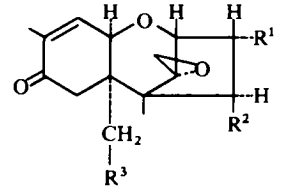


III

I II

	R^1	R^2	R^3	R^4
a:	OH	OAc	OAc	H
b:	OH	OAc	OAc	$O_2CCH_2CHMe_2$
c:	OH	OH	OH	OH
d:	OAc	OAc	OAc	$O_2CCH_2CHMe_2$

III

	R^1	R^2	R^3
a:	OAc	OAc	OAc
b:	OH	OH	OH

$C_{15}H_{18}O_2(OH)(OAc)_2$—$(O_2CCH_2CHMe_2)$ in which the nucleus is tetracyclic and contains one double bond and two ether-type oxygen atoms.

PMR spectra of some of the compounds so far described define fragments of the molecule. Apart from small shifts in the positions of corresponding signals, the spectrum at 60 Mc/s of toxin T-2(Ib) differs from that of diacetoxyscirpenol (Ia) mainly in the presence of a 6-proton doublet at δ 0·97 and a third signal (that at δ 5·20; see below) in the region $\delta > 5{\cdot}0$. The spectra at 100 Mc/s of toxin T-2 (Ib) and the tetraol (Ic) produced on hydrolysis are shown in Fig. 1. Signals present in the former but absent from the latter are a 6-proton doublet (splitting, 6 c/s, unchanged in the spectrum measured at 60 Mc/s) at δ 0·97 corresponding to the terminal Me groups of the isovaleric acid residue, and strong singlets at δ 1·99 and δ 2·09 which correspond to two acetate ester Me groups and are superimposed on multiplets due to 5 other protons including H7A, H7B and the methylene and methine protons of the isovaleric acid residue. Strong coupling between these methylene and methine protons is reflected in the filling between the components of the Me doublet at δ 0·97 as a result of "virtual" coupling.[6,7] Among the signals showing minor changes of position (partly due to change of solvent), the 3-proton singlet at δ 0·71 in the parent (δ 0·84 in the tetraol) is assigned to the angular Me group (C14), that at δ 1·74 in the parent (δ 1·80 in the tetraol) to the allylic Me group (C16) and the AB quartet ($J \sim 4$ c/s) at δ 2·97, 2·70 in the parent (δ 2·82, 2·69 in the tetraol) to the 13-methylene protons; epoxide protons are among the few absorbing in this region[8] and the geminal coupling constant is unexceptional for epoxides.[9] In the spectrum of dihydrotoxin T-2 (IIb), the allylic Me signal was replaced by a Me doublet near δ 1·0 superimposed on that due to the isovaleric acid residue, and the olefinic proton signal (H10; see below) was replaced by additional signals in the range δ 1·8–2·1.

The lowest field signal, a doublet of broadened lines ($J \sim 5$ c/s) at δ 5·72 in the parent or a doublet of doublets ($J \sim 6$ c/s, 1·5 c/s) at δ 5·50 in the tetraol is due to the olefinic proton (H10). Although the outer members of the expected quartets

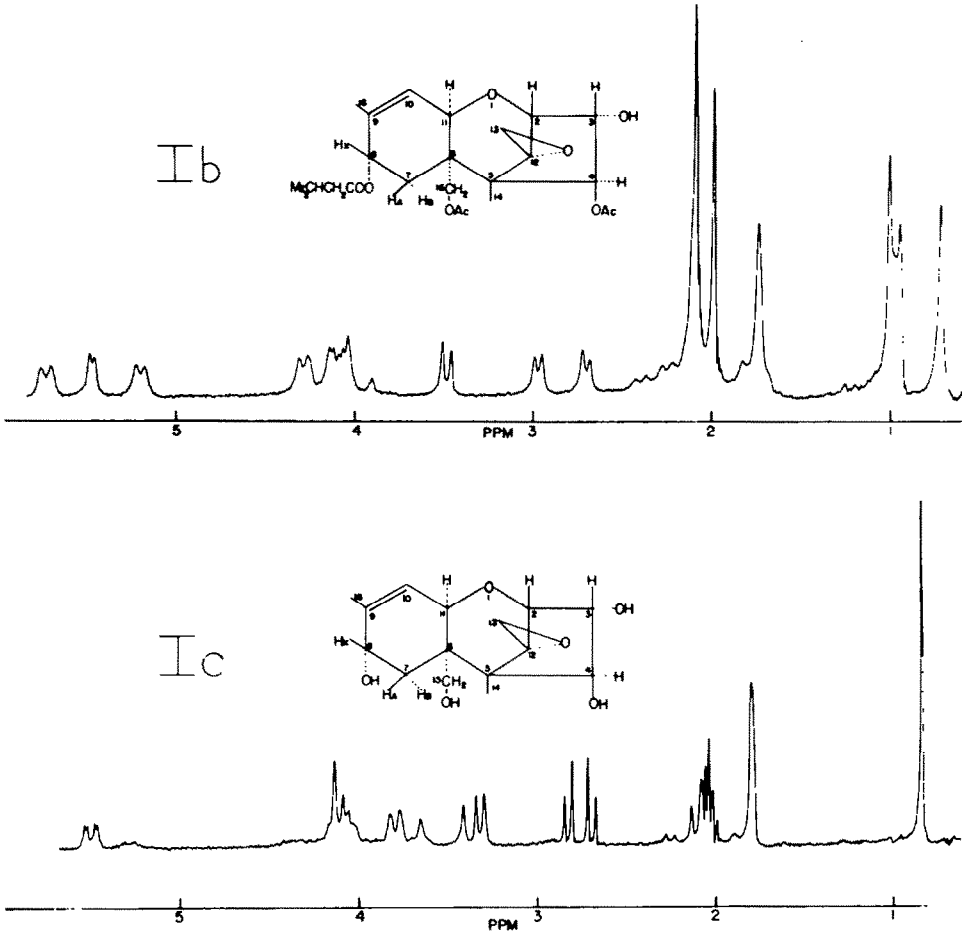


FIG. 1 PMR spectra of toxin T-2 (Ib) in carbon tetrachloride and alkaline hydrolysis product (Ic) in hexadeuteroacetone. Quintet at δ 2·1 in spectrum of Ic is due to residual pentadeuteroacetone.

appear only as weak shoulders, the smaller splitting (not resolved in the parent) is due to allylic coupling with the Me (C16) protons for, on irradiation of the tetraol at δ 1·80, the signal collapsed to a sharp doublet ($J \sim 6$ c/s); conversely, on irradiation at δ 5·50, the broad Me signal at δ 1·80 sharpened to a singlet approaching the peak height of the Me singlet at δ 0·84. At the same time the doublet ($J \sim 6$ c/s) at δ 3·80 due to H11 collapsed to a singlet; this signal is downfield at δ 4·29 in the parent, probably as a result of deshielding by the acetoxyl group on the *cis*-oriented 15-methylene group.

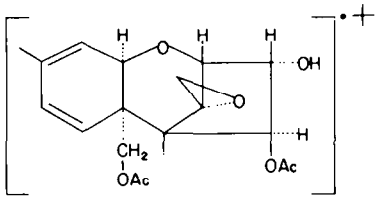
The doublet ($J \sim 2{\cdot}5$ c/s) at δ 5·47 in the parent is due to H4, that ($J \sim 5$ c/s) at δ 3·48 to H2, each coupled to H3 whose signal appears in the 3-proton multiplet around δ 4·1. Spin-decoupling of H2 and H4 and analysis of the multiplet patterns around δ 4·1 showed the presence in the latter of two strong peaks which must represent the inner lines of an AB quartet due to the 15-methylene protons; the outer high-field line of the quartet is visible near δ 3·9 (whence $J_{AB} = 12{\cdot}6$ c/s, $\nu_0 \delta_{AB} = 19{\cdot}8$);

the outer low-field line is hidden under the H11 doublet at δ 4·29 but was revealed when this doublet shifted upfield ca. 0·15 ppm in the spectrum of acetylated toxin T-2 (Id), apparently as a result of shielding by the new acetate function at position 3. Models show these groups in close proximity for that half-chair conformation of the cyclohexene ring having O1 pseudo-axial and C15 axial, H11 pseudo-equatorial and C5 equatorial. In the inverted half-chair conformation with C5 axial, H11 and the 3-acetoxyl group have moved well apart, and the 13-methylene group interferes strongly with the π-system of the 9,10-double bond; in that conformation the 13-methylene protons would be expected well upfield of the usual epoxide range, δ 2·5–3·0. The former fixed conformation is therefore much more likely, and this agrees also with the observed value of $J_{10,11}$ (5·5 c/s); if H11 were pseudo-axial, a value of ca. 2·3 c/s would be expected.[10]

The remaining low-field signal is the doublet of broad lines at δ 5·20 (splitting, ~4·7 c/s) due to H8, which is the X signal of an ABX system comprising also the 7-methylene protons. The signal due to A is visible as the doublet of doublets (splittings, 5·0 and 14·5 c/s) near δ 2·3 on the downfield tail of the multiplet around δ 2·1. On irradiation at δ 5·20 this signal collapsed to a doublet, splitting 14·5 c/s which is therefore the value of J_{AB}. The B signal is hidden upfield so that complete analysis of the ABX spectrum[11] is not possible. Failure to resolve the outer doublets of the X signal, however, indicates that J_{BX} is small, and J_{AX} must be greater than 5·0 c/s, the smaller splitting in the A signal. In agreement, although small differences of conformation and coupling constants may be expected between the systems examined, analysis[11] of the AB signals for the tetraol (Ic) gave (in c/s $\pm$0·1) $\nu_0\ \delta_{AB}$ = 16·4, J_{AB} = 14·4, J_{AX} = 5·5, J_{BX} = 0·2 in hexadeuteroacetone (part of the B signal was obscured by the quintet near δ 2·1 due to the small content of pentadeuteroacetone) and $\nu_0\ \delta_{AB}$ = 13·8, J_{AB} = 14·9, J_{AX} = 5·5, J_{BX} = 0·5 in deuterium oxide. In both cases the calculated X spectrum could not be checked directly because the signal had moved upfield (loss of the isovaleryl group) and was hidden under the multiplet near δ 4·1 due to H3 and H4. The vicinal coupling constants suggest[12] corresponding dihedral angles of 30–40° and 90–80°, respectively, which are found in the fixed conformation of the cyclohexene ring determined above if the 8-isovaleroxy group is in the pseudo-axial position downwards (i.e. α); H7A is then the axial member of the AB pair and the downfield position of its signal with respect to that for its equatorial counterpart (H7B) is probably to be ascribed to deshielding by its 1,3-diaxial opposition to O1 and its being nearly in the plane of the epoxide ring and in close proximity to it. In pentadeuteropyridine, $\nu_0\ \delta_{AB}$ became very small and the AB spectrum could not be analyzed. Importantly, however, the doublet due to H11 shifted to δ 4·40 and was clearly resolved from a broad signal due to H8 at δ 4·21. Irradiation of the latter sharpened up components of the olefinic proton quartet at δ 5·70 markedly whereas irradiation at various power levels of the AB protons (δ 2·4) sharpened the H8 signal, but had a negligible effect on the olefinic proton signal. The proton (X) on oxygenated carbon is therefore weakly coupled (allylically) to the olefinic proton and it must be H8.

With allowance for effects due to the additional acyloxy group in toxin T-2 (Ib), the close correspondence of its PMR spectrum to that of diacetoxyscirpenol (Ia) establishes a close structural and configurational relationship between these compounds. As the 7- and 8-methylene protons of Ia, however, give rise to a relatively

narrow multiplet (showing little or no resolved fine structure) under the acetate methyl signals near δ 2·0, and we were unable to resolve the H8–H11 allylic coupling, we have sought further evidence to distinguish between these positions as the location of the additional acyloxy group in toxin T-2 (Ib) and to define which acyloxy group is here located.



ION A

FIG. 2 The first product ion produced during the major mode of decomposition of the toxin T-2 (Ib) parent ion in the mass spectrometer.

In the mass spectrum of toxin T-2 (Ib) the base peak occurred at *m/e* 43 corresponding to acetyl ion with no doubt a contribution from isopropyl ion derived by fission of the isovaleric acid residue, for there were also strong peaks at *m/e* 57 (57%; isobutyl ion) and *m/e* 85 (37%; isovaleryl ion). Above *m/e* 400 the strongest peak lay in the parent-ion region at *m/e* 465 ± 1 (0·12%) and the only other peaks of greater than 0·05% relative intensity were at *m/e* 407 (0·07%) and 406 (0·09%) corresponding to loss of acetoxyl radical or acetic acid, respectively, from the parent ion which therefore has *m/e* 466 (calc., 466). The parent ion alternatively lost isopropylketene, isovaleroxy radical, or isovaleric acid to give peaks at *m/e* 382 (3·0%), 365 (2·7%) and 364 (12%), respectively. Corresponding metastable peaks were all very weak because of the low intensity of the parent-ion peak. The peak at *m/e* 364 was, however, the strongest above *m/e* 200 in the spectrum, and loss of isovaleric acid was the major mode of decomposition of the parent ion. The product ion A (Fig. 2) subsequently lost in part ketene or acetyl radical to give peaks at *m/e* 322 (1·2%) and 321 (0·7%), respectively, but mainly acetoxyl radical or acetic acid to give peaks at *m/e* 305 (3·7%) and 304 (4·6%), respectively. The corresponding broad, strong metastable peak appeared around *m/e* 254 (calc., *m/e* 255·6, 253·9, respectively). The remaining strong peaks in the spectrum are readily accounted for on the basis of fragmentation of ion A, but a discussion is not relevant to present purposes.

For dihydrotoxin T-2 (IIb) the base peak was again at *m/e* 43 with strong peaks at *m/e* 57 (47%) and 85 (38%). Again the parent-ion peak was weak (*m/e* 468; 0·02%), but the major mode of decomposition was now loss of acetic acid or acetic acid plus a hydrogen atom to give peaks at *m/e* 408 (1·6%) and 407 (6·0%). Loss of isovaleric acid was minor as a first stage, giving a relatively weak peak at *m/e* 366 (0·90%) but, as a second stage following loss of acetic acid, gave rise to a peak at *m/e* 306 (33%), the strongest peak above *m/e* 100 in the spectrum.

Loss of acetic acid and a hydrogen atom also occurred readily in the parent ion of diacetoxyscirpenol (Ia), the peak at highest *m/e* (305; 3·6% of base peak at *m/e* 43) corresponding to P-61. Preferential loss of isovaleric acid from the parent ion of toxin T-2 (Ib) is ascribed to the driving force associated with formation of the conjugated diene system in ion A (Fig. 2), a force that is lacking for the dihydrotoxin

(IIb) and diacetoxyscirpenol (Ia); it locates the isovaleroxy group as in the vicinty of the 9,10-double bond, but, as the elimination may be thermal, still does not distinguish between positions 7 and 8 for it. The final proof is based on chemical evidence.

Oxidation of acetylated toxin T-2 (Id) with selenium dioxide in 90% aqueous acetic acid yielded 3,4,15-triacetoxyscirpen-8-one (IIIa), identified by direct comparison of its m.p. and UV and IR absorption spectra with those of the product obtained[4] from diacetoxyscirpenol (Ia) by acetylation and oxidation. Compound IIIa was also hydrolyzed to 3,4,15-trihydroxyscirpen-8-one (IIIb) which showed R_f values (TLC in three different solvent systems) identical with those of a known sample.[4]

EXPERIMENTAL

Microanalyses were by Micro-Tech Laboratories Inc. Skokie, Illinois. Mass spectra were determined on an MS 9 instrument at the Mellon Institute, Pittsburgh, Pa. M.ps were determined on a hot stage instrument. Optical rotations were measured on a Perkin–Elmer polarimeter, model 141, in a one decimeter micro cell. IR spectra were determined on KBr pellets with a Beckman IR-5 spectrophotometer. UV spectra were measured in 95% EtOH on a Beckman DB spectrophotometer. PMR spectra were measured on Varian Associates instruments, either the A-60 or HA-100. Spin decoupling was done on the HA-100 spectrometer. TLC was done on either Silica Gel G or H (Brinkmann Instruments Inc.) developed in one of the following solvent systems: system A, 1:3 (v/v) toluene–EtOAc; system B, 1:4:4 (by volume) abs EtOH–EtOAc–acetone; system C, 2:1:1 (by volume) toluene–EtOAc–$CHCl_3$; system D, 1:1:1 (by volume) $CHCl_3$–MeOH–acetone; system E, 1:1 (v/v) toluene–abs EtOH. Brinkmann Instruments Silica Gel 0·05–0·20 mm was used for column chromatography. Spots on thin-layer plates were detected with concentrated H_2SO_4 spray followed by charring at 150°. Paper chromatograms were developed in solvent system F,[13] upper phase of a mixture of butanol (300 ml), water (300 ml), and aqueous 30% ethylamine soln (30 ml) equilibrated at 25°. Spots on the paper chromatograms were detected with bromcresol green spray. Gas chromatography was done on an Aerograph A-600-B Hy-Fi, equipped with a hydrogen flame ionization detector. A 5 ft × $\frac{1}{8}$ in column packed with 20% neopentylglycolsuccinate, 2% H_3PO_4 on 60–80 mesh firebrick was used. Operating parameters were: flow rate, 26 ml N_2 per min; column temp, 165°; H_2 flow rate to detector, 20 ml per min.

4,15-*Diacetoxyscirp*-9-*en*-3-*ol* (Ia). The isolation of this compound from *Fusarium tricinctum*, strain B-24 has been described.[1] The material formed white needles from benzene–hexane, m.p. 161–162°, unpressed when mixed with an authentic sample.* IR and PMR spectra were identical with those of diacetoxyscirpenol.

8α-(3-*Methylbutyryloxy*)4β,15-*diacetoxyscirp*-9-*en*-3α-*ol* (*toxin* T-2, Ib). *Fusarium tricinctum*, strain T-2 was grown at 8° in 500 ml Erlenmeyer flasks containing Gregory's medium (100 ml).[5] After 30 days 150 cultures (mycelium and broth) were blended and lyophilized. The powder obtained (300 g) was twice extracted in 10 g portions with EtOAc (250 ml each time), the combined extracts being concentrated *in vacuo* to give an oil (20 g). The oil was dissolved in EtOAc (100 ml) and washed with 0·5% H_2SO_4 (3 × 60 ml). The EtOAc layer was again concentrated *in vacuo* to an oil, which was dissolved in 180 ml MeOH–water 4:1 (v/v), extracted with Skellysolve B (b.p. 67–69°) (3 × 60 ml) and then treated with water (150 ml) to give a 1:1 (v/v) MeOH–water soln.

Extraction of the aqueous soln with 1:1 (v/v) $CHCl_3$–EtOAc (3 × 200 ml) and removal of these solvents *in vacuo* resulted in a brown oil (6·2 g). Chromatography of this oil on a silicic acid column (2 g oil per 150 g silicic acid) developed with 6:1 (v/v) $CHCl_3$–acetone yielded several 20 ml fractions containing toxic material as indicated by a rat skin bioassay.[1] This material, called toxin T-2, when crystallized from the concentrated fractions with benzene–Skellysolve B gave sheaves of white needles (1·5 g). The material was twice recrystallized from benzene–Skellysolve B: m.p. 151–152°; $[\alpha]_D^{26} = 15°$ (*c* 2·58 in 95% EtOH). (Found: C, 61·41; H, 7·29; O, 31·29; mol. wt. 466 ± 1 (mass spectrum). Calc. for $C_{24}H_{34}O_9$: C, 61·79; H, 7·35; O, 30·87%; mol. wt. 466·51); ν_{max}: 3400 (s), 2940 (s), 1720 (vs), 1635 (w), 1365 (s), 1240 (vs) cm^{-1}. UV spectrum showed end absorption only. PMR spectrum (Fig. 1) was measured in CCl_4.

* Kindly provided by J. F. Grove, Tropical Products Institute, London,[2] who suggested the possible identity of B-24 toxin[1] and diacetoxyscirpenol.

Derivatives of 8α-(3-*methylbutyryloxy*)4β,15-*diactoxyscirp*-9-*en*-3α-*ol*

Acetate (Id). T-2 toxin (Ib, 231 mg) was dissolved in pyridine (5 ml) and Ac_2O (5 ml). After standing 24 hr at room temp, the solvents were removed *in vacuo* to yield a pale yellow oil (263 mg). Chromatography of the oil on a 25 g silica gel column developed with solvent system A gave several 2 ml fractions containing the acetate as detected by TLC (R_f in system A, 0·59). The combined, concentrated fractions yielded a clear colorless oil (252 mg) that could not be obtained crystalline. An amorphous solid of Id was obtained from ether–pentane: $[\alpha]_D^{30} = 27°$ (*c* 0·9 in 95% EtOH). (Found: C, 61·82; H, 7·14; O, 32·00. Calc. for $C_{26}H_{36}O_{10}$: C, 61·40; H, 7·14; O, 31·46%); ν_{max} 3420 (s), 2950 (s), 1730 (s), 1635 (m), 1370 (s), 1240 (vs) cm^{-1}; only end absorption in the UV. PMR spectrum (100 Mc/s) in $CDCl_3$: δ 0·65 (3H, singlet: 14-C$\underline{H}_3$); 0·87 (6H, doublet, $J \sim 6·5$ c/s: —CH(C$\underline{H}_3$)$_2$); 1·66 (3H, broad singlet, sharpened by irradiation at δ 5·65: 16-C$\underline{H}_3$); 1·85–2·4 (14H, multiplet: —C$\underline{H}_2$C$\underline{H}$(CH$_3$)$_2$, 7-C$\underline{H}_2$, 3 × COC$\underline{H}_3$); 2·85 (2H, A$\underline{B}$ quartet, $J_{A\underline{B}} \sim 4·5$ c/s; $\delta_{A\underline{B}} \sim 0·2$ ppm: 13-C$\underline{H}_2$); 3·78 (1H, doublet, $J \sim 5$ c/s: $\underline{H}$2); 4·13 (2H, A$\underline{B}$ quartet, $J_{A\underline{B}} \sim 12·5$ c/s, $\delta_{A\underline{B}} \sim 0·28$: 15-C$\underline{H}_2$); 4·14 (1H, doublet, $J \sim 6$ c/s, collapsed to singlet by irradiation at δ 5·65: $\underline{H}$11); 5·07 (1H, doublet of doublets, $J \sim 3·5$, 5 c/s: $\underline{H}$3); 5·20 (1H, broad doublet, splitting ~6 c/s: $\underline{H}$8); 5·65 (1H, doublet of quartets, $J \sim 6$, 1·3 c/s: $\underline{H}$10); 5·85 (1H, doublet, $J \sim 3$ c/s: $\underline{H}$4).

Catalytic hydrogenation. Toxin T-2 (Ib, 50 mg) in 95% EtOH (10 ml) was hydrogenated at 25° and atmo press in the presence of PtO_2 (30 mg). Absorption of one molar equiv H_2 was complete after 1 hr. Concentration of the filtered soln gave a clear oil which formed plates from benzene–hexane of IIb, saturated to Br_2 in CCl_4: m.p. 140–141°; $[\alpha]_D^{30} = 54°$ (*c* 0·24 in 95% EtOH). (Found: C, 62·06; H, 7·85; O, 30·17; mol. wt. 468 ± 1 (mass spectrum). Calc. for $C_{24}H_{36}O_9$: C, 61·52; H, 7·75; O, 30·73%; mol. wt. 468·52); ν_{max}: 3440 (s), 2950 (s), 1730 (vs), 1460 (m), 1370 (s), 1240 (vs) cm^{-1}; no UV absorption. PMR spectrum (60 Mc/s) in $CDCl_3$: δ 0·70 (3H, singlet: 14-C$\underline{H}_3$); 0·90 (3H, doublet, $J \sim 6$ c/s: 16-C$\underline{H}_3$); 0·95 (6H, doublet, $J \sim 6$ c/s: —CH(C$\underline{H}_3$)$_2$); 1·6–2·3 (14H, multiplet: —C$\underline{H}_2$C$\underline{H}$(CH$_3$)$_2$, 7-C$\underline{H}_2$, 10-C$\underline{H}_2$, $\underline{H}$9, 2 × COC$\underline{H}_3$); 2·83 (2H, A$\underline{B}$ quartet, $J_{A\underline{B}} \sim 4·5$ c/s, $\delta_{A\underline{B}}$ 0·2 ppm: 13-C$\underline{H}_2$); 3·52 (1H, doublet, $J \sim 5$ c/s: $\underline{H}$2); 3·75–4·60 (4H, multiplet: $\underline{H}$3, $\underline{H}$11, 15-C$\underline{H}_2$); 4·9 (1H, multiplet: $\underline{H}$8); 5·25 (1H, doublet, $J \sim 2·5$ c/s: $\underline{H}$4).

Alkaline hydrolysis. Toxin T-2 (Ib, 14·6 mg) was dissolved in 0·0090N ethanolic (90%) NaOH (25 ml) and after 2 days an aliquot (5 ml) was titrated with 0·0107N HCl. The difference (1·73 ml) observed between sample and blank titrations corresponded to 2·95 moles of alkali consumed per mole of toxin T-2.

Toxin T-2 (Ib, 100 mg) was allowed to react with 1N ethanolic (90%) NaOH (5 ml) at room temp for 48 hr. Acidification with 0·1N HCl was followed by extraction with ether (3 × 15 ml). The ether soln was then extracted with a slightly alkaline (pH 8) water soln (3 × 20 ml). Concentration of the aqueous soln gave an oil. Part of this oil was dissolved in 0·5N HCl (10 ml) and one microliter of the sample was injected into the gas chromatograph. Retention times of the two solutes found in the soln were 1·65 and 4·15 min, corresponding to the retention times of acetic and isovaleric acids, respectively. The addition of a few drops of 30% ethylamine soln to the remaining oil and subsequent paper chromatography of the ethylamine salts in solvent system F[13] gave spots with R_f values of 0·18 and 0·55 which also correspond to acetic and isovaleric acids, respectively.

Scirp-9-*en*-3,4,8,15-*tetraol* (Ic). Toxin T-2 (Ib, 200 mg) was dissolved in 1N ethanolic (90%) NaOH and allowed to stand at 25° for 72 hr. The soln was then concentrated *in vacuo* to give a solid residue. Extraction of this residue with hot EtOAc, filtration through sintered glass and removal of the EtOAc *in vacuo* gave a yellow oil. Chromatography of the oil on a 25 g column of silica gel with solvent system B gave several 2 ml fractions containing the tetraol as detected by TLC (R_f in system B, 0·52). The tetraol-containing fractions were combined and concentrated *in vacuo* to give a clear, colorless oil. This product was soluble in highly polar solvents but came out of solution as an oil if combinations of polar and non-polar solvents were used in attempts to induce crystallization. However, it dissolved in hot EtOAc and cooling caused a white solid to come out of soln. The solid was dried at 65° for 48 hr *in vacuo* but the m.p. was very broad (70–85°) and analyses were unsatisfactory for $C_{15}H_{22}O_6$. PMR spectra of the solid showed the presence of EtOAc and upon removal of the PMR solvents (D_2O or C_5D_5N) *in vacuo* and redissolving the residue, a spectrum showing essentially pure tetraol was obtained; ν_{max}: 3400 (vs), 2930 (m), 1625 (m), 1450 (w), 1380 (w) cm^{-1}; UV end absorption only. PMR spectrum (Fig. 1) was measured in hexadeuteroacetone.

Selenium dioxide oxidation. Compound Id (231 mg) was dissolved in 90% AcOH (10 ml) and SeO_2 (68 mg) was added. After heating the soln to 80° for 72 hr, it was cooled and Na_2SO_3 was added to destroy excess SeO_2. Filtration of the soln through sintered glass was followed by removal of the solvents *in vacuo* and the remaining yellow oil was reacetylated by dissolving in pyridine (5 ml) and Ac_2O (5 ml) and allowing the soln to stand for 24 hr at 25°. The solvents were again removed *in vacuo* and the

remaining yellow oil chromatographed on a silica gel H preparative thin-layer plate developed twice with solvent system C. The edge of the plate was sprayed with H_2SO_4 and charred to develop the spots. Three bands corresponding to the spots (R_f values 0·10–0·22, 0·22–0·32, 0·32–0·45) were scraped off the plate and the material was eluted from the silica gel with 95% EtOH. An UV spectrum was taken of each eluate; only one band (R_f 0·21–0·32) showed any UV absorption. The EtOH was removed from this eluate *in vacuo* and a pale yellow oil (73 mg) remained. Attempted crystallization of this material from a variety of solvents yielded only an amorphous solid. The solid was chromatographed on an 8 g silica gel column developed with solvent system A. Fractions (1 ml) were collected and assayed for UV absorbing material having $\lambda_{max} \simeq 225$ nm. Fractions containing this material (31–37) were combined and concentrated *in vacuo*. Microcrystals of IIIa (12 mg) were obtained from ether–pentane: m.p. 136–138°; $[\alpha]_D^{22^\circ} = 71^\circ$ (*c* 0·142 in 95% EtOH); λ_{max}: 225 (log ε 3·94), 313 (log ε 1·31). An authentic sample of IIIa* showed: m.p. 137–138°; $[\alpha]_D^{22^\circ} = 75^\circ$ (*c* 0·141 in 95% EtOH); λ_{max}: 225 (log ε 3·95), 313 (log ε 1·31). IR spectra of the two samples were identical, and mixed m.p. was 135–138°.

8-*Oxoscirp*-9-*en*-3α,4β,15-*triol* (IIIb). Compound IIIa (2 mg) was dissolved in 0·5N ethanolic (80%) NaOH. After standing for 2 hr at 25°, samples of the soln were spotted on three different thin-layer plates next to a sample of authentic IIIb.* The R_f values for both samples were identical but varied with the solvent system (R_f: system B, 0·49; system D, 0·70; system E, 0·50).

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