error of the means are reported where appropriate.

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Registry No. 6, 55383-37-4; **7a**, 118102-34-4; **7b**, 865-21-4.

Structure–Activity Studies of Trichothecenes: Cytotoxicity of Analogues and Reaction Products Derived from T-2 Toxin and Neosolaniol

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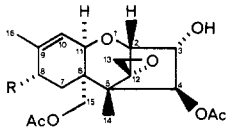
Forty-two analogues and reaction products derived from T-2 toxin or neosolaniol were assayed for their cytotoxicity to cultured mouse lymphoma cells. Structure–activity relationships confirmed the stereospecific nature of the cytotoxic action of T-2. Cytotoxicity was particularly susceptible to changes at C3, C4, C9, and C10 but was relatively unaffected by changes at C8, which appears to represent a region of steric tolerance in the interaction of T-2 with a cellular constituent. The most potent compounds were T-2, diacetoxyscirpenol, and a series of C8 ester analogues 11 and 31–35.

Trichothecene mycotoxins are toxic secondary metabolites produced by various species of fungi such as *Fusarium*, *Trichoderma*, *Trichothecium*, *Myrothecium*, *Stachybotrys*, *Cephalosporium*, and *Verticimonosporium*.[1] Chemically they are sesquiterpenoids, usually containing a relatively unreactive epoxide moiety. Trichothecenes, particularly those produced by species of *Fusarium* such as deoxynivalenol and T-2 toxin (1), have been implicated as the causative agents of various mycotoxicoses occurring in farm animals and man.[2] At the cellular level trichothecenes are potent inhibitors of protein synthesis in eukaryotic cells,[3] and one of the more potent naturally occurring metabolites, diacetoxyscirpenol (2) (DAS, anguidine), has undergone clinical trials as an anticancer agent.[4]



1 R = $-OCCH_2CH(CH_3)_2$

2 R = H

Structure–activity relationships of trichothecenes are therefore of interest with regard to the possible development of novel anticancer agents and to the etiology of mycotoxicoses occurring in animals and man. The first major structure–activity study was reported by Grove and Mortimer[5] following the isolation and characterization of diacetoxyscirpenol. Eighteen analogues and rearrangement products derived from diacetoxyscirpenol and nivalenol were assayed for their cytotoxicity toward HeLa cells. The main conclusion was that destruction of the epoxide moiety and rearrangement of the ring system virtually eliminated cytotoxicity. Further structure–activity studies, predominantly using trichothecenes isolated from laboratory cultures, have been reported from several laboratories.[6–11] More recently approximately 60 synthetic analogues derived from diacetoxyscirpenol were evaluated for their antitumor properties in mice.[12] Jarvis and co-workers have reported extensive investigations of the more complex macrocyclic trichothecenes.[13] All of these studies indicate a stereospecific interaction of trichothecenes with a receptor, presumed to be located on the ribosome. In this present paper we report additional structure–activity studies of synthetic trichothecenes, derived by modification of one of the most potent of the naturally occurring trichothecenes, T-2 toxin, or from its partial hydrolysis product neosolaniol (3). Some of the compounds reported herein were synthesized systematically as part of the structure–activity study; others were products of chemical investigations of T-2 toxin.

Chemistry

The natural metabolites T-2 toxin and neosolaniol were used as starting materials for this work. Analogues modified at C3, C4, or C15 are shown in Table I, those modified at C8 are shown in Table II, and those modified at C9–C10 are shown in Table III. New derivatives were characterized principally by NMR and mass spectroscopy. NMR as-

(1) *Trichothecenes–Chemical, Biological and Toxicological Aspects*; Ueno, Y., Ed.; Kodansha Scientific: Tokyo, 1983.
(2) Ueno, Y. *Adv. Nutr. Res.* **1980**, *3*, 301.
(3) McLaughlin, C. S.; Vaughan, M. H.; Campbell, I. M.; Wei, C. M.; Stafford, M. E.; Hansen, B. S. In *Mycotoxins in Human and Animal Health*; Rodricks, J. V., Hesseltine, C. W., Mehlman, M. A., Eds.; Pathotox Publishers Inc.: Park Forest South, IL, 1977; p 263.
(4) Yap, H. Y.; Murphy, W. K.; DiStefano, A. K.; Blumenshein, G. R.; Bodey, G. P. *Cancer Treat. Rep.* **1979**, *63*, 789.
(5) Grove, J. F.; Mortimer, P. H. *Biochem. Pharmacol.* **1969**, *18*, 1473.
(6) Ueno, Y. *Trichothecenes–Chemical, Biological and Toxicological Aspects*; Ueno, Y., Ed.; Kodansha Scientific: Tokyo, 1983; Chapters IV.3 and V.1.
(7) Ueno, Y.; Nakajima, M.; Sakai, K.; Ishii, K.; Sato, N. *J. Biochem.* **1973**, *74*, 285.
(8) Wei, C. M.; McLaughlin, C. S. *Biochem. Biophys. Res. Commun.* **1974**, *57*, 838.
(9) Carter, C. J.; Cannon, M. *Biochem. J.* **1977**, *166*, 399.
(10) Ehrlich, K. C.; Daigle, K. W. *Appl. Environ. Microbiol.* **1985**, *50*, 914.
(11) Thompson, W. J.; Wannemacher, R. W. *Toxicon.* **1986**, *24*, 985.
(12) Kaneko, T.; Schmitz, H.; Essery, J. M.; Rose, W.; Howell, H. G.; O'Herron, F. A.; Nachfolger, S.; Huftalen, J.; Bradner, W. T.; Patyka, R. A.; Doyle, T. W.; Davies, J.; Cundliffe, E. *J. Med. Chem.* **1982**, *25*, 579.
(13) Jarvis, B. B.; Eppley, R. M.; Mazzola, E. P. *Trichothecenes–Chemical, Biological and Toxicological Aspects*; Ueno, Y., Ed.; Kodansha Scientific: Tokyo, 1983; Chapter II.2.

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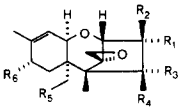
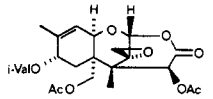
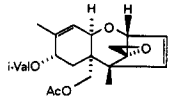
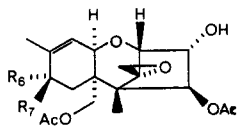
Table I. Structures of T-2 and Analogues Modified at C3, C4, or C15 and Their Cytotoxicity to Mouse Lymphoma Cells

compd	R_1	R_2	R_3	R_4	R_5	R_6	cytotoxicity:[a] LC_{50}, ng/mL
							
1	OH	H	H	OAc	OAc	Oi-Val[b]	1.8
4	OAc	H	H	OAc	OAc	Oi-Val	20
5	=O		H	OAc	OAc	Oi-Val	120
6	H	OH	H	OAc	OAc	Oi-Val	
7	H	OAc	H	OH	OAc	Oi-Val	
8	H	OAc	H	OAc	OAc	Oi-Val	1600
9	H	OH	H	OH	OH	OH	3000
10	OAc	H	H	OAc	OAc	OH	20
12	OAc	H	H	OAc	OAc	OAc	20
13	H	H	H	OAc	OAc	Oi-Val	20
14	H	H	H	OH	OAc	Oi-Val	1000
15	H	H	H	OH	OH	OH	9000
17	OH	H	H	OH	OAc	Oi-Val	3
18	OH	H	H	OH	OH	Oi-Val	100
19	OH	H	H	OH	OH	OH	100
20	OAc	H	H	OH	OAc	Oi-Val	20
21	OAc	H	=O		OAc	Oi-Val	2000
22	OAc	H	OH	H	OAc	Oi-Val	
23	OH	H	OAc	H	OAc	Oi-Val	120
24	OAc	H	H	H	OAc	Oi-Val	50
25	H	H	H	H	OAc	Oi-Val	2000
27	OMs[c]	H	H	OMs	OAc	Oi-Val	
28	=O		H	H	OH	OH	10000
29	=O		H	H	OAc	OAc	1100
30	OTBDMS[d]	H	H	OAc	OAc	OH	
44	OH	H	H	OAc	OH	OH	20
47	OAc	H	H	OAc	OH	OAc	80
48	OH	H	H	OAc	OH	OAc	60
16							2000
26							2000

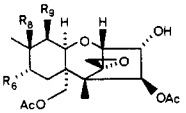
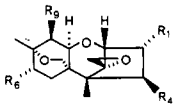
[a] Mouse lymphoma cells L5178Y. [b] i-Val = $COCH_2CH(CH_3)_2$. [c] Ms = SO_2Me. [d] TBDMS = $Si(CH_3)_2C(CH_3)_3$.

Table II. Structures of T-2 Analogues Modified at C8 and Their Cytotoxicity to Mouse Lymphoma Cells



compd	R_6	R_7	cytotoxicity: LC_{50}, ng/mL
2	H	H	2
3	OH	H	40
11	OAc	H	1.4
31	OCOEt	H	1.6
32	OCOPr	H	1.2
33	OCOBu	H	1.0
34	OCOPe	H	1.3
35	OCOPh	H	2.1
36	=O		6.9
37	H	OH	20

Table III. Structures of T-2 Analogues Modified at C9–C10 and Their Cytotoxicity to Mouse Lymphoma Cells

compd	R_1	R_4	R_6	R_8	R_9	cytotoxicity: LC_{50}, ng/mL
						
38			Oi-Val	H[a]	H	30
39			H	H[a]	H	50
40			Oi-Val			500
43			Oi-Val	OH	OH	85
						
41	OH	OH	OH		OH	2000
42	OAc	OAc	OAc		OAc	600
45	OH	OH	OH		Br	50000
46	OAc	OAc	OAc		Br	50000

[a] Mixture of C9 isomers.

signments were based on correlations that have been well established.[12,14,15] Selected ^1H and ^{13}C NMR data are shown in Tables IV and V. Elemental analyses were obtained for most new compounds; those isolated as solid foams or oils for which they were not obtained were shown to be >95% pure by ^{13}C NMR and TLC.

Isolation of T-2 and Neosolaniol. T-2 toxin and neosolaniol were isolated from cultures of *F. sporotrichioides* strain NRRL 3299 on rice, using culture condi-

(14) Cox, R. H.; Cole, R. J. *Trichothecenes–Chemical, Biological and Toxicological Aspects*; Ueno, Y., Ed.; Kodansha Scientific: Tokyo, 1983; Chapter II.3.
(15) Savard, M. E.; Blackwell, B. A.; Greenhalgh, R. *Can. J. Chem.* 1987, *65*, 2254.

Table IV. ^{1}H NMR Spectral Data (100 MHz) for T-2 Derivatives, Chemical Shifts, and Multiplicity (J in hertz)

	position										
compd	2	3	4	7	8	10	11	13	14	15	16
8	3.75 d (5.0)	5.4 dd (6.6)	6.1 d (6.6)	1.8, 2.4 ABX (5.5, 14.8)	5.25 ABX (5.5)	5.7 m (5.5)	4.05 d (5.5)	2.8, 3.1 AB (3.9)	0.8 s –	4.1, 4.3 AB (14.4)	1.75 s –
10	3.8 d (5.0)	5.15 dd (3.0, 4.5)	5.85 d (3.0)	1.8, 2.6 ABX (5.5, 14.0)	4.1 ABX –	5.6 m (5.5)	4.1 d –	2.8, 3.05 AB (4.0)	0.75 s –	4.3 AB –	1.85 s –
11	3.7 d (4.8)	4.2 dd (2.3, 4.8)	5.2 d (2.3)	2.0, 2.35 ABX (5.6, 12.9)	5.25 ABX (5.0)	5.8 m (5.8)	4.2 d (5.8)	2.8, 3.05 AB (4.0)	0.8 s –	4.1, 4.3 AB (12.2)	1.75 s –
12	3.85 d (5.4)	5.15 dd (3.2, 4.6)	5.85 d (3.2)	2.0, 2.3 ABX (5.7, 15.0)	5.25 ABX (5.7)	5.75 m (5.7)	4.15 d (5.7)	2.8, 3.05 AB (3.8)	0.75 s –	4.1, 4.35 AB –	1.75 s –
13	3.8 d (5.0)	2.55 m (7.5, 12.9)	5.95 d (7.6)	2.35 ABX (5.5, 15.0)	5.25 ABX (5.5)	5.7 m (5.6)	3.96 d (5.6)	2.85, 3.1 AB (3.6)	0.8 s –	4.3, 4.6 AB (12.0)	1.7 s –
14	3.8 d (5.0)	2.6 m (7.2, 15.4)	4.6 d –	2.3 ABX (5.2, 14.8)	5.25 ABX (5.2)	5.65 m (5.6)	3.8 d (5.6)	2.8, 3.1 AB (3.6)	0.85 s –	4.0, 4.25 AB (12.4)	1.7 s –
15	3.75 d (5.0)	2.5 m (7.2, 15.2)	4.35 d –	2.1 ABX –	– –	5.55 m (5.5)	– –	2.8, 3.1 AB (3.9)	0.95 s –	– –	1.85 s –
16	5.0 d (5.0)	– –	6.5 s –	1.75, 2.35 ABX (6.4, 15.0)	5.3 ABX –	5.7 m (6.0)	4.45 d (6.0)	2.9, 2.95 AB (3.7)	0.8 s –	4.1, 4.4 AB (14.5)	1.75 s –
20	3.75 d (4.5)	4.8 dd (3.0, 4.5)	4.4 d (3.0)	2.0, 2.3 ABX (5.0, 15.0)	5.25 ABX (5.0)	5.7 m (5.5)	3.85 d (5.5)	2.75, 3.0 AB (4.0)	0.8 s –	3.95, 4.2 AB (12.0)	1.7 s –
21	4.15 d (4.8)	5.3 d (4.8)	– –	2.0, 2.4 ABX (4.4, 14.6)	5.3 ABX (4.8)	5.7 m (5.0)	4.0 d (5.0)	3.0, 3.25 AB (3.8)	0.9 s –	3.95, 4.05 AB (12.5)	1.75 s –
24	3.75 d (4.5)	5.15 dd (5.2, 9.5)	2.0 m (4.5, 9.5)	– –	5.3 ABX (5.0)	5.75 m (5.5)	4.2 d (5.5)	2.85, 3.1 AB (3.8)	0.8 s –	3.95, 4.2 AB (12.0)	1.75 s –
25	3.7 d (3.6)	– –	– –	– –	5.3 ABX (5.4)	5.7 m (5.6)	3.9 d (5.6)	2.9, 3.15 AB (3.6)	0.8 s –	3.95, 4.2 AB (11.8)	1.75 s –
26	4.01 dd (0.8, 2.7)	6.1 dd (2.7, 5.9)	6.4 dd (0.8, 5.9)	2.3 ABX (5.1, 14.8)	5.35 ABX (5.1)	5.65 m (5.1)	4.05 d (5.1)	3.0, 3.2 AB (3.6)	0.95 s –	3.85, 4.15 AB (11.9)	1.75 s –
28	3.35 s –	– –	2.3, 2.7 d (19.0)	2.2 ABX (5.0)	4.15 ABX –	6.55 m (1.2, 5.5)	4.0 d –	3.2, 3.55 AB (4.0)	1.05 s –	3.6 AB –	1.95 s –
29	3.4 s –	– –	3.0, 6.3 d (18.5)	2.0, 2.4 ABX (5.5, 14.7)	5.3 ABX (4.5)	5.7 m (5.5)	4.1 d (5.5)	3.1, 3.25 AB (3.8)	1.0 s –	4.0, 4.2 AB (12.0)	1.75 s –
30	3.5 d (4.6)	4.3 dd –	5.7 d (2.6)	1.8, 2.3 ABX (5.3, 14.2)	4.1 ABX (5.3)	5.6 m (5.5)	4.3 d (5.5)	2.75, 3.0 AB (4.0)	0.7 s –	4.3 AB –	1.85 s –
34	3.65 d (4.8)	4.2 dd (2.8, 4.8)	5.3 d (2.8)	1.9, 2.3 ABX –	5.25 ABX (5)	5.75 m (5.5)	4.35 d (5)	2.8, 3.05 AB (3.8)	0.8 s –	4.1, 4.3 AB (12.2)	1.75 s –
35	3.7 d (4.7)	4.2 dd (3, 5)	5.5 d (3)	2.45 ABX (4.5, 14.5)	5.15 ABX (4.5)	5.9 m (5.5)	4.3 d (5.5)	2.85, 3.1 AB (3.9)	0.8 s –	4.1, 4.4 AB (12.3)	1.8 s –
36	3.8 d (4.8)	4.25 dd (2.8, 4.8)	5.15 d (2.8)	2.5, 2.9 AB (15.2)	– –	6.65 m (1.4, 5.6)	4.55 d (5.3)	2.85, 3.1 AB (3.7)	0.9 s –	4.2 AB (12.2)	1.85 s –
37	3.6 d (4.8)	4.1 dd –	5.15 d (2.8)	2.0 ABX –	4.1 ABX –	5.5 m (5.1)	4.1 d –	2.6, 2.9 AB (3.9)	0.75 s –	3.9, 4.15 AB (12.2)	1.7 s –
38	3.7 d (4.6)	4.1 dd (2.4, 4.6)	5.25 d (2.4)	2.0 ABX –	5.0 m (2.4)	– –	– –	2.8, 3.1 AB (3.8)	0.75 s –	4.0, 4.5 AB (12.0)	0.9 s –
39	3.65 d (4.6)	4.1 dd (2.8, 4.6)	5.35 d (2.8)	2.0 m –	– –	– –	4.1 m –	2.75, 3.1 AB (3.8)	0.75 s –	4.2 AB (13.3)	0.9 s –
40	3.75 d (4.5)	4.15 dd (2.0, 4.5)	5.25 d (2.0)	2.1 ABX –	5.25 ABX (5.0)	3.3 d (5.5)	4.3 d (5.5)	2.7, 3.05 AB (4.0)	0.7 s –	3.3, 4.0 AB (12.5)	1.3 s –
42	3.9 d (5.0)	5.1 dd (3.2, 5.0)	5.8 d (3.5)	2.4 ABX (3.0, 8.0)	5.1 ABX (3.0)	5.1 d (8.0)	4.2 d (8.0)	2.75, 3.0 AB (3.5)	0.6 s –	4.0 AB (9.5)	1.15 s –
44	3.65 d (4.5)	4.8 dd (2.8, 5.5)	5.05 d (2.8)	2.1 ABX –	4.1 ABX –	5.6 m (5.5)	4.1 d –	2.8, 3.05 AB (4.0)	0.85 s –	3.5 AB (12.4)	1.85 s –
45	3.55 d (5.0)	4.2 dd (5.0)	4.2 d –	1.55, 2.4 ABX –	4.2 ABX (7.6)	4.2 d –	4.2 d –	2.75, 2.9 AB (4.2)	0.7 s –	3.7, 3.9 AB (8.9)	1.3 s –
46	4.0 d (4.8)	5.3 dd (3.7, 4.8)	5.6 d (3.7)	1.45, 2.35 ABX (8.4, 14.5)	5.4 ABX (8.4)	4.35 d (8.2)	4.15 d (8.2)	2.75, 3.1 AB (3.8)	0.6 s –	3.8, 4.0 AB (9.2)	1.3 s –
47	3.8 d (4.8)	5.2 dd (3.4, 4.5)	6.05 d (3.4)	1.2, 2.3 ABX (5.6, 14.5)	5.3 ABX (5.5)	5.8 m (1.0, 6.0)	4.3 d (6.0)	2.3, 3.05 AB –	0.8 s –	3.65, 4.0 AB (15.0)	1.75 s –
48	3.55 d (4.6)	4.25 dd (3.1, 4.8)	5.45 d (3.0)	1.8, 2.3 ABX (5.5, 14.7)	5.35 ABX (5.5)	5.85 m (1.0, 5.6)	4.3 d (5.6)	2.8, 3.05 AB (3.9)	0.85 s –	3.6, 3.9 AB (12.6)	1.75 s –

tions modified from those reported by Burmeister[16] and Chi et al.[17] A simplified isolation procedure was adopted, which allowed the facile and consistent isolation of gram quantities of metabolites from the cultures. Previously reported procedures have utilized blending[16] or drying and grinding[17] of the moldy solid substrate prior to extraction of the metabolites, both potentially hazardous operations that also release large amounts of extraneous material. Extraction was accomplished quite simply by gentle shaking of the broken up substrate with dichloromethane. Crystalline T-2 toxin and neosolaniol were then obtained after one or two chromatographic purifications of the extract. Isolated yields of T-2 toxin were 2.5–3.5 g per kg of rice when cultured at 15 °C, together with 0.5–0.7 g per kg of neosolaniol. Yields of neosolaniol could be increased to 1 g per kg by raising the incubation temperature to 27 °C but at the expense of lower T-2 production.

Modification at C3. Acetylation[18] and oxidation[19] of the C3 hydroxyl group in T-2 to **4** and the labile ketone **5**, respectively, have been reported. Reduction of **5** with sodium borohydride gave T-2 toxin plus an unresolved mixture of the 3β epimer of T-2 (**6**) and the transesterified compound **7**. The common stereochemistry in **6** and **7** was confirmed by treatment with acetic anhydride–pyridine to give a single acetylation product (**8**). Hydrolysis gave

(16) Burmeister, H. R. *Appl. Microbiol.* **1971**, *21*, 739.
(17) Chi, M. S.; Mirocha, C. J.; Kurtz, H. J.; Weaver, G.; Bates, F.; Shimoda, V.; Burmeister, H. R. *Poult. Sci.* **1977**, *56*, 103.
(18) Bamburg, J. R.; Riggs, N. V.; Strong, F. M. *Tetrahedron* **1968**, *24*, 3329.
(19) Wallace, E. M.; Pathre, S. V.; Mirocha, C. J.; Robison, T. S.; Fenton, S. W. *J. Agric. Food. Chem.* **1977**, *25*, 836.

Table V. ^{13}C NMR Spectral Data (60 MHz) for T-2 Derivatives, Chemical Shifts

	position														
compd	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16
8	80.9	73.7	73.3	49.2	42.6	28.1	67.8	136.9	123.1	67.8	64.4	46.2	6.6	64.4	20.5
10	79.1	-	77.2	48.7	43.4	29.6	67.6	139.8	120.7	66.5	64.1	47.2	6.3	64.7	20.7
11	78.1	78.7	84.2	48.6	42.9	27.2	68.3	136.0	123.7	67.2	64.3	47.1	6.9	64.3	20.2
12	79.0	78.0	77.0	48.7	43.2	27.6	68.3	136.4	123.6	67.5	64.4	47.2	6.8	64.4	20.4
13	78.8	36.5	74.7	48.3	42.1	27.8	68.0/66.0[a]	136.5	123.3	68.0/66.0	65.2	47.8	6.6	64.4	20.3
14	78.3	39.6	74.0	48.8	41.4	27.5	68.0	136.5	123.4	65.7	65.2	47.4	7.1	64.4	20.2
15	78.4	39.4	74.1	49.0	43.5	28.0	67.1/66.5	139.6	121.6	67.1/66.5	65.3	47.4	7.0	61.9	20.4
16	99.4	-	-	48.4	41.9	28.9	-	-	121.4	67.4	55.1	48.4	8.5	63.6	-
20	83	78	77	48.6	42.4	27.4	67.8	136.4	123.3	67.3	63.9	46.7	6.7	64.4	20.0
21	75.5	73.2	209.1	53.8	45.5	25.9	67.2	136.6	122.7	67.6	60.8	48.3	6.6	62.9	20
24	77.7	71.0	39.0	45.1	41.7	27.2	68.2/67.5	135.9	124.1	68.3/67.5	64.5	48.6	12.1	64.5	20.3
25	79.7	31.6	-	49.4	43.6	25.7	68.4	136.2	124.2	66.0	64.7	45.0	12.0	66.0	20.4
26	81.0	146.5	126.2	49.9	42.5	27.1	66.9	135.7	124.7	69.2	70.2	51.2	9.0	65.0	21.1
28	79.1	212.2	46.0	48.7	44.3	27.7	69.4	139.7	121.9	66.7	64.2	47.9	12.1	62.0	20.5
29	79.3	211.7	48.1	42.5	45.6	27.0	68.3	136.9	123.6	68.3	64.1	48.8	11.6	64.3	20.6
30	77.6	79.2	83.3	48.8	43.4	30.3	67.2	139.6	120.9	66.5	64.5	46.8	6.3	64.5	20.4
34	78.2	78.7	84.3	48.3	42.9	27.7	68.1	136.2	123.6	67.2	64.3	47.1	6.9	64.5	21.0
35	78.1	78.7	84.3	48.7	42.8	26.6	69.2	136.2	123.9	67.5	64.2	47.0	7.1	64.2	20.4
36	83.5	78.9	78.1	47.5	48.9	38.1	196.5	138.8	136.8	68.2	64.4	46.6	6.2	64.4	15.4
37	84.3	79.1	78.1	48.8	45.9	31.5	68.2/67.8	142.6	120.8	68.2/67.7	64.2	47.0	6.8	63.7	18.7
38	84	79	78	49.2	43.8	28.6	72.0/69.6	27.8	30.5	72.0/69.6	64.6	47.5	6.7	65.1	17.3
39	84	79	78	49.2	44.3	35.0	30.3	21.9	25.0	70.2	64.7	47.7	6.6	64.1	21
40	77.7	77.1	83.7	47.0	42.6	25.8	68.9	57.8	58.9	67.6	63.7	48.6	6.5	65.5	18.7
42	68	-	78	45.8	41.3	29.6	68	-	-	78	63.7	46.6	5.6	66	19.7
44	84	78	77	49.2	44.9	28.2	68.6/66.2	-	-	68.6/66.2	64.4	47.0	6.8	62.1	20.5
45	80.8	80.8	80.0	45.8	42.3	30.9	68.0/67.5	74.7	55.0	68.0/67.5	66.0	46.6	6.7	64.7	20.8
46	78.3	77.6	77.6	45.8	41.7	28.3	70.1/67.6	73.6	52.0	70.1/67	65.9	46.4	5.6	63.4	20.0
47	79.7	77.8	77	47.2	44.2	27.6	68.2/67.3	135.8	124.0	68.2/67.3	64.2	48.4	6.1	63.3	20.1
48	84.3	78.7	77.6	47.2	44.4	27.3	68.3/67.2	135.8	124.3	68.3/67.2	64.4	48.7	6.7	63.2	20.3

[a] Assignments uncertain.

the tetrahydroxy compound **9**. Acetylation of neosolaniol with 1 equiv of acetic anhydride afforded selective acetylation at the C3 hydroxyl to give **10**, together with minor amounts of the C8 acetylated product **11** and the tetraacetate **12**. Deoxygenation of T-2 toxin at C3 was achieved by using the reagent of Barton and McCombie[20,21] to give **13**. Additional modification of **13** was obtained by partial hydrolysis with ammonium hydroxide to give **14** and **15**. Oxidation of ketone **5** with *m*-chloroperoxybenzoic acid gave a single lactone (**16**) via a regiospecific Baeyer–Villiger rearrangement. The structural assignment was confirmed by NMR decoupling experiments, which indicated that the proton on the carbon attached to two oxygens coupled to H11. Thus decoupling of H11 indicated H2 to be at δ 5.0. Selective decoupling at this frequency collapsed the low-field ^{13}C signal at δ 99.5.

Modification at C4. Hydrolysis of T-2 to a mixture of HT-2 (**17**), T-2 triol (**18**), and T-2-tetraol (**19**) (65:15:20) was achieved as reported previously.[22] HT-2 was selectively acetylated to the isomer of T-2 (**20**), with only minor amounts of T-2 and the triacetate (**4**) being formed. Oxidation of **20** with chromium trioxide–pyridine afforded the stable crystalline ketone **21**. Reduction of **21** with sodium borohydride proceeded stereospecifically at the β-face of the molecule, but the product was not the expected carbinol **22** but the transesterified isomer **23**. Support for the structural assignment was obtained by the failure of the product to oxidize back to **21**, unstable oxidation products being formed consistent with the C3 assignment. The isomer of T-2 (**20**) was deoxygenated under standard conditions to give **24**.

Modification at C3 and C4. Deoxygenation of HT-2 (**17**) using the Barton procedure afforded a mixture of the simple C3–C4 dideoxygenated product **25** plus the diolefin **26**, resulting from monodeoxygenation and elimination. Treatment of the dimesylate **27** with sodium methoxide gave the C3 deoxygenated ketone **28** in a reaction analogous to that reported for DAS by Sigg et al.[23] Acetylation of **28** gave **29**.

Modification at C8. In order to prepare a series of C8-modified analogues of T-2, neosolaniol was used as starting material. Selective protection of the C3 hydroxyl was achieved by silylation with *tert*-butyldimethylsilyl chloride and imidazole in dimethylformamide to give **30**.[24] Only minor amounts of the C8-silylated and C3,C8-disilylated products were formed. Acylation of **30** with a series of anhydrides or acid chlorides, followed by removal of the protective group with tetra-*n*-butylammonium fluoride, gave the C8-modified T-2 analogues **11** and **31–35**. Oxidation of neosolaniol with pyridinium dichromate in dichloromethane afforded the enone **36**, which on reduction gave a mixture of neosolaniol and its C8 β-OH epimer **37**.

Modification at C9–C10. Catalytic reduction of T-2 over 5% palladium on charcoal gave the expected reduction product **38**[18] plus the C8-deoxygenated product **39**. Although this reaction implies the intermediacy of DAS, attempts to modify the reaction conditions to achieve the direct conversion of T-2 to DAS were unsuccessful. An alternative procedure for achieving this conversion has been reported elsewhere.[25] Oxidation of T-2 with *m*-chloroperoxybenzoic acid gave the diepoxide **40**. Confirmation of the assignment of β stereochemistry to the ep-

(20) Barton, D. H. R.; McCombie, S. W. *J. Chem. Soc., Perkin Trans. I* **1975**, 1574.
(21) Tulshian, D. B.; Fraser-Reid, B. *Tetrahedron Lett.* **1980**, *21*, 4549.
(22) Wei, R.-D.; Strong, F. M.; Smalley, E. B.; Schnoes, H. K. *Biochem. Biophys. Res. Commun.* **1971**, *45*, 396.
(23) Sigg, H. P.; Mauli, R.; Flury, E.; Hauser, D. *Helv. Chim. Acta* **1965**, *48*, 962.
(24) Anderson, D. W.; Black, R. M.; Leigh, D. A.; Stoddart, J. F. *Tetrahedron Lett.* **1987**, *28*, 2653.
(25) Anderson, D. W.; Black, R. M.; Leigh, D. A.; Stoddart, J. F.; Williams, N. E. *Tetrahedron Lett.* **1987**, *23*, 2661.

oxide was obtained by treatment of **40** with base to give the cyclized product **41**, formed by intramolecular rearside attack at C9 by the C15 hydroxyl function. An analogous reaction was reported for a derivative of diacetoxyscirpenol.[23] Acetylation of **41** gave the tetraacetate **42**. Reaction of T-2 with a catalytic amount of osmium tetraoxide in aqueous acetone containing *N*-methylmorpholine *N*-oxide resulted in cis hydroxylation of the double bond to give **43**.

Modification at C15. Treatment of neosolaniol with aqueous ammonia afforded regiospecific hydrolysis of the primary ester function at C15 to give triol **44**. Acetylated analogues **47** and **48** were synthesized via the intermediacy of the bridged compound **45**, following the methodology reported by Roush.[26] Acylation of **45** gave **46**, which on treatment with a zinc–silver couple gave two products identified as **47** and **48**. Attempts to deoxygenate at C15, e.g., in compound **47** using the Barton method, were unsuccessful.

Cytotoxicity Testing

Compounds were assayed for cytotoxicity to mouse lymphoma cells L5178Y as described in the Experimental Section. The results are shown in Tables I–III. Results are expressed in terms of ng/mL since variations in molecular weight are generally small in relation to the accuracy of the assay.

Results and Discussion

Modification at C3. The most cytotoxic of the natural metabolites amongst the simple trichothecenes are T-2 and DAS, both of which possess a 3α-hydroxyl substituent. Acetylation of C3 (**4**, **12**, **20**) generally decreased activity by an order of magnitude, the exception being 3-acetylneosolaniol (**10**), which did not differ significantly in activity from neosolaniol; deoxygenation at C3 decreased activity by 1 to 2 orders of magnitude (**13**, **14**, **15**), the decrease being more pronounced with an adjacent 4β-hydroxyl group as in **14**. Oxidation of the 3α-hydroxyl to the ketone **5** or altering the stereochemistry to a 3β configuration (**8**, **9**) resulted in similar reductions in activity. A free hydroxyl group in the natural α configuration therefore appears optimum for cytotoxicity in the simple nonmacrocyclic trichothecenes (it should be noted, however, that very potent macrocyclic trichothecenes such as verrucarin A[27] are unsubstituted at C3).

Modification at C4. T-2 and DAS possess a 4β-acetoxy substituent. Selective hydrolysis of the 4β-acetoxy function (**17**, **20**) made little difference to activity in the presence of neighboring 3-hydroxy or acetoxy groups, as observed previously for HT-2 (**17**) in other in vitro systems.[6,11] A significant reduction in activity was observed when there was no adjacent substituent at C3 as in **14**. Deoxygenation at C4 reduced activity by up to an order of magnitude (**24**), and oxidation (**21**) or alteration of the stereochemistry at C4 (**23**) led to substantial decreases in activity. The optimum substitution at C4 therefore appears to be an acetoxy or hydroxyl function in the β configuration. Simultaneous modification of substituents at C3 and C4 generally produced marked decreases in activity (**16**, **25**, **26**, **28**, **29**).

Modification at C8. The fact that T-2 possesses a relatively bulky isovaleroyloxy substituent at C8, whereas diacetoxyscirpenol is unsubstituted, suggests that C8 corresponds to a region of steric tolerance in the interaction of trichothecenes with their receptor. This was supported by the homologous series of C8 esters **11** and **31–35**, which showed no significant differences in cytotoxicity from T-2 or DAS. Similarly the 8-keto analogue **36** showed only a small reduction in activity as found in other in vitro test systems.[11,12] The only compound modified at C8 that showed significantly reduced activity (20 fold) was the 8α-hydroxy analogue neosolaniol (**3**). This may be due to reduced transport through the cell membrane rather than to stereoelectronic effects at the receptor. In contrast to alterations at C3 and C4, alteration of the stereochemistry as in the 8β-hydroxyl compound **37** made no significant difference to activity.

Modification at C9–C10. In line with Grove and Mortimer's observation[5] with diacetoxyscirpenol, hydrogenation of the olefinic bond in T-2 led to a 10-fold reduction in cytotoxicity (**38**). Epoxidation or hydroxylation of the double bond resulted in greater losses in activity (**40**, **43**). Linkage of C15 to C9, which makes conformational as well as simple steric alterations to the molecule, substantially reduced or abolished activity (**41**, **42**, **45**, **46**).

Modification at C15. T-2 and diacetoxyscirpenol each contain an acetoxy function at C15. Because of difficulties encountered in deoxygenation at C15, the only modification studied was that of hydrolysis to leave a free hydroxyl. This generally reduced activity (**47**, **48**, **18**), except in the case of the neosolaniol analogue **44**. Kaneko et al.[12] reported that oxidation to give an aldehyde function at C15 increased protein synthesis inhibitory activity in HeLa cells.

Conclusions

It appears that for the simple trichothecenes natural evolution has produced the metabolites T-2 toxin and diacetoxyscirpenol with maximum cytotoxicity. Modifications at C3, C4, C15, and C9–C10 generally decreased activity, marked dependence on stereochemistry being shown for substituents at C3 and C4. The only compounds synthesized that were equipotent with T-2 were a series of C8-modified ester analogues, suggesting that C8 corresponds to a region of steric tolerance in the interaction of trichothecenes with a cellular receptor. However, it is not known to what extent mouse lymphoma cells metabolize trichothecenes and this could involve hydrolysis at C8. Lipophilicity is likely to effect the access of the compounds to the cell, and decreased activity was generally observed with compounds containing more than two free hydroxyl functions.

Experimental Section

Melting points were determined in capillary tubes on a Gallenkamp apparatus and are uncorrected. ^{1}H NMR spectra were determined in deuteriochloroform unless otherwise stated with tetramethylsilane as internal standard on a JEOL JNM-MH-100 spectrometer. ^{13}C NMR spectra were obtained using a JEOL FX60Q spectrometer with deuteriochloroform as internal standard. IR spectra were measured in KBr disks on a Perkin-Elmer 157 spectrophotometer. Mass spectra were obtained on a VG 7070 EQ mass spectrometer. Optical rotations were measured in ethanol unless otherwise stated on a Bellingham and Stanley P-70 polarimeter. Column chromatography was performed under slight pressure (hand bellows) using Merck silica gel 60G (230–400 mesh). Petroleum ether refers to the fraction with bp 60–80 °C. Trichothecenes may cause necrotic lesions of the skin at μg doses. All operations were performed in an efficiently ventilated fumecupboard using protective gloves. Glassware was decontaminated by standing in sodium hypochlorite solution overnight. Solid waste was sealed in polyethylene bags and incinerated.

Production of T-2 Toxin and Neosolaniol. Spores from a soil tube of *F. sporotrichioides* NRRL 3299 were plated onto Petri

(26) Roush, W. R.; Russo-Rodriguez, S. *J. Org. Chem.* **1985**, *50*, 3224.

(27) The macrocyclics verrucarin A and roridin A gave LC_{50} values of 1 and 2 ng/mL, respectively, in the cytotoxicity assay.

dishes containing potato agar and grown at 25 °C for 3 days. Subcultures were made onto potato agar slopes (7–10 mL) contained in 1-oz Mckartney bottles. After incubating for 1 week the slope cultures were transferred, one per flask, into 64 × 250 mL conical flasks each containing rice (50 g dry weight), previously soaked in an equal weight of distilled water for 30 min at room temperature, strained through a sieve, and autoclaved at 15 psi for 30 min. The flasks were incubated statically at 15 °C for 4 weeks, after which time a mass of white mycelium with varying amounts of red and yellow pigmentation was visible. The matted rice and mycelium was broken up with a glass rod and extracted by gentle shaking (135 rpm) overnight with dichloromethane (150 mL per flask), using a New Brunswick incubator–shaker. The extracts were decanted through Whatman 113v fluted filter paper, periodically changing the paper to ensure rapid filtration. The flasks were again extracted with a further portion of dichloromethane (80 mL), and the contents were filtered through Celite contained in a Buchner funnel. The residue was washed several times with dichloromethane, and the combined filtrates were concentrated to an oil on a rotary evaporator. The residual oil was extracted with warm ether, monitoring by TLC to indicate complete extraction of the T-2 and neosolaniol. The extracts were dried ($MgSO_4$), concentrated, and purified by flash chromatography. The concentrate was dissolved in the minimum volume of dichloromethane and loaded onto a column (2 in. × 8 in.) containing silica gel (130 g) and 1:1 ethyl acetate–petroleum ether as solvent. Elution with 1:1 ethyl acetate–ether yielded a crude fraction containing T-2 toxin, which crystallized on concentration and addition of petroleum ether. Recrystallization from ether–petroleum ether yielded T-2 toxin, 10.7 g, mp 150–152 °C (lit.[18] mp 151–152 °C), pure by TLC and NMR: yield 3.35 g per kg of rice (dry wt).

Further elution of the column with 7:3 ethyl acetate–petroleum ether yielded a fraction containing neosolaniol. This was concentrated and rechromatographed on silica gel (40 g) using 7:3 ethyl acetate–petroleum ether as eluant. Neosolaniol was crystallized from the appropriate fraction after concentration and addition of diisopropyl ether. Recrystallization from ethyl acetate–petroleum ether yielded neosolaniol, 2.5 g, mp 169–170 °C (lit.[28] mp 171–172 °C), pure by TLC and NMR: yield 0.66 g per kg of rice. Alteration of the culture conditions to 27 °C for 3 weeks increased the isolated yield of neosolaniol to 1.04 g per kg of rice, but with decreased isolated yields of T-2 (0.4–0.5 g per kg of rice).

4β,15-Diacetoxy-3β-hydroxy-8α-[(3-methylbutyryl)oxy]-12,13-epoxytrichothec-9-ene (6). To a solution of 4β,15-diacetoxy-8α-[(3-methylbutyryl)oxy]-12,13-epoxytrichothec-9-en-3-one (**5**)[19] (250 mg, 0.54 mmol) in 2-propanol (30 mL) was added sodium borohydride (4.9 mg, 0.13 mmol) in 2-propanol (4.9 mL), and the reaction was stirred at room temperature for 30 min. Acetone (5 mL) was then added, and the mixture was concentrated and partitioned between diethyl ether and water. The combined ether extracts were dried ($MgSO_4$) and concentrated, and the residue was chromatographed (eluant 0.5% 2-propanol in benzene) to give 53 mg of **1** plus 47 mg (19%) of an unresolved mixture of **6** and its transesterified isomer **7**.

3β,4β,15-Triacetoxy-8α-[(3-methylbutyryl)oxy]-12,13-epoxytrichothec-9-ene (8). A mixture of **6** and **7** (40 mg) was treated with acetic anhydride (1 mL) and pyridine (1 mL) at room temperature overnight. After concentration, the residue was chromatographed (eluant 4:1 petroleum ether–acetone) to give 33 mg (82%) of **8**: $[\alpha]_D$ +10.2° (*c* 0.23): IR 1750 cm^{-1}; MS, *m/z* 526 (MNH_4^+), 509 (MH^+). Anal. ($C_{26}H_{36}O_{10}$) C, H.

3β,4β,8α,15-Tetrahydroxy-12,13-epoxytrichothec-9-ene (9). The reduction product from the ketone **5** (400 mg) was stirred in methanol–water (4:1, 50 mL) with potassium hydroxide (1 g) at room temperature for 4 h. After concentration, the residue was chromatographed (initial eluant 5% MeOH in $CHCl_3$, final eluant 15% MeOH in $CHCl_3$) to give 75 mg of **9** (29%): $[\alpha]_D$ −58.6° (*c* 0.13); IR 3350 cm^{-1}; MS, *m/z* 316 (MNH_4^+), 299 (MH^+).

3α,4β,15-Triacetoxy-8α-hydroxy-12,13-epoxytrichothec-9-ene (10). Neosolaniol (**3**, 220 mg, 0.58 mmol) was treated with acetic anhydride (58 mg, 0.57 mmol) and pyridine (1 mL) at room temperature overnight. The residue was chromatographed (eluant 1% MeOH in $CHCl_3$) to give 216 mg (86%) of **10** as an oil: $[\alpha]_D$ +35.4° (*c* 0.7); IR 3540, 1750 cm^{-1}; MS, *m/z* 442 (MNH_4^+), 425 (MH^+). Anal. ($C_{21}H_{28}O_9$) C, H. The isomeric compound **11** and tetraacetate **12** were also obtained in 2% and 8% yield, respectively.

4β,15-Diacetoxy-8α-[(3-methylbutyryl)oxy]-12,13-epoxytrichothec-9-ene (13). T-2 toxin (280 mg, 0.6 mmol) and *N,N*-thiocarbonyldiimidazole (260 mg, 1.44 mmol) in dichloromethane (5 mL) were heated under reflux for 1.5 h. After cooling, the mixture was concentrated and chromatographed (eluant $CHCl_3$) to give 4β,15-diacetoxy-3-[(1-imidazolylthiocarbonyl)oxy]-8α-[(3-methylbutyryl)oxy]-12,13-epoxytrichothec-9-ene (335 mg, 97%); IR 1750 cm^{-1}. A solution of this compound (270 mg, 0.48 mmol) in anhydrous toluene (18 mL) was added dropwise over a period of 90 min to a refluxing solution of tri-*n*-butyltin hydride (390 mg, 1.34 mmol) in anhydrous toluene (12 mL) containing a catalytic amount (0.04 mmol) of α-azoisobutyrylnitrile. After refluxing an additional hour, the reaction was cooled, concentrated, and chromatographed (initial eluant petroleum ether, final eluant 20% acetone in petroleum ether) to give 270 mg of **13** (62%): mp 105–6 °C; $[\alpha]_D$ −19.7° (*c* 0.6); IR 1750 cm^{-1}, MS, *m/z* 468 (MNH_4^+), 451 (MH^+). Anal. ($C_{24}H_{34}O_8 \cdot 0.25H_2O$) C, H.

15-Acetoxy-4β-hydroxy-8α-[(3-methylbutyryl)oxy]-12,13-epoxytrichothec-9-ene (14). **13** (90 mg, 0.2 mmol) was added to a solution of 2% ammonia in methanol–water (4:1, 45 mL), and the solution was stirred at room temperature for 48 h. Concentration and chromatography (initial eluant $CHCl_3$, final eluant 5% MeOH in $CHCl_3$) gave 10 mg of **14** (16%): $[\alpha]_D$ −31.6° (*c* 0.1): IR 3500, 1740 cm^{-1}; MS, *m/z* 426 (MNH_4^+), 409 (MH^+). Further elution yielded the triol **15** (11 mg, 25%): mp 171–172 °C; $[\alpha]_D$ −76.0° (*c* 0.5); IR 3500 cm^{-1}; MS, *m/z* 300 (MNH_4^+), 283 (MH^+). Anal. ($C_{15}H_{22}O_5$) C, H.

Lactone 16. *m*-Chloroperoxybenzoic acid (35 mg, 0.2 mmol) was added to a solution of the ketone **5** (90 mg, 0.19 mmol) in dichloromethane (7 mL), and the mixture was stirred at room temperature for 5 h. The solution was washed with 20% sodium thiosulfate solution and then with 2 N sodium bicarbonate. After drying (Na_2SO_4) and removal of the solvent, the crude product was chromatographed (eluant 1% MeOH in $CHCl_3$) to give 67 mg of **16** (72%): $[\alpha]_D$ −19.6° (*c* 0.7); IR 1750 cm^{-1}; MS, *m/z* 498.2337 (498.2339) (MNH_4^+), 481 (MH^+).

3α,15-Diacetoxy-4β-hydroxy-8α-[(3-methylbutyryl)oxy]-12,13-epoxytrichothec-9-ene (20) was prepared from HT-2 (**17**)[22] and 1 equiv of Ac_2O in pyridine: yield 64%; mp 147.5–148.5; $[\alpha]_D$ −24.9° (*c* 0.5); IR 3490, 1750 cm^{-1}; MS, *m/z* 484 (MNH_4^+). Anal. ($C_{24}H_{34}O_9$) C, H.

3α,15-Diacetoxy-8α-[(3-methylbutyryl)oxy]-12,13-epoxytrichothec-9-en-4-one (21). Pyridine (1.4 mL) was added dropwise to a suspension of chromium trioxide (800 mg, 8 mmol) in dichloromethane (8 mL) at 0 °C, and the mixture was stirred at this temperature for a further 20 min. A solution of **20** (110 mg, 0.24 mmol) in dichloromethane (2 mL) was added, and the reaction was allowed to warm to room temperature. After 1 h TLC indicated complete absence of starting material. Diethyl ether was added and the resulting suspension was filtered through Celite. The filtrate was concentrated and the residue was crystallized from ether to give 90 mg of **21**: yield 82%; mp 148–149 °C; $[\alpha]_D$ +43.2° (*c* 0.5); IR 1750 cm^{-1}; MS, *m/z* 482 (MNH_4^+), 465 (MH^+). Anal. ($C_{24}H_{32}O_9 \cdot 0.25H_2O$) C, H.

4α,15-Diacetoxy-3α-hydroxy-8α-[(3-methylbutyryl)oxy]-12,13-epoxytrichothec-9-ene (23) was prepared from **21** by reduction with sodium borohydride in 2-propanol: yield 31%; IR 3490, 1750 cm^{-1}; MS, *m/z* 484 (MNH_4^+), 467 (MH^+).

3α,15-Diacetoxy-8α-[(3-methylbutyryl)oxy]-12,13-epoxytrichothec-9-ene (24) was prepared from **20** by Barton deoxygenation: yield 73%; mp 122.5–123.5 °C; $[\alpha]_D$ +2.5° (*c* 0.5); IR 1750 cm^{-1}; MS, *m/z* 468 (MNH_4^+), 451 (MH^+). Anal. ($C_{24}H_{34}O_8$) C, H.

15-Acetoxy-8α-[(3-methylbutyryl)oxy]-12,13-epoxytrichothec-9-ene (25) was prepared from HT-2 (**17**) by Barton deoxygenation and isolated by chromatography (eluant 8:2 petroleum ether–acetone): yield 31%; $[\alpha]_D$ −58.3° (*c* 0.12); IR 1740 cm^{-1}; MS, *m/z* 410.2520 (410.2543) (MNH_4^+), 393 (MH^+). The diene **26** was also obtained in 10% yield: $[\alpha]_D$ +129.8° (*c* 0.6); IR 1730 cm^{-1}; MS, *m/z* 408.2432 (408.2386) (MNH_4^+), 391 (MN^+).

(28) Harri, E.; Loeffler, W.; Sigg, H. P.; Stahelin, H.; Stoll, C.; Tamm, C.; Wiesinger, E. *Helv. Chim. Acta* **1962**, *45*, 839.

15-Acetoxy-3α,4β-bis(mesyloxy)-8α-[(3-methylbutyryl)oxy]-12,13-epoxytrichothec-9-ene (27). To a solution of HT-2 (**17**) (50 mg, 0.12 mmol) in anhydrous pyridine at 0 °C was added methanesulfonyl chloride (0.5 mL), and the mixture was stirred at this temperature for 2 h. Concentration and chromatography (eluant $CHCl_3$) yielded 60 mg of **27** (88%): IR 1730 cm^{-1}; MS, m/z 598 (MNH_4^+).

8α,15-Dihydroxy-12,13-epoxytrichothec-9-en-3-one (28). To a solution of 60% sodium hydride (40 mg, 1 mmol) in methanol (3 mL) was added the dimesylate **27** (55 mg, 0.09 mmol) in methanol (3 mL), and the mixture was refluxed under argon for 3 h. After cooling, the reaction was quenched with a few drops of water and concentrated and the residue was chromatographed (initial eluant CH_2Cl_2, final eluant 21% MeOH in CH_2Cl_2) to give the ketone **28**, 20 mg (75%): mp 160.5–161.5 °C; $[\alpha]_D$ −35.2° (*c* 0.5); IR 3450, 1750 cm^{-1}; MS, m/z 298 (MNH_4^+), 281 (MH^+). Anal. ($C_{15}H_{20}O_5$) C, H.

8α,15-Diacetoxy-12,13-epoxytrichothec-9-en-3-one (29) was prepared from **28** by acetylation with excess Ac_2O: yield 76%; $[\alpha]_D$ −54.9° (*c* 0.5); IR 3000, 1740 cm^{-1}; MS, m/z 382 (MNH_4^+), 365 (MH^+).

3α-(*tert*-Butyldimethylsiloxy)-4β,8α,15-triacetoxy-12,13-epoxytrichothec-9-ene (30). A mixture of neosolaniol (**3**) (250 mg, 0.66 mmol), *tert*-butyldimethylsilyl chloride (300 mg, 1.99 mmol), and imidazole (135 mg, 1.99 mmol) in anhydrous dimethylformamide (8 mL) was stirred at room temperature, and the reaction was monitored by TLC. After 3 days the solvent was removed and the residue was chromatographed (initial eluant $CHCl_3$, final eluant 5% MeOH in $CHCl_3$) to give the title compound, 242 mg (74%): mp 157.5–158.5 °C; $[\alpha]_D$ +20.5° (*c* 0.6); IR 3500, 1735, 1250 cm^{-1}; MS, m/z 514 (MNH_4^+), 497 (MH^+). Anal. ($C_{25}H_{40}O_8Si$) C, H.

Also obtained were 8α-(*tert*-butyldimethylsiloxy)-4β,15-diacetoxy-3α-hydroxy-12,13-epoxytrichothec-9-ene (23 mg, 7%) [mp 173–174 °C; $[\alpha]_D$ −16.3° (*c* 0.5); IR 3480, 1735, 1250 cm^{-1}; MS, m/z 514 (MNH_4^+), 497 (MH^+). Anal. ($C_{25}H_{40}O_8Si$) C, H.] and 3α,8α-bis(*tert*-butylsiloxy)-4β,15-diacetoxy-12,13-epoxytrichothec-9-ene (60 mg, 15%) [mp 116–118 °C; $[\alpha]_D$ +12.6° (*c* 0.6); IR 3490, 1745, 1250 cm^{-1}; MS, m/z 628 (MNH_4^+), 611 (MH^+). Anal. ($C_{31}H_{54}O_8Si_2$) C, H.].

3α-Hydroxy-4β,8α,15-triacetoxy-12,13-epoxytrichothec-9-ene (11). To a solution of **30** (150 mg, 0.3 mmol) in anhydrous dichloromethane (3 mL) were added acetic anhydride (61 mg, 0.60 mmol), triethylamine (61 mg, 0.60 mmol), and a catalytic amount of 4-(dimethylamino)pyridine (5 mg, 0.04 mmol). The reaction was stirred overnight at room temperature, concentrated and chromatographed (eluant 10% acetone in petroleum ether) to give 3α-(*tert*-butyldimethylsiloxy)-4β,8α,15-triacetoxy-12,13-epoxytrichothec-9-ene (134 mg, 82%); IR 1735, 1250 cm^{-1}. A solution of this compound (134 mg, 0.25 mmol) was stirred with tetra-*n*-butylammonium fluoride (0.25 mL of a 1 M solution in THF, 0.25 mmol) in anhydrous tetrahydrofuran (2 mL) at room temperature for 1 h. The solvent was removed, and the residue was taken up in ether, washed with water, and dried (Na_2SO_4). Concentration and chromatography (initial eluant $CHCl_3$, final eluant 1% MeOH in $CHCl_3$) gave 94 mg of **11** (89%): mp 182–183 °C; $[\alpha]_D$ −3.6° (*c* 0.5); IR 3350, 1730, 1250, 1225 cm^{-1}; MS, m/z 442 (MNH_4^+), 425 (MH^+). Anal. ($C_{21}H_{28}O_9$) C, H.

The following 8α-acylated trichothecenes were prepared similarly from **30**.

3α-Hydroxy-4β,15-diacetoxy-8α-(propionyloxy)-12,13-epoxytrichothec-9-ene (31): overall yield 56%; mp 179.5–180 °C; $[\alpha]_D$ +14.1° (*c* 0.4); IR 3400, 1740, 1250 cm^{-1}; MS, m/z 456 (MNH_4^+), 439 (MH^+). Anal. ($C_{22}H_{30}O_9$) C, H.

3α-Hydroxy-4β,15-diacetoxy-8α-(butyryloxy)-12,13-epoxytrichothec-9-ene (32): overall yield 64%; mp 148–149 °C; $[\alpha]_D$ +12.9° (*c* 0.45); IR 3400, 1730, 1240 cm^{-1}; MS, m/z 470 (MNH_4^+), 453 (MH^+). Anal. ($C_{23}H_{32}O_9$) C, H.

3α-Hydroxy-4β,15-diacetoxy-8α-(pentanoyloxy)-12,13-epoxytrichothec-9-ene (33): overall yield 58%; mp 152.5–153 °C; $[\alpha]_D$ +13.3° (*c* 0.55); IR 3460, 1740, 1250 cm^{-1}; MS, m/z 484 (MNH_4^+), 467 (MH^+). Anal. ($C_{24}H_{34}O_9 \cdot 0.25H_2O$) C, H.

3α-Hydroxy-4β,15-diacetoxy-8α-(hexanoyloxy)-12,13-epoxytrichothec-9-ene (34): overall yield 55%; mp 146–147 °C; $[\alpha]_D$ +13.8° (*c* 0.5); IR 3450, 1735, 1250 cm^{-1}; MS, m/z 498 (MNH_4^+), 481 (MH^+). Anal. ($C_{25}H_{36}O_9 \cdot 0.25H_2O$) C, H.

3α-Hydroxy-4β,15-diacetoxy-8α-(benzoyloxy)-12,13-epoxytrichothec-9-ene (35). To a solution of **30** (102 mg, 0.21 mmol) in anhydrous acetonitrile (3 mL) were added benzoyl chloride (89 mg, 0.63 mmol), triethylamine (81 mg, 0.8 mmol), and a catalytic amount of 4-(dimethylamino)pyridine (5 mg, 0.04 mmol). The reaction was heated under reflux for 7 h and concentrated, and the residue was chromatographed (eluant 2% MeOH in $CHCl_3$) to give 8α-(benzoyloxy)-3α-(*tert*-butyldimethylsiloxy)-4β,15-diacetoxy-12,13-epoxytrichothec-9-ene (111 mg, 90%); IR 1740, 1720, 1270, 1250 cm^{-1}. Desilylation was carried out as described above to give **35** (68%): mp 204–205 °C; $[\alpha]_D$ −71.1° (*c* 0.5); IR 3450, 1740, 1720, 1250 cm^{-1}; MS, m/z 504 (MNH_4^+), 487 (MH^+). Anal. ($C_{26}H_{30}O_9 \cdot 0.5H_2O$) C, H.

4β,15-Diacetoxy-3α-hydroxy-12,13-epoxytrichothec-9-en-8-one (36) was prepared from neosolaniol and pyridinium dichromate: yield 90%; $[\alpha]_D$ +81.2° (*c* 0.40); IR 3510, 1755 cm^{-1}; MS, m/z 398 (MNH_4^+), 381 (MH^+).

4β,15-Diacetoxy-3α,8β-dihydroxy-12,13-epoxytrichothec-9-ene (37) was prepared by reduction of **36** with $NaBH_4$ in 2-propanol: yield 13%; $[\alpha]_D$ +72.5° (*c* 0.13); IR 3400, 1740 cm^{-1}; MS, m/z 400.1944 (400.1971) (MNH_4^+).

4β,15-Diacetoxy-3α-hydroxy-8α-[(3-methylbutyryl)oxy]-12,13-epoxytrichothecane (38). A solution of T-2 (90 mg, 0.19 mmol) in methanol (2 mL) was added to a suspension of 10% palladium on charcoal (10 mg), and the mixture was stirred at room temperature under an atmosphere of hydrogen for 5 h. After filtration and concentration the residue was chromatographed (initial eluant $CHCl_3$, final eluant 5% MeOH in $CHCl_3$) to yield 37 mg (41%) of **38** as a mixture of isomers: mp 132–4 °C; $[\alpha]_D$ +56.9° (*c* 0.5); IR 3500, 1725 cm^{-1}; MS, m/z 486 (MNH_4^+). Anal. ($C_{24}H_{36}O_9$) C, H.

Further elution yielded the C8-deoxygenated hydrogenolysis product **39** (35 mg, 49%): mp 137–8 °C; $[\alpha]_D$ +44.5° (*c* 0.5) IR 1748 cm^{-1}; MS, m/z 386 (MNH_4^+), 369 (MH^+). Anal. ($C_{19}H_{28}O_7 \cdot 0.25H_2O$) C, H.

4β,15-Diacetoxy-3α-hydroxy-8α-[(3-methylbutyryl)oxy]-9β,10β:12,13-diepoxytrichothecane (40). To a solution of T-2 (130 mg, 0.28 mmol) in ethanol-free chloroform (10 mL) was added *m*-chloroperoxybenzoic acid (160 mg, 0.93 mmol), and the mixture was heated under reflux for 48 h. After cooling, the solution was washed sequentially with 20% aqueous sodium thiosulfate and 2 N aqueous sodium bicarbonate. After drying (Na_2SO_4) and concentration, chromatography (initial eluant $CHCl_3$, final eluant 2% MeOH in $CHCl_3$) yielded 100 mg of **40** (76%): mp 161–2 °C; $[\alpha]_D$ +62.4° (*c* 0.6); IR 1730 cm^{-1}; MS, m/z 500 (MNH_4^+). Anal. ($C_{24}H_{34}O_{10}$) C, H.

3α,4β,8α,10β-Tetrahydroxy-9α,15:12,13-diepoxytrichothecane (41) was prepared from **40** by hydrolysis with KOH in aqueous methanol as used for the preparation of **9**: yield 56%; $[\alpha]_D$ +9.3° (*c* 0.4); MS, m/z 332 (MNH_4^+), 315 (MH^+). Acetylation gave 3α,4β,8α,10β-tetraacetoxy-9α,15:12,13-diepoxytrichothecane (**42**): yield 56%; mp 159–160 °C; $[\alpha]_D$ +73.7° (*c* 0.55); IR 1740 cm^{-1}; MS, m/z 500 (MNH_4^+). Anal. ($C_{23}H_{30}O_7$) C, H.

4β,15-Diacetoxy-8α-[(3-methylbutyryl)oxy]-3α,9β,10β-trihydroxy-12,13-epoxytrichothecane (43). T-2 toxin (50 mg, 0.11 mmol) was dissolved in 50% aqueous acetone (3 mL) and a catalytic amount (one crystal) of osmium tetraoxide was added followed by *N*-methylmorpholine *N*-oxide (20 mg, 0.17 mmol). The reaction was stirred at room temperature and monitored by TLC. Two further similar amounts of reagents were added at intervals of 6 h. After column chromatography (elution with 5% MeOH in $CHCl_3$) the triol **43** was obtained (26 mg, 48%): $[\alpha]_D$ +62.4° (*c* 0.13); IR 3480, 1745 cm^{-1}; MS, m/z 518.2601 (518.2713) (MNH_4^+), 501 (MH^+).

4β-Acetoxy-3α,8α,15-trihydroxy-12,13-epoxytrichothec-9-ene (44) was prepared by treatment of neosolaniol with aqueous ammonia: yield 67%; mp 163–4 °C; $[\alpha]_D$ −2.1° (*c* 0.5); IR 3400, 1745 cm^{-1}; MS, m/z 358 (MNH_4^+), 341 (MH^+). Anal. ($C_{17}H_{24}O_7$) C, H.

10β-Bromo-3α,4β,8α-trihydroxy-9α,15:12,13-diepoxytrichothecane (45). To a solution of T-2 tetraol (**19**) (90 mg, 0.30 mmol) in anhydrous acetonitrile (87 mL) was added *N*-bromosuccinimide (59 mg, 0.33 mmol), and the mixture was stirred at room temperature overnight. Chromatography (initial eluant $CHCl_3$, final eluant 5% MeOH in $CHCl_3$) yielded the pure bromide

45 (111 mg, 97%): IR 3450 cm^{-1}; MS, m/z 396, 394 (MNH_4^+), 378, 376 (MH^+).

10β-Bromo-3α,4β,8α-triacetoxy-9α,15:12,13-diepoxytrichothecane (46) was prepared from **45** and excess Ac_2O: yield 84%; $[\alpha]_D$ +27.1° (c 0.56); IR 1745 cm^{-1}; MS, m/z 522, 520.1240 (520.1182) (MNH_4^+), 503, 501 (MH^+).

3α,4β,8α-Triacetoxy-15-hydroxy-12,13-epoxytrichothecene (47). The zinc–silver couple was prepared immediately before use as described previously.[26] To a suspension of the couple (prepared from zinc dust (30 g) and silver acetate (175 mg)) in ether (73 mL) was added **46** (942 mg, 1.87 mmol) in a tetrahydrofuran–ethanol mixture (175 mL, 5:1). The mixture was heated under reflux for 16 h. The solvents were removed in vacuo, and the residue taken up in acetone and filtered through Celite. After concentration, the residue was chromatographed (initial eluant $CHCl_3$; final eluant 2% MeOH in $CHCl_3$) to give the alcohol **47** (155 mg, 20%): $[\alpha]_D$ +30.2° (c 0.44); IR 3480, 1735 cm^{-1}; MS, m/z 442.2124 (442.2077) (MNH_4^+), 425 (MH^+). Also obtained was 4β,8α-diacetoxy-3α,15-dihydroxy-12,13-epoxytrichothec-9-ene (**48**) (151 mg, 18%): $[\alpha]_D$ +17.4° (c 0.41); IR 3500, 1730 cm^{-1}; MS, m/z 400.1992 (400.1971) (MNH_4^+).

Cytotoxicity Assay. Cultures of mouse lymphoma cells (L5178Y) were prepared from frozen stocks derived from an original culture supplied by the Central Toxicology Laboratory, ICI (Alderley Edge). Eagle's Minimum Essential Medium supplemented with 10% or 3% horse serum (10:90 HS–EMEM, 3:97 HS–EMEM) and containing sodium pyruvate (200 μg/mL) was used for routine subculture and cytotoxicity experiments, respectively. Cells were cultured in suspension in 1-oz polystyrene Universal bottles (20 mL per bottle) at 37 °C with intermittent agitation. Cells selected for use in cytotoxicity experiments were subcultured 24 h previously to ensure that immediately prior to use in an experiment the cells were in log phase growth and at a final cell density not exceeding 5×10^5 cells/mL. All media contained penicillin (100 units/mL), streptomycin (100 μg/mL), and amphotericin B (2.5 μg/mL).

Stock solutions of the compounds were made up in DMSO at initial concentrations of 1×10^{-2} g/mL. Tenfold serial dilutions were then made in DMSO to achieve concentrations down to 1×10^{-7} g/mL. These solutions (100 μL) were then diluted 200-fold with 10:90 HS–EMEM (20 mL) to achieve final concentrations in the culture media of (5×10^{-5})–(5×10^{-10}) g/mL. L5178Y cells in log-phase growth were seeded at 2×10^5 cells per 20 mL of culture medium containing the test compound or solvent control and grown up in suspension culture for 3 days. At the end of the growth period, final cell numbers were determined by plating efficiency tested on washed cells from each culture. The cells were suspended in 5 mL of 3:97 HS–EMEM (phenol red free), a further 1 in 10 dilution of the cell suspension was made in the same medium, and the crude cell concentration was determined from the standard absorbance curve for L5178Y (wavelength 540 nm). A nominal 1×10^5 cells were then transferred to 10 mL of fresh 3:97 HS–EMEM, from which 0.1 mL (ca. 1000 cells) was transferred to 23 mL of prewarmed (37 °C) 20:80 HS–EMEM. Molten 5% Noble Agar (1.6 mL) was added to the dilute cell suspension, which was then mixed vigorously, poured into a 90-mm plastic culture dish (one dish per original treated cell suspension), and allowed to gel for 15 min on an ice-cooled tray. The soft agar cultures were then incubated at 37 °C in a humidified incubator gassed with 5% CO_2 in air. The cell colonies were counted at the end of a 10-day incubation period by using an automated colony counter (System III Image Analyser, Micromeasurements Ltd.). The total viable cell yield from each original culture was calculated on the basis of the final plating efficiency in agar, and the cytotoxicity was expressed as a percentage relative to the cell yield in solvent controls. LC_{50} values were interpolated from the dose–response data for each compound. Since 10-fold serial dilutions were used in order to cover a wide range of activities, differences <0.25 log are not significant. T-2 was used as a positive control with each batch of determinations.

Registry No. 1, 21259-20-1; 1 (R_1 = OC(S)imidazole), 118143-05-8; **2**, 2270-40-8; **3**, 36519-25-2; **4**, 21259-21-2; **5**, 63148-31-2; **6**, 115589-76-9; **7**, 118142-89-5; **8**, 118142-90-8; **9**, 118204-86-7; **10**, 68165-53-7; **11**, 65041-92-1; **12**, 65725-06-6; **13**, 99127-69-2; **14**, 118142-91-9; **15**, 74516-69-1; **16**, 118142-92-0; **17**, 26934-87-2; **18**, 34114-98-2; **19**, 34114-99-3; **20**, 34084-03-2; **21**, 118142-93-1; **22**, 118142-94-2; **23**, 118204-87-8; **24**, 118142-95-3; **25**, 118142-96-4; **26**, 118142-97-5; **27**, 118142-98-6; **28**, 118142-99-7; **29**, 118143-00-3; **30**, 113706-92-6; **30** (R_1 = OH, R_6 = OTBDMS), 118143-06-9; **30** (R_6 = OTBDMS), 113728-56-6; **30** (R_6 = OAc), 118143-07-0; **30** (R_6 = OCOEt), 118169-52-1; **30** (R_6 = OCOPr), 118170-26-6; **30** (R_6 = OCOBu), 118143-08-1; **30** (R_6 = OCOPe), 118143-09-2; **30** (R_6 = OCOPh), 118143-10-5; **31**, 111112-47-1; **32**, 98813-18-4; **33**, 116163-74-7; **34**, 118143-01-4; **35**, 118143-02-5; **36**, 77620-47-4; **37**, 77620-53-2; 9α-**38**, 118243-15-5; 9β-**38**, 118204-88-9; 9α-**39**, 118243-16-6; 9β-**39**, 118204-89-0; **40**, 118204-90-3; **41**, 118170-22-2; **42**, 118170-23-3; **43**, 118143-03-6; **44**, 76348-84-0; **45**, 118170-24-4; **46**, 118170-25-5; **47**, 118143-04-7; **48**, 65180-29-2.

Long Acting Dihydropyridine Calcium Antagonists. 2. 2-[2-Aminoheterocycloethoxy]methyl Derivatives

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A series of [(2-aminoheterocycloethoxy)methyl]dihydropyridines were prepared as selective coronary vasodilators. Results showed that a wide variety of five- and six-membered heterocycles were acceptable at the 2-position of the dihydropyridine ring and in vitro potency and tissue selectivity was independent of the basicity of these heterocycles. The SAR indicated that activity was optimum when the largest ester group was placed at the 3 rather than 5 position. 2-[[2-[(3-Amino-1*H*-1,2,4-triazol-5-yl)amino]ethoxy]methyl]-4-(2,3-dichlorophenyl)-3-(ethoxycarbonyl)-5-(methoxycarbonyl)-6-methyl-1,4-dihydropyridine (**3b**) (UK-52,831) emerged as a potent (IC_{50} = 6.3×10^{-9} M) and tissue-selective calcium channel blocker with a duration of action >7 h in the anaesthetized dog.

In an earlier publication,[1] we reported the preparation and structure–activity relationship (SAR) of a series of novel dihydropyridine (DHP) calcium antagonists substituted in the 2-position by various basic side chains. The aim of our work was to modify the physicochemical properties of the DHP system such that bioavailability and duration of action were superior to currently available agents. These studies led to the identification of amlodipine (**1a**), which is presently in late stage clinical de-

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(1) Arrowsmith, J. E.; Campbell, S. F.; Cross, P. E.; Stubbs, J. K.; Burges, R. A.; Gardiner, D. G.; Blackburn, K. J. *J. Med. Chem.* **1986**, *29*, 1696.

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