magnesium sulfate. Concentration and silica gel chromatography (hexanes/ethyl acetate, 1:1) gave **43** (246 mg, 57%): ^{1}H NMR ($CDCl_3$) δ 1.16 (s, 3 H), 1.40–2.20 (m, 8 H), 2.30–3.45 (m, 4 H); ^{13}C NMR ($CDCl_3$) δ 6.8 (q), 20.7 (t), 27.8 (t), 27.9 (t), 31.1 (t), 36.2 (t), 49.9 (t), 50.7 (s), 61.1 (s), 207.9 (s); IR ($CHCl_3$ solution) 1700 cm^{-1}; MS, *m/e* (relative intensity) 165 (M^+, 77), 150 (32), 137 (50), 122 (100), 110 (82), 96 (11).

3-(4-Azidobutyl)-2-butylcyclohex-2-en-1-one (45). To a solution of **44**[14a] (290 mg, 0.77 mmol) in dimethylformamide (10 mL) was added sodium azide (280 mg, 4.3 mmol) and potassium iodide (20 mg). The mixture was stirred at room temperature for 12 h. After the solvent was removed under vacuum, the residue was taken up in ether (20 mL). The ether layer was washed with brine and dried over anhydrous magnesium sulfate. Concentration gave product **45** (148 mg, 78%): ^{1}H NMR ($CDCl_3$) δ 0.9 (t, 3 H), 1.0–2.5 (m, 18 H), 3.3 (t, 2 H); MS, *m/e* (relative intensity) 221 (M^+ - 28, 25), 178 (58), 85 (67), 83 (100).

7-*n*-Butyl-6-azatricyclo[4.5.0.0^{1,6}]undecan-8-one (46). A solution of comound **45** (214 mg, 0.86 mmol) in *m*-xylene (20 mL) was heated to reflux for 6 h. Concentration and silica gel chromatography (hexanes/ethyl acetate, 5:1) gave **46** (138 mg, 86%): ^{1}H NMR ($CDCl_3$) δ 0.9 (t, 3 H), 1.0–2.8 (m, 18 H), 3.0–3.4 (m, 2 H); ^{13}C NMR (C_6D_6) δ 14.9 (q), 18.8 (t), 19.1 (t), 23.2 (t), 24.1 (t), 24.3 (t), 25.9 (t), 29.1 (t), 33.0 (t), 38.8 (t), 42.1 (t), 43.4 (s), 52.0 (s), 206.8 (s); IR (neat) 2950, 1700 cm^{-1}. MS, *m/e* (relative intensity) 221 (M^+, 20). Anal. Calcd for $C_{14}H_{23}NO$: C, 75.97; H, 10.47; N, 6.33. Found: C, 76.10; H, 10.48; N, 6.63.

(6*SR*,7*SR*)-7-*n*-Butyl-1-azaspiro[5.5]undecan-8-one (47). To a solution of **46** (180 mg, 0.81 mmol) in acetone (5 mL) was added a solution (4 mL) of freshly prepared chromous chloride.[20] The mixture was stirred at room temperature for 5 min, and acetone was removed. The aqueous layer was then basified with sodium carbonate. The aqueous solution was extracted with dichloromethane (3 × 10 mL). The organic layer was dried over anhydrous magnesium sulfate. Concentration and silica gel chromatography (methanol/ethyl acetate, 1:2) gave **47** (134 mg, 74%): ^{1}H NMR ($CDCl_3$) δ 0.9 (t, 3 H), 1.0–2.8 (m, 22 H); MS, *m/e* (relative intensity) 223 (M^+, 80), 97 (100); IR (neat) 3350, 2950, 1710 cm^{-1}.

(6*SR*,7*SR*)-7-*n*-Butyl-*N*-benzyl-1-azaspiro[5.5]undecan-8-one (48). To a solution of **47** (66.7 mg, 0.3 mmol) and benzyl bromide (600 mg, 3.5 mmol) in tetrahydrofuran (15 mL) was added potassium carbonate (500 mg, 3.6 mmol). The reaction mixture was heated to reflux for 24 h and then poured into 5% hydrochloric acid (15 mL). The acidic solution was extracted with ether (10 mL) and then basified with sodium carbonate and extracted with dichloromethane (3 × 10 mL). Concentration and silica gel chromatography (hexanes/ethyl acetate, 3:1) gave **48** (79.7 mg, 85%): ^{1}H NMR ($CDCl_3$) δ 0.87 (t, 3 H), 1.1–1.8 (m, 16 H), 2.1–2.7 (m, 5 H), 3.50, 3.66 (AB q, 2 H), 7.1–7.4 (m, 5 H); IR (neat) 2910, 1705 cm^{-1}.

Acknowledgment. We thank the National Science Council of the Republic of China for the support of this work.

Registry No. (±)-**2**, 55228-77-8; **7**, 74056-07-8; **15**, 1006-51-5; **16**, 91891-64-4; **17**, 82026-14-0; **18**, 91891-61-1; **22**, 91891-66-6; **23**, 70156-98-8; **24**, 91891-62-2; **25**, 91891-63-3; **29**, 91891-67-7; **34**, 20643-20-3; (±)-**35**, 100466-69-1; **36**, 100466-70-4; **37**, 100466-71-5; **38**, 91891-59-7; (±)-**43**, 100466-72-6; **44**, 83562-30-5; **45**, 100466-73-7; (±)-**46**, 100466-74-8; (±)-**47**, 82260-14-8; (±)-**48**, 83562-34-9; I(CH_2)$_4$Cl, 10297-05-9; 3-(4-hydroxybutyl)-2-cyclohexen-1-one, 78877-14-2; 1-bromo-3-(1-ethoxyethoxy)propane, 34399-67-2.

Hypochlorite-Promoted Transformations of Trichothecenes. Verrucarol[1]

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Treatment of verrucarol in methanol with hypochlorite bleach containing added sodium hydroxide gave rise to two chlorine-containing rearrangement products **2a,b**. Their structures were established by ^{13}C and ^{1}H NMR spectroscopies and mass spectrometry. Both products possess an unusual pentacyclic ring system resulting from selective oxidation at C-4 followed by hemiketal formation and a haloform-like reaction in addition to rearrangement and are resistant to further oxidation.

Trichothecenes are an important class of mycotoxins produced in nature by a number of taxonomically unrelated genera of fungi and have attracted widespread interest because of their biological effects, most notably toxicity, in man and animals.[2] The skeleton of the tetracyclic sesquiterpenoid unit present in all trichothecenes, macrocyclic and nonmacrocyclic, was established by X-ray crystallography,[3] and chemical transformations of the simpler nonmacrocyclic trichothecenes have been extensively studied.[2a,4] The 12,13-epoxide, generally believed to be a crucial element for biological activity, was resistant to attack by a number of reagents including nucleophiles. Under acidic conditions, however, rearrangement to a tricyclic structure (termed apotrichothecene) readily occurred.[2a,5]

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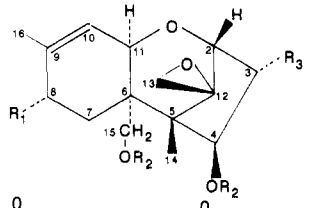
Table I. ^{13}C and ^{1}H NMR Spectral Data of Major Transformation Product

assignmnt (carbon no.)	^{13}C chemical shift (ppm)[a,b] (mult)	^{1}H chemical shift (ppm)[c,d] (mult)	J (Hz)[e]	T_1 (s)[f]
14	8.7 (q)	1.10 (s)		
16	27.6 (q)	1.17 (s)		
7	27.7 (t)	1.2–1.8 (m)		
8	31.7 (t)			
13	31.0 (t)	1.42 (dd)	14.1, 5.4	
		1.82 (dd)	14.1, 11.8	
10	46.4 (d)	2.15 (m)		
6	51.1 (s)			1.8
5	57.7 (s)			4.7
9	74.0 (s)			0.9
11	74.2 (d)	3.67 (d)	3.9	
12	74.8 (s)			2.5
15	77.4 (t)	3.68 (d)	8.4	
		3.86 (d)	8.4	
2	87.3 (d)	3.99 (s)		
3	99.9 (s)			5.5
4	117.5 (s)			7.7

[a] 334 mg in CD_3OD, sweep width 11 KHz, pulse width 5 μs (34°), repetition rate 4 s, accumulation time 33 min. [b] ±0.1 ppm. [c] 162 mg in CD_3OD, sweep width 2600 Hz, pulse width 7 μs (58°), repetition rate 6 s, accumulation time, 24 s. [d] ±0.01 ppm. [e] ±0.2 Hz. [f] Determined by an inversion–recovery experiment.

We were intrigued by an observation that solutions resulting from treatment of T-2 toxin (**1a**) with hypochlorite



1a R_1=OCCH$_2$CH(CH$_3$)$_2$, R_2=CCH$_3$, R_3=OH

1b R_1=R_2=R_3=H

1c R_1=H, R_2=CCH$_3$, R_3=OCCH$_3$

bleach containing added sodium hydroxide no longer exhibited the characteristic properties of T-2 in a variety of biological assays: dermal sensitivity, cytotoxicity, inhibition of protein synthesis, mouse LD_{50}, and radioimmunoassay.[6] Thus, alkaline hypochlorite treatment was recommended as a decontamination procedure for T-2 and other trichothecenes, but no attempts were made to characterize the products. Indeed, T-2, with the complications presented by probable hydrolysis of one or more of the three ester groups in addition to rearrangement, was not an appealing candidate for a pioneering product study. Instead, we chose verrucarol (**1b**) as a similar but simpler type A[7] trichothecene prototype.

Results and Discussion

Treatment of verrucarol in methanol with alkaline hypochlorite for 20 h resulted in complete conversion to a single major product (83%) and a minor product (17%). The molecular formula of the major product, $C_{15}H_{20}Cl_2O_5$, was apparent from its low resolution mass spectrum (MS). Relative intensities of both the isotope cluster for the molecular ion at m/z 350, 352, and 354 and the cluster due to loss of methyl at m/z 335 (base peak), 337, and 339 were in agreement with calculated values for two chlorine atoms, and the intensities for the cluster at m/z 315 and 317, corresponding to loss of chlorine from the molecular ion, showed the presence of one chlorine atom. The mass spectrum of the minor product showed a similar molecular ion cluster 14 amu higher, at m/z 364, 366, and 368. Again, its relative intensities and those of the cluster due to loss of methyl at m/z 349, 351, and 353 showed the presence of two chlorine atoms. The fragments corresponding to loss of chlorine from the molecular ion, at m/z 329 and 331, with intensities indicating one chlorine atom, were also observed. The molecular weight indicated one of two possible molecular formulae, $C_{15}H_{18}Cl_2O_6$ or $C_{16}H_{22}Cl_2O_5$. The pure compounds, in yields of 51% and 10%, respectively, were isolated by column chromatography on silica gel.

The ^{13}C and ^{1}H spectra observed for the predominant product (in methanol) are summarized in Table I. Carbon multiplicities were determined from an APT experiment[8] and a DEPT experiment;[9] proton–proton coupling information was obtained from a two-dimensional (2D) homonuclear correlated experiment (COSY);[10] and assignments of carbons with attached protons were facilitated by a proton–carbon correlated 2D spectrum.[11]

Evaluation of the ^{13}C NMR spectrum showed the presence of two methyl groups (δ 8.7 and 27.6), three alkyl methylene groups (δ 27.7, 31.0, and 31.7), one oxymethylene moiety (δ 77.4), one alkyl methine carbon (δ 46.4), two oxy methine moieties (δ 74.2 and 87.3), and six quaternary carbons (δ 51.1, 57.7, 74.0, 74.8, 99.9, and 117.5). In corroboration, the ^{1}H NMR spectrum displayed two three-proton singlets (δ 1.10 and 1.17), a methylene envelope (δ 1.2–1.8) integrating for four protons, two strongly coupled resonances (δ 1.42 and 1.82, J = 14.1 Hz) indicating an alkyl methylene group with nonequivalent protons, an alkyl methine multiplet (δ 2.15), an oxy methine doublet (δ 3.67, J = 3.9 Hz), an oxy methine singlet (δ 3.99),

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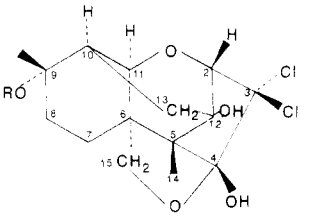
(11) Bax, A.; Morris, G. A. *J. Magn. Reson.* **1981**, *42*, 501.

Table II. ^{13}C and ^{1}H NMR Spectral Data of Minor Transformation Product

assignmnt (carbon no.)	^{13}C chemical shift (ppm)[a,b] (mult)		^{1}H chemical shift (ppm)[c,d] (mult)		
	CD_3OD[e]	$CDCl_3$[f]	CD_3OD[e]	$CDCl_3$[f]	J (Hz)[g]
14	8.7	8.0 (q)	1.11	1.04 (s)	
16	21.1	20.6 (q)	1.11	1.07 (s)	
7	27.5	26.3 (t)	1.2–1.6	1.26–1.60 (m)	
8	27.8	27.4 (t)			
13	30.8	29.0 (t)	1.45	1.35 (dd)	14.3, 5.7
			1.83	1.80 (dd)	14.3, 11.8
10	44.0	41.6 (d)	2.25	2.30 (m)	12.0, 4.2, 3.9
OCH_3	48.9	48.5 (q)	3.14	3.07 (s)	
6	50.9	49.9 (s)			
5	57.7	56.6 (s)			
11	74.0	72.5 (d)	3.57	3.58 (d)	3.7
12	74.8	74.3 (s)			
9	78.9	76.6 (s)			
15	77.4	77.4 (t)	3.68	3.69 (d)	8.8
			3.84	3.87 (d)	8.8
2	87.4	85.6 (d)	3.99	4.05 (s)	
3	99.9	98.5 (s)			
4	117.5	115.6 (s)			

[a] Sweep width 11 KHz, pulse width 5 μs (34°), repetition rate 3s, accumulation time 17 h. [b] ±0.1 ppm. [c] Sweep width 2600 Hz, pulse width 12 μs (90°), repetition rate 3 s, accumulation time 12 s. [d] ±0.01 ppm. [e] 40 mg. [f] 20 mg. [g] ±0.2 Hz.

and an AB pattern for an oxy methylene moiety with nonequivalent protons (δ 3.68 and 3.86, J = 8.4 Hz). Thus it was clear from both ^{13}C and ^{1}H spectra that the C-9,10 double bond was not present in the product. The absence of the AB quartet centered at δ 2.95 ± 0.05 with a coupling constant of 4.0 ± 0.1 Hz, characteristic of epoxide protons in all trichothecenes measured,[12] demonstrated that the epoxide was not present either. Counts of protons attached to carbon by integration and by carbon multiplicities both totaled 17; thus three exchangeable protons were present. From these data and the established molecular formula, a unique structure (**2a**) was deduced for the major rearrangement product.



2a R=H

2b R=CH_3

The relationships between H-11, H-10, and H-13 were documented by a COSY experiment, which showed also the obvious coupling between the resonances of the H-15 AB pattern (δ 3.68 and 3.86). Thus, the doublet at δ 3.67 (assigned to H-11) was coupled to the multiplet at δ 2.15 (assigned to H-10), and that multiplet was also coupled to the resonances at δ 1.42 and 1.82 (assigned to the C-13 methylene protons) which were, in turn, strongly coupled to each other.

The proton–carbon correlated 2D experiment served to demonstrate and verify the ^{13}C–^{1}H relationships for all carbons with attached protons except for the two methylene-bearing carbons, 7 and 8. Assignments of the latter were made on the basis of a selective decoupling experiment in which irradiation of the C-15 protons resulted in sharpening of the triplet at δ 27.7 but had no effect on the triplet at δ 31.7. A similar demonstration of long-range coupling was utilized for assignment of the high frequency quaternary signals at δ 99.9 and δ 117.5 to C-3 and C-4, respectively. Irradiation of the C-15 protons collapsed the latter resonance to a small quartet, and irradiation of the C-14 proton singlet at δ 1.10 collapsed the signal at δ 117.5 to a triplet. This conclusively identified δ 117.5 as C-4 and confirmed the hemiketal linkage between C-4 and C-15. The resonance at δ 99.9, on the other hand, was a sharp singlet showing no long-range coupling and was assigned to C-3. The chemical shift agrees fairly well with that reported for the dichloro-substituted carbon of endrin (δ 108.7).[13]

Assignments of the four remaining quaternary carbons were made on the basis of chemical shifts and spin–lattice relaxation time (T_1) measurements (Table I). According to chemical shift, the resonances at δ 51.1 and 57.7 arise from C-5 and C-6 and the pair at δ 74.0 and 74.8 arise from the oxygen-bearing C-9 and C-12. Further assignments were based on the qualitative observation that T_1 of a quaternary carbon is expected to decrease with increasing number of protons attached to carbons α to the quaternary carbon.[14] Thus the signal at δ 51.1, with T_1 of 1.8 s, was assigned to C-6 (five protons on α carbons), and the signal at δ 57.7, with T_1 of 4.1 s, to C-5 (three protons on α carbons). Assignments to C-6 and C-5 in verrucarol (δ 43.7 and 48.7, respectively), made on the basis of comparison with certain derivatives,[15] also support this conclusion. In a similar manner, the resonance at δ 74.0, with T_1 of 0.9 s, was assigned to C-9 (six protons on α carbons), and the resonance of δ 74.8, with T_1 of 2.5 s, to C-12 (three protons on α carbons). The T_1 values observed for the resonances previously assigned to C-3 and C-4 (5.5 and 7.7 s, respectively) also substantiate the assignments.

Both the ^{13}C and ^{1}H spectra of the minor product (summarized in Table II) were strikingly similar in chemical shift and identical in multiplicity with those of **2a**,

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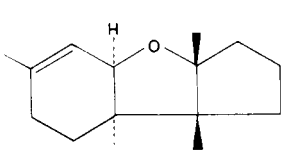
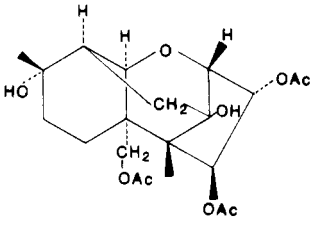
except for the presence of an additional signal in each spectrum: a carbon quartet at δ 48.9 and a three-proton singlet at δ 3.14. Accordingly, the minor product was assigned the methyl ether structure **2b**. It was sufficiently soluble in chloroform for spectral determination to confirm the presence of the methoxy carbon resonance in the absence of methanol solvent (Table II).

This unusual pentacyclic ring system was resistant to further oxidation, and the same products were recovered after the reaction mixture was allowed to stand several days longer. While the vast majority of previously described rearrangements of trichothecenes have given products with the apotrichothecene skeleton (**3**),[2a,5] there



3



4

is a published precendent for nucleophilic attack at C-9 resulting in opening of the epoxide and formation of the C-10,C-13 bond, thereby establishing the stereochemistry at C-9 and C-10. Rearrangement product **4** was obtained after prolonged refluxing of scirpenetriol triacetate (**1c**) in chloroform/water.[16] We envision a similar process in addition to preferential oxidation at C-4 by chlorine followed by α-chlorination, in the manner of a haloform reaction, and cyclization to the hemiketal.[17]

The behavior of **2a** with derivatization reagents is worthy of note. Before we discovered that **2a** and **2b** are readily separated and analyzed by GC without derivatization, we undertook the preparation of trimethylsilyl and trifluoroacetyl derivatives. With a powerful silylating reagent, Tri-Sil TBT,[19] the predominant products for **2a** and **2b** were the tri- and ditrimethylsilyl ethers (M_r 566 and 508), respectively, in addition to small amounts of the respective di- and monotrimethylsilyl ethers (M_r 494 and 436). While **2b** was inert to treatment with trifluoroacetic anhydride (TFAA), **2a** gave rise to mixtures which on GC/MS showed a prominent product with a molecular ion cluster at m/z 332, 334, and 336, 18 amu below that of **2a**. While dehydration of tertiary alcohols derived from trichothecenes under mild TFAA derivatization conditions has been observed by one of us[20] and by others,[21] NMR analysis of the mixture showed no evidence for such a product. Rather, the mixture appeared to consist of **2a** and its 9-trifluoroacetyl derivative in approximately 1:1 ratio. Signals for the trifluoromethyl and carbonyl carbons were observed at δ 115.8 and 156.8 with C–F coupling constants of 286 and 41 Hz, respectively. The position of the trifluoroacetate was evident from its effect on the adjacent C-16 and C-10 and their attached protons, and on C-8 and H-11. Thus, C-16 and C-10 were shifted upfield to δ 22.4 (–5.2 ppm) and δ 43.7 (–2.7 ppm), respectively, and their attached protons were shifted downfield to δ 1.61 (+0.44 ppm) and δ 2.77 (+0.62 ppm), respectively. Upfield shifts for C-8 (δ 28.5, –3.2 ppm) and H-11 (δ 3.46, –0.21 ppm) were also observed. The new signal at δ 93.5 (+19.5 ppm) was therefore assigned to C-9. Other carbon resonances were either coincident (C-4 and C-5) or within 1 ppm or less of those of **2a**. Thus, the apparent dehydration product observed on GC/MS may be attributed to thermal loss of trifluoroacetic acid, most probably in the GC injection port, which was kept at 250 °C.

We are currently investigating the course of reaction of hypochlorite with other trichothecenes.

Experimental Section

^{13}C and ^1H NMR spectra were determined with a Varian XL-200 Superconducting FTNMR spectrometer system at 50.3 and 200 MHz, respectively; other parameters are footnoted in Tables I and II. High resolution mass spectra (HRMS) were determined with a VG Model 70E-HFQ MS/MS instrument, low resolution MS with a Hewlett Packard 5985B GC/MS/DS in electron impact mode at 70 eV, source temperature 200 °C. A 25 m × 0.2 mm i.d. fused silica capillary column (cross-linked OV-1 or DB-5) was interfaced directly to the source and was programmed from 160 °C or 180 °C at 20°/min to 250 °C after an initial hold of 1 min. Samples were injected directly or treated for 1.5 to 2 h at room temperature with 1:1 CH_2Cl_2/TFAA or 1:1 acetonitrile/TFAA or 1:1 $CHCl_3$/Tri-Sil TBT for derivatization. TLC conditions were silica gel, 19:1 $CHCl_3$/MeOH, visualization with 0.5% *p*-anisaldehyde in 85:10:5 MeOH/HOAc/concentrated H_2SO_4.[22]

Hypochlorite Treatment of Verrucarol. To a stirred solution of verrucarol (100 mg, 0.38 mmol, Sigma Chemical Co., St. Louis, MO) in methanol (200 mL) was added a 10% solution of sodium hypochlorite (1 L, Robinson Chemical Co., Cambridge, MD) in which 20 g of sodium hydroxide had been freshly dissolved. The mixture was cooled in an ice bath intermittently to achieve an internal temperature at or below 30 °C during the addition and was stirred at least 20 h at room temperature. It was again cooled while 6 N HCl (84 mL) was added and was saturated with NaCl and extracted with three portions of EtOAc (combined vol 750–800 mL). An aliquot of the extract showed no verrucarol by TLC or GC/MS of the TFA derivative, and direct injection of an aliquot showed two products in a ratio of 17:83. The extracts were dried ($MgSO_4$) and evaporated to dryness, and the residue was extracted with methanol. The methanol extracts were combined with those from four similarly run reactions and evaporated to dryness, and the residue (600 mg) was dissolved in $CHCl_3$ and subjected to chromatography on silica gel (25 g, Bio-Sil A, Bio-Rad Laboratories, Richmond, CA). Elution with $CHCl_3$/acetone mixtures of increasing polarity and rechromatography of certain of the combined fractions gave **2a** (334 mg, 51%) and **2b** (67 mg, 10%) as pure solids. Characteristics of **2a**: mp 192–194 °C dec; HRMS, calcd for $C_{15}H_{20}Cl_2O_5$, 350.0688, found, 350.0672. Characteristics of **2b**: mp 208–209 °C; HRMS, calcd for $C_{16}H_{22}Cl_2O_5$, 364.0844, found, 364.0819.

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Registry No. **1b**, 2198-92-7; **2a**, 101199-68-2; **2b**, 101199-69-3.

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