Chemical Deoxygenation of the Trichothecenes Diacetoxyscirpenol and Deoxynivalenol

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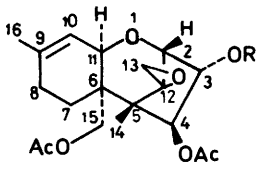
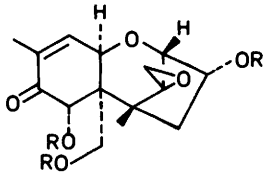
Based on a model study using the bicyclo[3.2.1]octane epoxy acetates (**14**) and (**15**), an efficient one-step procedure for the selective removal of the 12,13-epoxide ring of the trichothecene mycotoxins diacetoxyscirpenol (**1**; R = H) and deoxynivalenol (**2**; R = H) has been devised. The key to success proved to be use of the lower-valent tungsten deoxygenation system of Sharpless *et al.*

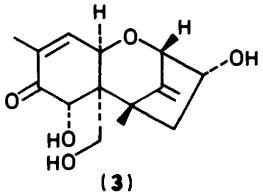
The trichothecenes[1] are a group of some 80 complex fungal sesquiterpenoid secondary metabolites produced by various species of Fungi imperfecti, in particular by *Fusarium* species. Most exhibit a range of significant biological properties such as cytostatic activity, but are also highly toxic. They act as potent yet reversible inhibitors of protein synthesis in eukaryotes[2] and have been implicated in a number of diseases of plants, animals, and humans. For example, diacetoxyscirpenol (anguidine) (**1**; R = H) has been the subject of phase II clinical trials[3] as an anti cancer agent, whereas others, such as deoxynivalenol (**2**; R = H), have been implicated[4] in the 'Yellow Rain' chemical warfare controversy. Deoxynivalenol is produced naturally[5] when cereal grains are infected with *Fusarium* species: consumption of feedstuffs infected with this toxin causes emesis (hence its trivial name 'vomitoxin') and sub-lethal toxicoses in animals.



(**1**)



(**2**)



(**3**)

Despite the toxicological importance of the trichothecenes, relatively little is known about their metabolic fate[2,6] in mammals. A characteristic feature of all trichothecenes is a spiro-12,13-epoxide function. Its presence seems to be essential for manifestation of biological effects: activity is lost when this function is removed.[7,8] Indeed, studies with rumen micro-organisms *in vitro*[9] and with rats *in vivo*[5] have revealed that the predominant biological transformation, and presumed detoxification route of deoxynivalenol (**2**; R = H) is one of deoxygenation to form the 9,12-diene (**3**). This has added to speculation[10] that the mode of action may involve the epoxide group acting as a bio-alkylating agent, through some form of intramolecularly assisted nucleophilic ring opening, induced by interaction of the epoxide with an electrophilic site on an enzyme.

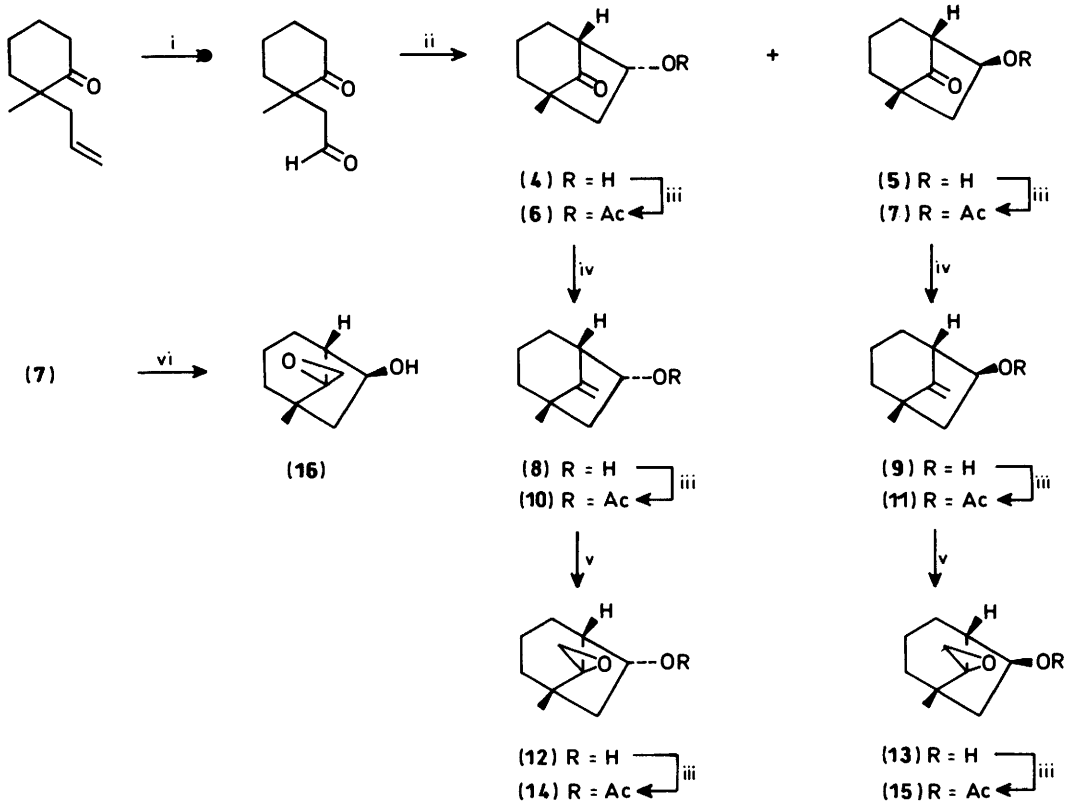
Stimulated by these remarkable biological deoxygenation findings, and continuing our interest in the synthesis[11–13] and synthetic interconversions of the trichothecenes, we embarked on a programme to develop an efficient chemical method for such deoxygenation. It is the result of this study which we now report in detail.[14] The lack of reactivity of the 12,13-epoxide function towards external nucleophilic attack,[15] and its contrasting high lability towards intramolecular rearrangement under acidic conditions, imposes severe constraints on potential epoxide deoxygenating systems.[16] At the outset, model studies were carried out on the readily accessible bicyclo[3.2.1]octane epoxides (**14**) and (**15**), which were prepared in racemic form as shown (Scheme 1). Ozonolysis of 2-allyl-2-methylcyclohexanone to the keto aldehyde, followed by aldol cyclisation using sodium methoxide in methanol, gave the epimeric alcohols (**4**) and (**5**), in a ratio of 2:1 in favour of the β-epimer (**5**). Chromatographic separation, conversion into the acetates (**6**) and (**7**), and treatment with an excess of methylenetriphenylphosphorane gave the hydroxy alkenes (**8**) and (**9**). Acetylation then provided the respective acetates (**10**) and (**11**).

Epoxidation of the hydroxy alkenes (**8**) and (**9**) with *m*-chloroperbenzoic acid (MCPBA) to the epoxy alcohols (**12**) and (**13**) followed by acetylation gave, in each case, a single epoxy acetate, namely (**14**) and (**15**). The rigid geometry of the bicyclo[3.2.1]octane framework ensures attack by the reagent from the side of the two-carbon bridge, regardless of the epimeric alcohol stereochemistry. Indeed, treatment of the keto acetate (**7**) with an excess of dimethylsulphonium methylide[17] again resulted in the same sense of attack, producing an epoxy alcohol (**17**) epimeric with (**13**). This ability to control the relative stereochemistry of the epoxide by either alkene epoxidation or by direct ketone transformation in such bicyclic systems had been observed earlier in the synthesis of trichodermin.[11]

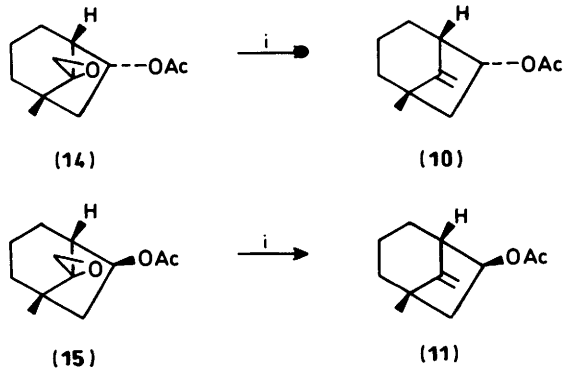
Preliminary investigations on epoxy acetates (**14**) and (**15**) using dimethyl diazomalonate with rhodium(II) acetate catalysis, a promising deoxygenating system,[18] proved unsatisfactory. Attention was then focussed on the lower-valent tungsten method of Sharpless,[19] using WCl_6–BuLi, a system which has seen surprisingly little use.[20] This method proved excellent in the model series (Scheme 2), both epimeric acetates undergoing efficient deoxygenation to give the acetoxy alkenes (**10**) and (**11**) in high yield. Optimum conditions involved treatment of 1 equiv. of the epoxide in tetrahydrofuran (THF) at reflux with the reagent generated from 2 equiv. of WCl_6 and 6 equiv. of butyl-lithium; reflux was necessary, in that operation at 25 °C resulted in epoxide ring opening and resulting chlorohydrin formation.

A limited range of natural trichothecenes is commercially available, but at high cost. Using a suitable *Fusarium* species[21,*]

* We thank Professor B. W. Bycroft (Nottingham) for advice and provision of the culture.

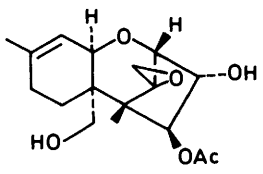


Scheme 1. *Reagents:* i, O_3; ii, NaOMe, MeOH; iii, Ac_2O, pyridine; iv, $Ph_3P{=}CH_2$; v, MCPBA; vi, $Me_2S^+{-}CH_2^-$



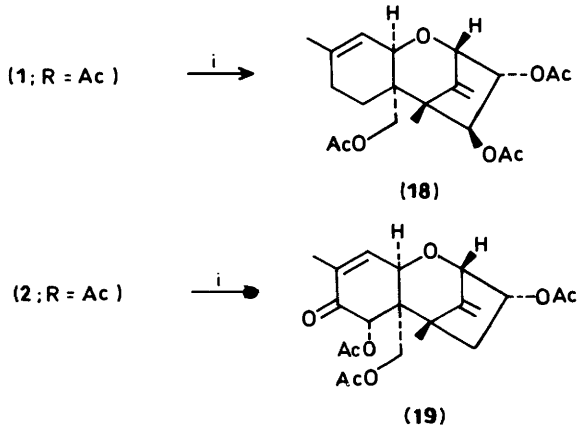
Scheme 2. *Reagents and conditions:* i, BuLi (6 mol equiv.), WCl_6 (2 mol equiv.), THF, reflux, 6 h

and a shake culture procedure, we have been able to obtain substantial amounts (250—300 mg l^{-1}) of anguidine (**1**; R = H). In our hands, an incubation period of 7 days proved to be ideal for this compound: interestingly, longer periods of incubation, 11 days being optimal, led to the production of equal amounts of 4β-acetoxy-3α,15-dihydroxy-12,13-epoxytrichothec-9-ene (**17**), a compound otherwise obtainable only with some difficulty.[22]



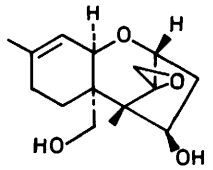
(17)

Anguidine (**1**; R = H) was converted into the triacetate (**1**; R = Ac). Treatment of this compound with the Sharpless reagent resulted in clean and selective deoxygenation to afford the diene (**18**) in excellent yield (Scheme 3). Reaction of deoxynivalenol triacetate (**2**; R = Ac) under the same conditions gave the corresponding diene (**19**) in more modest yield: the lower yield is probably due to the scale of this particular reaction, and relates to the inaccessibility of larger amounts of deoxynivalenol.



Scheme 3. *Reagents and conditions:* i, BuLi (6 mol equiv.), WCl_6 (2 mol equiv.), THF, reflux, 6 h

Apart from a very early report,[23] two other methods for trichothecene epoxide deoxygenation have been reported. A three-step procedure,[15] based upon nucleophilic opening of the 12,13-epoxide ring with benzenethiolate ion, has been successfully applied to protected forms of anguidine (**1**; R = H)



(20)

and verrucarol (**20**). However, the vigorous conditions necessary in this procedure are unlikely to be compatible with the sensitive enone functionality of deoxynivalenol, or with the various trichothecene ester groupings. An alternative method[24] for the deoxygenation of deoxynivalenol, involving Zn–AcOH reduction of a derived bromohydrin, proceeds in an overall yield of 9%, and cannot be applied to the triacetate (**1**; R = Ac).[25]

Diene (**18**) proved to be essentially devoid of meaningful toxicity, as detected by determining the lowest concentration which completely inhibited cell division of human epithelial tumour cells.[26] This provides further evidence for the mandatory presence of the 12,13-epoxide function for biological activity.

Experimental

M.p.s were determined on a Kofler hot-stage apparatus, and are uncorrected. Bulb-to-bulb distillations were carried out on a Büchi GKR-50 Kugelrohr, with recorded b.p.s referring to the indicated air-bath temperature. I.r. spectra were taken on a Perkin-Elmer 580 spectrometer, and optical rotations were determined on an Optical Activity AA-100 auto-digital polarimeter.

^{1}H N.m.r. spectra were recorded on a 90 MHz Perkin-Elmer R32 or a 100 MHz Varian XL100 or a 200 MHz Bruker WP200 SY spectrometer. ^{13}C N.m.r. spectra were recorded on either a Varian XL100 operating at 25 MHz or a Bruker WP200 SY spectrometer operating at 50 MHz; deuteriochloroform was used as solvent with Me_4Si as internal standard. Chemical shifts are reported relative to Me_4Si using Me_4Si or the δ 7.25 residual chloroform peak and the δ 77.0 deuteriochloroform peak as internal references for ^{1}H and ^{13}C n.m.r. spectra respectively. The ^{1}H n.m.r. signals for 2-, 3-, 4-, 5-, and 7-H in the model bicyclo[3.2.1]octane series generally appear as a multiplet between δ 1.5 and 2.3, and are omitted for clarity. The multiplicities of the 50 MHz ^{13}C n.m.r. resonances were determined using DEPT spectra with pulse angles of θ 90° and 135°. The multiplicities of the 25 MHz ^{13}C n.m.r. resonances were determined by use of ^{13}C–^{1}H couplings in the off-resonance spectra.

Low- and high-resolution molecular weights were determined from mass spectra, measured with VG updated A.E.I. MS12 and MS902 spectrometers, respectively. Elemental analyses were performed using a Carlo Erba 1106 elemental analyser.

Reactions were normally performed in an atmosphere of nitrogen. THF and diethyl ether (ether) were freshly distilled from sodium benzophenoneketyl. Dichloromethane was distilled from P_2O_5, then filtered through Grade I basic alumina. Light petroleum (b.p. 60—80 °C), hexane, and pentane were distilled from CaH_2. With the exception of THF and ether, all the above were stored over 4 Å molecular sieves. 4-Dimethylaminopyridine (DMAP) was recrystallised from cyclohexane. Organic extracts were dried over $MgSO_4$. Dry column flash chromatography[27] and flash chromatography[28] refer to techniques previously described.

(±)-6α-*Hydroxy*-1-*methylbicyclo*[3.2.1]*octan*-8-*one* (**4**) *and* (±)-6β-*Hydroxy*-1-*methylbicyclo*[3.2.1]*octan*-8-*one* (**5**).—Ozone was bubbled through a solution of 2-allyl-2-methylcyclohexanone (3.97 g, 26.1 mmol) in dichloromethane (50 cm^3), cooled to −78 °C, until the blue colour of excess of ozone had appeared. The solution was then purged with nitrogen, and the ozonide was reduced by the addition of triethylamine (6.9 cm^3, 52.2 mmol) at −78 °C followed by stirring overnight at 25 °C. The solution was filtered through a short column of chromatographic silica gel and concentrated under reduced pressure to yield the keto aldehyde (3.69 g) as a pale yellow oil, which was used immediately without purification.

A solution of the keto aldehyde (2.81 g, 18 mmol) in methanol (90 cm^3) was added to a freshly prepared solution of sodium methoxide [from sodium (2.87 g, 124 mmol) in methanol (200 cm^3)]. The solution was heated under reflux for 0.5 h, cooled, and then poured onto ice–water (200 cm^3). Most of the methanol was removed by concentration under reduced pressure, and the residual aqueous portion was extracted with ether (3 × 100 cm^3). The combined extracts were dried, and concentrated under reduced pressure to give a mixture of the α- and β-alcohol epimers in the ratio 1:2 (^{1}H n.m.r.). Purification by dry column flash chromatography yielded pure α-alcohol (**4**) (351 mg), pure β-alcohol (**5**) (760 mg), and a mixed fraction (589 mg), in a total yield of 60%.

The *α-alcohol* (**4**) was obtained as a white crystalline solid, m.p. 95—96 °C (from ether–light petroleum); $\nu_{max.}$(CCl_4) 3 630 and 1 750 cm^{-1}; δ_H(90 MHz) 4.45 (1 H, ddd, *J* 10, 5, and 5 Hz, 6-H) and 0.95 (3 H, s, Me); δ_C(25 MHz) 221.9 (C-8), 65.9 (C-6), 52.2 (C-5), 48.7 (C-1), 45.2 (C-2), 42.3 (C-7), 31.8 (C-4), and 19.3 (C-3 and Me) (Found: C, 70.2; H, 9.25%; M^+, 154.0995. $C_9H_{14}O_2$ requires C, 70.1; H, 9.15%; *M*, 154.0994).

The *β-alcohol* (**5**) was also obtained as a white crystalline solid, m.p. 69—71 °C (from ether–light petroleum); $\nu_{max.}$(CCl_4) 3 620, 3 450br, and 1 750 cm^{-1}; δ_H(90 MHz) 4.25 (1 H, dd, *J* 9 and 4 Hz, 6-H) and 1.00 (3 H, s, Me); δ_C(25 MHz) 221.4 (C-8), 69.4 (C-6), 55.9 (C-5), 47.9 (C-1), 43.9 (C-2), 43.8 (C-7), 34.0 (C-4), and 19.1 (C-3 and Me) (Found: C, 70.1; H, 9.15%; M^+, 154.1001).

(±)-1-*Methyl*-8-*oxobicyclo*[3.2.1]*octan*-6α-*yl Acetate* (**6**).—To a solution of the keto alcohol (**4**) (270 mg) in ether (5 cm^3) were added acetic anhydride (2 cm^3) and pyridine (1 cm^3). The mixture was kept at 25 °C overnight, then excess of acetic anhydride and pyridine were removed azeotropically under reduced pressure using toluene (×4) and then CCl_4 (×2). Purification by dry column flash chromatography gave the *keto acetate* (**6**) (314 mg, 91%) as an oil, b.p. 85 °C/0.5 mmHg; $\nu_{max.}$(NaCl) 1 750 cm^{-1}; δ_H(90 MHz) 5.2 (1 H, ddd, *J* 10, 5, and 5 Hz, 6-H), 2.1 (3 H, s, MeCO), and 0.98 (3 H, s, Me); δ_C(25 MHz) 219.6 (C-8), 170.6 (Me*C*O), 68.8 (C-6), 50.0 (C-5), 48.0 (C-1), 45.1 (C-2), 39.4 (C-7), 32.4 (C-4), 20.9 (*Me*CO), and 19.1 (C-3 and Me) (Found: C, 67.2; H, 8.0%; M^+, 196.1102. $C_{11}H_{16}O_3$ requires C, 67.3; H, 8.2%; *M*, 196.1099).

(±)-1-*Methyl*-8-*oxobicyclo*[3.2.1]*octan*-6β-*yl Acetate* (**7**).—Keto alcohol (**5**) was acetylated and purified as above to give the *keto acetate* (**7**) as an oil (87%), b.p. 85 °C/0.5 mmHg; $\nu_{max.}$(CCl_4) 1 750 cm^{-1}; δ_H(90 MHz) 5.08 (1 H, dd, *J* 9 and 4 Hz, 6-H), 2.00 (3 H, s, MeCO), and 1.02 (3 H, s, Me); δ_C(25 MHz) 220.2 (C-8), 170.5 (Me*C*O), 71.7 (C-6), 52.3 (C-5), 47.6 (C-1), 43.8 (C-2), 40.8 (C-7), 34.0 (C-4), 21.1 (*Me*CO), and 19.0 (C-3 and Me) (Found: C, 67.1; H, 8.3%; M^+, 196.1094).

(±)-1-*Methyl*-8-*methylenebicyclo*[3.2.1]*octan*-6α-*ol* (**8**).—To a stirred suspension of methyltriphenylphosphonium bromide (1.43 g, 4 mmol) in THF (35 cm^3) was added butyl-lithium (2.2M) in hexane (2 cm^3). The mixture was stirred for 0.25 h at 25 °C, and then a solution of the keto acetate (**6**) (314 mg, 1.6 mmol) in THF (10 cm^3) was added. The mixture was then heated under reflux for 1 h, when it was cooled, diluted with

water, and extracted with ether. The organic extracts were washed successively with 1M HCl and brine, and were then dried, and concentrated under reduced pressure. Purification by dry column flash chromatography gave the *hydroxy alkene* (**8**) (130 mg, 53%) as a white crystalline solid, m.p. 70—72 °C (from light petroleum); $\nu_{max.}(CCl_4)$ 3 625, 3 070, 1 660, and 940 cm^{-1}; δ_H(90 MHz) 4.65 (1 H, s, $=CH_2$), 4.53 (1 H, s, $=CH_2$), 4.3 (1 H, ddd, *J* 10, 5, and 5 Hz, 6-H), and 1.00 (3 H, s, Me); δ_C(25 MHz) 162.3 (C-8), 98.7 ($=CH_2$), 71.3 (C-6), 48.7 (C-5), 45.2 (C-7), 43.2 (C-2), 42.6 (C-1), 29.7 (C-4), 23.1 (Me), and 20.3 (C-3) (Found: C, 78.8; H, 10.7%; M^+, 152.1204. $C_{10}H_{16}O$ requires C, 78.9; H, 10.6%; *M*, 152.1201).

(±)-1-*Methyl*-8-*methylenebicyclo*[3.2.1]*octan*-6β-*ol* (**9**).—Keto acetate (**7**) was treated as above to give the *hydroxy alkene* (**9**) (50%) as a white crystalline solid, m.p. 49—52 °C (from light petroleum); $\nu_{max.}(CCl_4)$ 3 610, 3 070, 1 665, and 885 cm^{-1}; δ_H(90 MHz) 4.75 (1 H, s, $=CH_2$), 4.68 (1 H, s, $=CH_2$), 4.05 (1 H, dd, *J* 8 and 4 Hz, 6-H), and 1.08 (3 H, s, Me); δ_C(25 MHz) 161.4 (C-8), 99.6 ($=CH_2$), 73.7 (C-6), 53.5 (C-5), 48.5 (C-7), 43.8 (C-1), 41.9 (C-2), 32.7 (C-4), 23.0 (Me), and 20.1 (C-3) (Found: C, 78.75; H, 10.65%; M^+, 152.1197).

(±)-1-*Methyl*-8-*methylenebicyclo*[3.2.1]*octan*-6α-*yl Acetate* (**10**).—The hydroxy alkene (**8**) was acetylated in the normal manner to give the *acetate* (**10**) (91%) as an oil, b.p. 85 °C/1 mmHg; $\nu_{max.}(CCl_4)$ 3 080, 1 740, 1 670, and 890 cm^{-1}; δ_H(90 MHz) 5.05 (1 H, ddd, *J* 10, 5, and 5 Hz, 6-H), 4.7 (1 H, s, $=CH_2$), 4.6 (1 H, s, $=CH_2$), 2.05 (3 H, s, MeCO), and 1.04 (3 H, s, Me); δ_C(25 mHz) 170.8 (Me*C*O), 160.6 (C-8), 99.6 ($=CH_2$), 73.6 (C-6), 46.5 (C-5), 42.9 (C-2), 42.1 (C-7), 42.0 (C-1), 30.3 (C-4), 22.9 (*Me*CO), 20.9 (Me), and 19.9 (C-3) (Found: C, 74.0; H, 9.4%; M^+, 194.1319. $C_{12}H_{18}O_2$ requires C, 74.2; H, 9.3%; *M*, 194.1307).

(±)-1-*Methyl*-8-*methylenebicyclo*[3.2.1]*octan*-6β-*yl Acetate* (**11**).—The hydroxy alkene (**9**) was acetylated in the normal manner to give the *acetate* (**11**) (87%) as an oil, b.p. 50 °C/0.05 mmHg; $\nu_{max.}(CCl_4)$ 3 040, 1 730, 1 670, and 890 cm^{-1}; δ_H(90 MHz) 4.9 (1 H, dd, *J* 9 and 4 Hz, 6-H), 4.7 (1 H, s, $=CH_2$), 4.6 (1 H, s, $=CH_2$), 1.95 (3 H, s, MeCO), and 1.04 (3 H, s, Me); δ_C(25 MHz) 170.8 (Me*C*O), 161.0 (C-8), 98.9 ($=CH_2$), 76.6 (C-6), 50.0 (C-5), 45.0 (C-7), 43.8 (C-1), 42.1 (C-2), 32.8 (C-4), 21.4 (*Me*CO), and 20.1 (C-3) (Found: C, 74.1; H, 9.6%; M^+, 194.1314).

(±)-1-*Methylspiro*(*bicyclo*[3.2.1]*octane*-exo-8,2′-*oxiran*)-6α-*ol* (**12**).—A mixture of the hydroxy alkene (**8**) (225 mg, 1.48 mmol), MCPBA (85% pure; 490 mg, 2.41 mmol), and Na_2HPO_4 (2.28 g) in dichloromethane (70 cm^3) was stirred for 24 h at 25 °C. The mixture was then poured into water and extracted thoroughly with ether. The combined extracts were washed successively with saturated aq. sodium hydrogen carbonate and brine, and dried. Concentration under reduced pressure and purification by dry column flash chromatography gave the *epoxy alcohol* (**12**) (217 mg, 87%) as an oil, b.p. 120 °C/0.1 mmHg; $\nu_{max.}(CCl_4)$ 3 620 and 3 500br cm^{-1}; δ_H(90 MHz) 4.65 (1 H, ddd, *J* 10, 5, and 5 Hz, 6-H), 2.72 (2 H, s, 2′-H_2), and 0.73 (3 H, s, Me); δ_C(25 MHz) 72.6 (C-8), 71.2 (C-6), 46.6 (C-5), 45.8 (C-2′), 44.3 (C-7), 40.2 (C-2), 39.9 (C-1), 26.9 (C-4), 19.6 (C-3), and 18.7 (Me) (Found: M^+, 168.1148. $C_{10}H_{16}O_2$ requires *M*, 168.1150).

(±)-1-*Methylspiro*(*bicyclo*[3.2.1]*octane*-exo-8,2′-*oxiran*)-6β-*ol* (**13**).—Epoxy alcohol (**13**) was prepared as above from hydroxy alkene (**9**) (250 mg, 1.64 mmol), MCPBA (540 mg, 2.66 mmol), and Na_2HPO_4 (2.55 g) in dichloromethane (70 cm^3). Isolation and purification gave the *epoxy alcohol* (**13**) (242 mg, 88%) as a white amorphous solid, m.p. 95—98 °C (from light petroleum); $\nu_{max.}(CCl_4)$ 3 600 and 3 500br cm^{-1}; δ_H(90 MHz) 4.1 (1 H, dd, *J* 8 and 4 Hz, 6-H), 2.75 (2 H, s, 2′-H_2), and 0.80 (3 H, s, Me); δ_C(25 MHz) 74.1 (C-6), 72.1 (C-8), 50.7 (C-5), 47.6 (C-7), 44.8 (C-2′), 41.6 (C-1), 38.9 (C-2), 29.3 (C-4), 19.3 (C-3), and 18.6 (Me) (Found: 71.4; H, 9.6%; M^+, 168.1150. $C_{10}H_{16}O_2$ requires C, 71.4; H, 9.6%).

(±)-1-*Methylspiro*(*bicyclo*[3.2.1]*octane*-exo-8,2′-*oxiran*)-6α-*yl Acetate* (**14**).—The epoxy alcohol (**12**) (122 mg, 0.73 mmol) was acetylated in the normal manner to give the *epoxy acetate* (**14**) (125 mg, 82%) as an oil, b.p. 110 °C/2 mmHg; $\nu_{max.}(CCl_4)$ 1 740 cm^{-1}; δ_H(90 MHz) 5.33 (1 H, ddd, *J* 9, 5, and 5 Hz, 6-H), 2.73 (2 H, s, 2′-H_2), 2.08 (3 H, s, MeCO), and 0.75 (3 H, s, Me); δ_C(25 MHz) 170.8 (Me*C*O), 73.8 (C-6), 71.8 (C-8), 45.7 (C-2′), 44.7 (C-5), 41.4 (C-7), 40.0 (C-2), 39.5 (C-1), 27.4 (C-4), 20.9 (*Me*CO), 19.3 (C-3), and 18.5 (Me) (Found: M^+, 210.1254. $C_{12}H_{18}O_3$ requires *M*, 210.1256).

(±)-1-*Methylspiro*(*bicyclo*[3.2.1]*octane*-exo-8,2′-*oxiran*)-6β-*yl Acetate* (**15**).—Similar acetylation of the epoxy alcohol (**13**) (153 mg, 0.91 mmol) gave the *epoxy acetate* (**15**) (155 mg, 81%) as an oil, b.p. 100 °C/1.0 mmHg; $\nu_{max.}(CCl_4)$ 1 730 cm^{-1}; δ_H(90 MHz) 5.0 (1 H, dd, *J* 8 and 4 Hz, 6-H), 2.75 (2 H, s, 2′-H_2), 2.04 (3 H, s, MeCO), 0.82 (3 H, s, Me); δ_C(25 MHz) 170.0 (Me*C*O), 75.2 (C-6), 70.5 (C-8), 46.7 (C-5), 43.8 and 43.0 (C-7 and C-2′), 40.6 (C-1), 38.0 (C-2), 28.4 (C-4), 20.3 (*Me*CO), 18.3 (C-3), and 17.3 (Me) (Found: C, 68.6; H, 8.7%; M^+, 210.1241. $C_{12}H_{18}O_3$ requires C, 68.5; H, 8.6%).

(±)-1-*Methylspiro*(*bicyclo*[3.2.1]*octane*-endo-8,2′-*oxiran*)-6β-*ol* (**16**).—To a stirred suspension of trimethylsulphonium iodide (833 mg, 4.08 mmol) in THF (15 cm^3) at 0 °C was added butyl-lithium (2.4M) in hexane (1.91 cm^3). The mixture was stirred at 0 °C for 1 h, and then a solution of the keto acetate (**7**) (200 mg, 1.02 mmol) in THF (10 cm^3) was added. After 1 h at 0 °C, the mixture was diluted with ether, washed successively with saturated aq. ammonium chloride and brine, and dried. Concentration under reduced pressure and purification by dry column flash chromatography gave the *epoxy alcohol* (**16**) (64 mg, 37%) as an oil; $\nu_{max.}(CCl_4)$ 3 620 and 3 450br cm^{-1}; δ_H(90 MHz) 4.15 (1 H, dd, *J* 8 and 3.5 Hz, 6-H), 2.97 and 2.75 (2 H, ABq, $J_{obs.}$ 4.5, 2′-H_2), and 0.74 (3 H, s, Me); δ_C(25 MHz) 71.9 (C-6), 68.7 (C-8), 52.5 (C-2′), 49.1 (C-5), 46.4 (C-7), 39.2 (C-1), 36.0 (C-2), 26.3 (C-4), 20.9 (Me), and 19.6 (C-3) (Found: M^+, 168.1149. $C_{10}H_{16}O_2$ requires *M*, 168.1150).

Deoxygenation of Epoxides.—General procedure. To WCl_6 (793 mg, 2 mmol), pre-cooled to −196 °C (liquid nitrogen), was added THF (8 cm^3) slowly. The contents of the flask were allowed to warm to −78 °C, and were stirred while butyl-lithium (2.5M) in hexane (2.4 cm^3) was added. The cooling bath was removed, and the stirred mixture was allowed to warm to 25 °C, when it became dark brown and homogeneous. It was then re-cooled to −78 °C, and a solution of the epoxide (1 mmol) in THF (12 cm^3) was added. The cooling bath was removed, and the reaction mixture was heated under reflux for 6 h. On cooling to 25 °C, it was diluted with hexane, washed once with aqueous NaOH (2M)–sodium tartrate (1.5M), and once with water. The organic solution was dried, concentrated under reduced pressure, and the residue was purified by flash chromatography.

Deoxygenation of epoxy acetate (**14**). Following the general procedure, epoxy acetate (**14**) (117 mg, 0.56 mmol) was deoxygenated to give alkene (**10**) (98 mg, 91%).

Deoxygenation of epoxy acetate (**15**). Following the general procedure, epoxy acetate (**15**) (77 mg, 0.37 mmol) was deoxygenated to give alkene (**11**) (65 mg, 91%).

12,13-*Epoxytrichothec*-9-*ene*-3α,4β,15-*triol*-4,15-*Diacetate* (*Anguidine*) (**1**; R = H) *and* 12,13-*Epoxytrichothec*-9-*ene*-3α,4β,

Seed medium		Production medium	
Malt extract	2 g	$NH_4H_2PO_4$	1 g
Yeast	2 g	$K_2HPO_4 \cdot 3H_2O$	3.93 g
Peptone (Oxoid)	2 g	$MgSO_4 \cdot 7H_2O$	0.2 g
KH_2PO_4	2 g	NaCl	5 g
$MgSO_4 \cdot 7H_2O$	2 g	Sucrose	40 g
$FeSO_4 \cdot 7H_2O$	0.2 g	Glycerol	10 g
NH_4Cl	3 g	Deionized water	1 l
Glucose	20 g		
Deionized water	1 l		

15-*triol* 4-*Acetate*(4-*Acetoxyscirpenediol*) (**17**).—*Culture procedure.* Wide-necked conical flasks (500 cm^3) each containing seed medium (100 cm^3) were inoculated with the *Fusarium* species[21] (strain C37410-90, Bristol-Myers Company) which had been maintained on slants of Sabouraud dextrose agar for 7 days. The seed culture was shaken for 2 days in the dark (27 °C, 160 rpm) and was then used to inoculate conical flasks (35) containing production medium (100 cm^3). The production culture was then fermented for 11 days in the dark (27 °C, 160 rpm). Continuous extraction of the whole culture (3.5 l) with ethyl acetate, without altering the pH, for 24 h, followed by drying, and concentration under reduced pressure, gave a brown oily residue (3—4 g). Purification by dry column flash chromatography gave typically 200—300 mg l^{-1} each of anguidine (**1**; R = H) and 4-acetoxyscirpenediol (**17**).

Anguidine (**1**; R = H) was characterised as a white crystalline solid, m.p. 161—163 °C (from ether) (lit.,[29] 162—164 °C); $[\alpha]_D^{20}$ −24.5 (*c* 1.16 in $CHCl_3$) {lit.,[29] $[\alpha]_D^{24}$ −27° (*c* 1.28 in $CHCl_3$)}; $\nu_{max.}(CCl_4)$ 3 560, 1 750, and 1 725 cm^{-1}; δ_H(100 MHz) 5.47 (1 H, dq, *J* 5.6 and 1.8 Hz, 10-H), 5.14 (1 H, d, *J* 2.9 Hz, 4-H), 4.13 (1 H, dd, *J* 5 and 2.9 Hz, 3-H), 4.05 (1 H, br d, *J* 5 Hz, 11-H), 4.14 and 4.0 (2 H, ABq, J_{obs} 12.2 Hz, 15-H_2), 3.64 (1 H, d, *J* 4.9 Hz, 2-H), 3.01 and 2.72 (2 H, ABq, J_{obs} 3.9 Hz, 13-H_2), 2.09 (3 H, s, MeCO), 2.0 (3 H, s, MeCO), 2.01—1.80 (4 H, m, 7- and 8-H_2), 1.67 (3 H, br s, 16-H_3), and 0.76 (3 H, s, 14-H_3); δ_C(25 MHz) 172.5 (Me*C*O), 170.5 (Me*C*O), 140.5 (C-9), 118.6 (C-10), 84.8 (C-4), 79.0 (C-2), 78.4 (C-3), 68.0 (C-11), 64.4 (C-12), 63.7 (C-15), 48.8 (C-5), 47.2 (C-13), 44.0 (C-6), 28.0 (C-8), 23.2 (C-16), 21.3 (C-7), 21.0 (2 × *Me*CO), and 6.9 (C-14) (Found: *m/z* 306.1473. Calc. for $C_{19}H_{26}O_7$–AcOH: *m/z* 306.1467).

4-Acetoxyscirpenediol (**17**) was characterised as a glassy solid which resisted crystallisation, $[\alpha]_D^{20}$ +9.4° (*c* 1.3 in acetone) {lit,[22] $[\alpha]_D^{20}$ +10.3° (*c* 1.2 in acetone)}; $\nu_{max.}(CCl_4)$ 3 630, 3 490br, and 1 720 cm^{-1}; δ_H(200 MHz) 5.56 (1 H, dq, *J* 5.5 and 1.4 Hz, 10-H), 5.52 (1 H, d, *J* 3.2 Hz, 4-H), 4.25 (1 H, dd, *J* 4.9 and 3.2 Hz, 3-H), 4.19 (1 H, br d, *J* 5.5 Hz, 11-H), 3.79 and 3.61 (2 H, ABq, J_{obs} 12.3 Hz, 15-H_2), 3.67 (1 H, d, *J* 4.9 Hz, 2-H), 3.05 and 2.77 (2 H, ABq, J_{obs} 4 Hz, 13-H_2), 2.15 (3 H, s, MeCO), 2.10—1.96 (4 H, m, 7- and 8-H_2), 1.72 (3 H, br s, 16-H_3), and 0.84 (3 H, s, 14-H_3); δ_C(50 MHz) 173.0 (Me*C*O), 140.3 (C-9), 118.8 (C-10), 84.4 (C-4), 79.0 (C-2), 77.5 (C-3), 68.0 (C-11), 64.6 (C-12), 62.6 (C-15), 48.7 (C-5), 47.3 (C-13), 44.8 (C-6), 28.0 (C-8), 23.3 (C-16), 21.2 (C-7), 21.0 (*Me*CO), and 6.6 (C-14) (Found: *m/z* 306.1467. Calc. for $C_{17}H_{24}O_6$–H_2O: *m/z* 306.1467).

12,13-*Epoxytrichothec*-9-*ene*-3α,4β,15-*triyl Triacetate* (**1**; R = Ac).—Anguidine (**1**; R = H) (315 mg, 0.86 mmol) was acetylated in the normal manner. Purification by dry column flash chromatography gave the triacetate (**1**; R = Ac) (332 mg, 95%) as a white crystalline solid, m.p. 122—123 °C (from benzene–hexane) (lit.,[29] 123—125 °C); $[\alpha]_D^{20}$ +36.7° (*c* 0.99 in acetone) {lit.,[29] $[\alpha]_D^{23}$ +44° (*c* 1.0 in acetone)}; $\nu_{max.}(CCl_4)$ 1 750 cm^{-1}; δ_H(200 MHz) 5.75 (1 H, d, *J* 3.3 Hz, 4-H), 5.49 (1 H, dq, *J* 5.6 and 1.3 Hz, 10-H), 5.19 (1 H, dd, *J* 4.9 and 3.3 Hz, 3-H), 4.26 and 4.06 (2 H, ABq, J_{obs} 12.3 Hz, 15-H_2), 3.99 (1 H, br d, *J* 5.6 Hz, 11-H), 3.87 (1 H, d, *J* 4.9 Hz, 2-H), 3.08 and 2.81 (2 H, ABq, J_{obs} 4 Hz, 13-H_2), 2.15 (3 H, s, MeCO), 2.11 (3 H, s, MeCO), 2.07 (3 H, s, MeCO), 2.0—1.80 (4 H, m, 7- and 8-H_2), 1.73 (3 H, br s, 16-H_3), and 0.77 (3 H, s, 14-H_3); δ_C (50 MHz) 170.5 (2 × Me*C*O), 170.0 (Me*C*O), 140.7 (C-9), 118.2 (C-10), 79.3 (C-2), 78.3 (C-3), 77.5 (C-4), 67.9 (C-11), 64.1 (C-12), 63.4 (C-15), 48.7 (C-5), 47.1 (C-13), 44.0 (C-6), 27.8 (C-8), 23.1 (C-16), 21.2 (C-7), 20.9 (*Me*CO), 20.8 (2 × *Me*CO), and 6.5 (C-14) (Found: *m/z* 348.1588. Calc. for $C_{21}H_{28}O_8$–AcOH: *m/z* 348.1573.

Trichotheca-9,12-*diene*-3α,4β,15-*triyl Triacetate* (**18**).—Following the general deoxygenation procedure, the triacetate (**1**; R = Ac) (100 mg, 0.245 mmol) was deoxygenated to give the *diene* (**18**) (93 mg, 97%) as a white crystalline solid, m.p. 120—121 °C (from ether–hexane); $\nu_{max.}(CCl_4)$ 1 750 and 915 cm^{-1}; δ_H(200 MHz) 5.75 (1 H, d, *J* 3.1 Hz, 4-H), 5.42 (1 H, dq, *J* 5.4 and 1.4 Hz, 10-H), 5.21 (1 H, s, 13-H), 4.83 (1 H, s, 13-H), 4.81 (1 H, dd, *J* 4.8 and 3.1 Hz, 3-H), 4.42 (1 H, d, *J* 4.8 Hz, 2-H), 4.22 and 4.02 (2 H, ABq, J_{obs} 12.2 Hz, 15-H_2), 4.00 (1 H, br d, *J* 5.4 Hz, 11-H), 2.12 (3 H, s, MeCO), 2.06 (3 H, s, MeCO), 2.04 (3 H, s, MeCO), 2.0—1.80 (4 H, m, 7- and 8-H_2) 1.66 (3 H br s, 16-H_3), and 0.97 (3 H, s, 14-H_3); δ_C(50 MHz) 170.7 (Me*C*O), 170.2 (2 × Me*C*O), 149.0 (C-12), 140.3 (C-9), 118.5 (C-10), 109.1 (C-13), 79.4 (C-2), 78.6 (C-3), 77.4 (C-4), 67.6 (C-11), 63.8 (C-15), 52.0 (C-5), 43.8 (C-6), 27.8 (C-8), 23.2 (C-16), 21.0 (*Me*CO), 20.9 (*Me*CO), 20.8 (*Me*CO), 20.5 (C-7), and 10.9 (C-14) (Found: C, 64.3; H, 7.1%; *m/z* 332.1613 $C_{21}H_{28}O_7$ requires C, 64.3; H, 7.2%. $C_{21}H_{28}O_7$–AcOH requires *m/z* 332.1624).

12,13-*Epoxy*-8-*oxotrichothec*-9-*ene*-3α,7α,15-*triyl Triacetate* (**2**; R = Ac).—To a solution of 3-*O*-acetyldeoxynivalenol (34 mg, 0.101 mmol) in dichloromethane (3 cm^3) were added an excess of triethylamine (1.13 cm^3) and acetic anhydride (0.8 cm^3) and a catalytic amount of DMAP (5 mg). The mixture was stirred for 2 days at 25 °C, then was diluted with ether, and washed once with saturated aq. sodium hydrogen carbonate and once with water. The organic phase was dried, concentrated under reduced pressure, and the residue was purified by flash chromatography, to give *deoxynivalenol triacetate* (**2**; R = Ac) (40 mg, 94%) as a white crystalline solid, m.p. 153—157 °C (from ethyl acetate–light petroleum) (lit.,[24] 156—157 °C); $[\alpha]_D^{20}$ 75° (*c* 0.2 in $CHCl_3$); $\nu_{max.}(CCl_4)$ 1 755 and 1 705 cm^{-1}; δ_H(200 MHz) 6.53 (1 H, dq, *J* 5.9 and 1.6 Hz, 10-H), 6.03 (1 H, s, 7-H), 5.19 (1 H, ddd, *J* 11, 4.5, and 4.5 Hz, 3-H), 4.72 (1 H, d, *J* 5.9 Hz, 11-H), 4.37 and 4.26 (2 H, ABq, J_{obs} 12.2 Hz, 15-H_2), 3.88 (1 H, d, *J* 4.5 Hz, 2-H), 3.10 and 2.78 (2 H, ABq, J_{obs} 3.5 Hz, 13-H_2), 2.34 (1 H, dd, *J* 15.2 and 4.5 Hz, 4-H_α), 2.18 (3 H, s, MeCO), 2.12 (3 H, s, MeCO), 1.88 (3 H, s, MeCO), 1.82 (3 H, br s, 16-H_3), and 0.93 (3 H, s, 14-H_3); δ_C(50 MHz) 191.9 (C-8), 170.1 (Me*C*O), 170.0 (Me*C*O), 169.6 (Me*C*O), 136.9 (C-9), 136.8 (C-10), 78.7 (C-7), 74.6 (C-2), 70.6 (C-11), 70.2 (C-3), 64.5 (C-12), 62.3 (C-15), 50.0 (C-5), 47.6 (C-13), 45.6 (C-6), 40.7 (C-4), 20.8 (2 × *Me*CO), 20.5 (*Me*CO), 15.3 (C-16), and 13.6 (C-14) (Found: C, 59.8; H, 6.1%; M^+, 422.1557. $C_{21}H_{26}O_9$ requires C, 59.7; H, 6.2%; *M*, 422.1557).

8-*Oxatrichotheca*-9,12-*diene*-3α,7α,15-*triyl Triacetate* (**19**).—To WCl_6 (190 mg, 0.479 mmol), pre-cooled to −196 °C (liquid nitrogen) was added THF (2 cm^3) slowly. The contents of the flask were allowed to warm to −78 °C, and were stirred while butyl-lithium (2.5M) in hexane (0.57 cm^3) was added. The cooling bath was removed and the mixture was stirred and allowed to warm to 25 °C. One half (1.25 cm^3) of this solution was taken up in a syringe and was added to a solution of the epoxide (**2**; R = Ac) (50 mg, 0.118 mmol) in THF (3 cm^3), cooled to −78 °C. The reaction mixture was then heated under reflux for 6 h, and processed as before. The crude product was

shown by ^{1}H n.m.r. spectroscopy to consist of the desired diene (**19**) and starting material in the ratio 3:2; chromatographic separation proved impossible. The crude product was recycled through the above procedure. Purification by flash chromatography gave the *diene* (**19**) (19 mg, 40%) as a white solid, which resisted recrystallisation; $\nu_{max.}(CCl_4)$ 1 750, 1 705, and 920 cm^{-1}; δ_H(200 MHz) 6.50 (1 H, dq, *J* 5.7 and 1.5 Hz, 10-H), 5.87 (1 H, s, 7-H), 5.28 (1 H, s, 13-H), 4.95 (1 H, ddd, *J* 11.5, 4.5 and 4.5 Hz, 3-H), 4.88 (1 H, s, 13-H), 4.69 (1 H, br d, *J* 5.7 Hz, 11-H), 4.49 (1 H, d, *J* 4.5 Hz, 2-H), 4.37 and 4.25 (2 H, Abq, $J_{obs.}$ 12.0, 15-H_2), 2.37 (1 H, dd, *J* 14.9 and 4.5 Hz, 4-H_α), 2.21 (3 H, s, MeCO), 2.15 (3 H, s, MeCO), 1.91 (3 H, s, MeCO), 1.83 (3 H, dd, *J* 1.5 and 0.7 Hz, 16-H_3), and 1.23 (3 H, s, 14-H_3); δ_C(50 MHz) 192.5 (C-8), 170.2 (Me*C*O), 170.0 (Me*C*O), 169,0 (Me*C*O), 149.9 (C-12), 137.1 (C-9), 136.8 (C-10), 110.0 (C-13), 79.0 (C-7), 74.9 (C-2), 71.0 (C-11), 70.4 (C-3), 62.4 (C-15), 51.1 (C-5), 47.8 (C-6), 41.5 (C-4), 21.0 (*Me*CO), 20.9 (*Me*CO), 20.6 (*Me*CO), 19.1 (C-16), and 15.6 (C-14) (Found: M^+, 406.1603. $C_{21}H_{26}O_8$ requires *M*, 406.1628).

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References

1 'Trichothecenes,' ed. Y. Ueno, Elsevier, Amsterdam, 1984; Ch. Tamm and M. Tori, 'Trichothecenes,' ch. 8, in 'Mycotoxins—Production, Isolation, Separation and Purification,' ed. V. Betina, Elsevier, Amsterdam, 1984; R. J. Cole and R. H. Cox, 'Handbook of Toxic Fungal Metabolites,' Academic Press, New York, 1981; P. G. McDougal and N. R. Schmuff, *Fortschr. Chem. Org. Naturst.*, 1985, **47**, 153; *Synform.*, 1984, **4**, 2229, *Chem. Abstr.*, 1985, **103**, 71517e—71522c; J. F. Grove, *Nat. Prod. Rep.*, 1988, 187.
2 F. Hernandez and M. Cannon, *J. Antibiot.*, 1982, **35**, 875, and references therein.
3 S. S. Adler, S. Lowenbraun, B. Birch, R. Jarrell, and J. Garrard, *Cancer Treat. Rep.*, 1984, **68**, 423.
4 See, for example, L. R. Ember, *Chem. Eng. News*, 1984, 8.
5 T. Yoshizawa, H. Takeda, and T. Ohi, *Agric. Biol. Chem.*, 1983, **47**, 2133, and references therein.
6 W. R. Roush, M. A. Marletta, S. Russo-Rodriguez, and J. Recchia, *J. Am. Chem. Soc.*, 1985, **107**, 3354.
7 J. R. Grove and P. H. Mortimer, *Biochem. Pharmacol.*, 1969, **18**, 1473.
8 M. D. Grove, H. R. Burmeister, S. L. Taylor, D. Weisleder, and R. Plattner, *J. Agric. Food Chem.*, 1984, **32**, 541.
9 R. R. King, R. E. McQueen, D. Levesque, and R. Greenhalgh, *J. Agric. Food Chem.*, 1984, **32**, 1181.
10 B. B. Jarvis and E. P. Mazzola, *Acc. Chem. Res.*, 1982, **15**, 388, and references therein.
11 E. W. Colvin, S. Malchenko, R. A. Raphael, and J. S. Roberts, *J. Chem. Soc., Perkin Trans. 1*, 1973, 1989.
12 E. W. Colvin, S. Malchenko, R. A. Raphael, and J. S. Roberts, *J. Chem. Soc., Perkin Trans. 1*, 1978, 658.
13 E. W. Colvin and I. G. Thom, *Tetrahedron*, 1986, **42**, 3137.
14 E. W. Colvin and S. Cameron, *J. Chem. Soc., Chem. Commun.*, 1986, 1084.
15 W. R. Roush and S. Russo-Rodriguez, *J. Org. Chem.*, 1985, **50**, 5465.
16 *Org. Synth.*, 1981, **60**, 29, and references therein.
17 E. J. Corey and M. Chaykovsky, *J. Am. Chem. Soc.*, 1965, **87**, 1353.
18 M. G. Martin and B. Ganem, *Tetrahedron Lett.*, 1984, **25**, 251.
19 K. B. Sharpless, M. A. Umbreit, M. T. Nieh, and T. C. Flood, *J. Am. Chem. Soc.*, 1972, **94**, 6538; K. B. Sharpless, A. Y. Teranishi, and J.-E. Bäckvall, *ibid.*, 1977, **99**, 3120.
20 A. Bélanger, D. J. F. Berney, H.-J. Borschberg, R. Brousseau, A. Doutheau, R. Durand, H. Katayama, R. LaPalme, D. M. Leturc, C.-C. Liao, F. N. MacLachlan, J.-P. Maffrand, F. Marazza, R. Martino, C. Moreau, L. Saint-Laurent, R. Saintonge, P. Soucy, L. Ruest, and P. Deslongchamps, *Can. J. Chem.*, 1979, **57**, 3348; A. Sattar, J. Forrester, M. Moir, J. S. Roberts, and W. Parker, *Tetrahedron Lett.*, 1976, 1403; M. L. Hill and R. A. Raphael, *ibid.*, 1986, **27**, 1293.
21 N. C. P. Baldwin, B. W. Bycroft, P. M. Dewick, and J. Gilbert, *Z. Naturforsch., Teil C*, 1986, **41**, 845.
22 W. R. Roush and S. Russo-Rodriguez, *J. Org. Chem.*, 1985, **50**, 3224.
23 J. Gutzwiller, R. Mauli, H. P. Sigg, and Ch. Tamm, *Helv. Chim. Acta*, 1964, **47**, 2234.
24 R. R. King and R. Greenhalgh, *Can. J. Chem.*, 1985, **63**, 1089.
25 Ref. 15, footnote 24.
26 J. Robb and M. Norval, *Appl. Environ. Microbiol.*, 1983, **46**, 948.
27 L. M. Harwood, *Aldrichimica Acta*, 1985, **18**, 25.
28 W. C. Still, M. Kahn, and A. Mitra, *J. Org. Chem.*, 1978, **43**, 2923.
29 H. P. Sigg, R. Mauli, E. Flury, and Ch. Tamm, *Helv. Chim. Acta*, 1965, **48**, 962.

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