Studies in Terpenoid Biosynthesis. Part 39.[1] The Stereochemistry of the Relationship Between Substrate and Oxidant in the Hydroxylation of Aphidicolin at C-18 by *Cephalosporium aphidicola*

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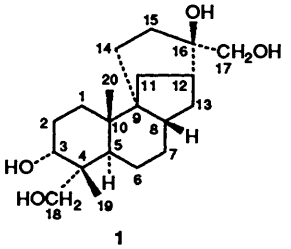
The preparation of some 3α,18-oxetane analogues of intermediates in the biosynthesis of aphidicolin is described. Their stereoselective labelling at C-18 with deuterium is reported. Biotransformation studies using these substrates with *Cephalosporium aphidicola* have shown that the 18-*pro*-*R*-hydrogen is removed in hydroxylation at this centre suggesting a *Re* stereochemical relationship between the substrate and oxidant for the normal hydroxylation at C-18. The X-ray crystallographic structure of 3α,18-oxetane **6** is presented and compared with that of aphidicolin 17-nor-16-ketone **13**.

The enzymatic hydroxylation of a methyl group plays an important role in many biosyntheses particularly in the terpenoid and steroid area.[2–4] Knowledge of the stereochemical relationship between the substrate and the oxidant in these steps is useful in defining the geometry of the active site particularly as some of the steps are the target for inhibitors.[5] The diterpenoid fungal metabolite, aphidicolin **1**[6] is biosynthesized from aphidicolan-16β-ol **2**[7] by a series of steps involving methyl group hydroxylations including that at C-18.[8] In this paper we describe the preparation of the 3α,18-oxetanes **3** and **4**, their stereochemical comparison with aphidicolin **1**, their stereoselective labelling at C-18 and the results of their biotransformation at C-18 by *Cephalosporium aphidicola*. Since the oxetane ring locks C-18 into a particular conformation, this provided an opportunity for determining the stereochemical relationship between the substrate and the hydroxylase at this centre.



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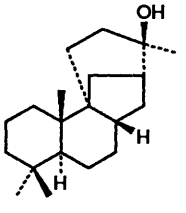

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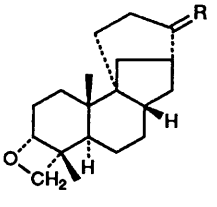
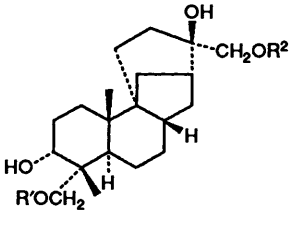
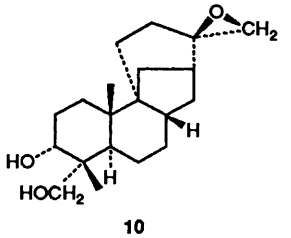


3 R = α-CH_3, β-OH
4 R = 
5 R = O
6 R = α-CH_2OH, β-OH



7 R^1 = H, R^2 = Ts
8 R^1 = R^2 = Ts
9 R^1 = Ts, R^2 = H

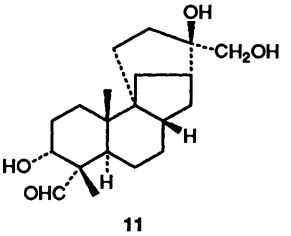


10

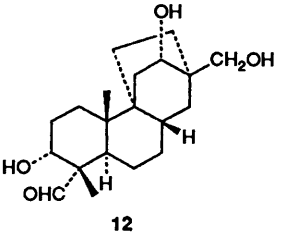
The selective acylation of aphidicolin **1**[6] with toluene-*p*-sulfonyl chloride affords a mono- **7** and a di-toluene-*p*-sulfonate **8**. At low temperature the 17-mono-toluene-*p*-sulfonate is formed whereas the use of 2.75 equiv. of toluene-*p*-sulfonyl chloride at room temperature favours the formation of the 17,18-ditoluene-*p*-sulfonate. On one occasion the 18-mono-toluene-*p*-sulfonate **9** was obtained from this preparation after chromatography. It is possible that hydrolysis of the 17-toluene-*p*-sulfonate occurred on the column. Treatment of the 17-mono-toluene-*p*-sulfonate **7** with sodium hydrogen carbonate in DMSO (dimethyl sulfoxide) leads to the formation of the 16β,17-epoxide **10** which was identical with the material obtained by the Corey–Chaykovsky reaction on the 17-nor-16-ketone.[6] Thus the mono-toluene-*p*-sulfonate is located at C-17.

Treatment of the 17,18-ditoluene-*p*-sulfonate **8** with sodium hydrogen carbonate in DMSO gives the 16β,17-epoxy 3α,18-oxetane **4**. The oxetane structure followed from the absence of hydroxy absorption in the IR spectrum and the presence of ^{1}H NMR signals at δ_H 3.87 and 4.30 (*J* 5)† attributable to the 18-H. A signal at δ_H 4.68 was assigned to 3-H. A number of other 3α,18-oxetanes including **3**, **5** and **6**, were prepared from the corresponding 18-toluene-*p*-sulfonates.

Incubation of both the 16β-hydroxy 3α,18-oxetane **3** and the 16β,17-epoxy 3α,18-oxetane **4** with *Cephalosporium aphidicola* gave the 18-aldehyde **11** as the major metabolite. Its structure followed from the ^{1}H NMR spectrum which contained a signal



11



12

† *J* Values are given in Hz throughout.

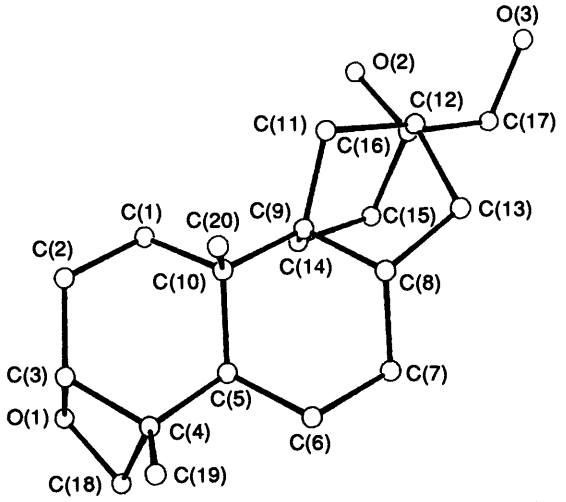


Fig. 1 X-Ray molecular structure of compound **6**

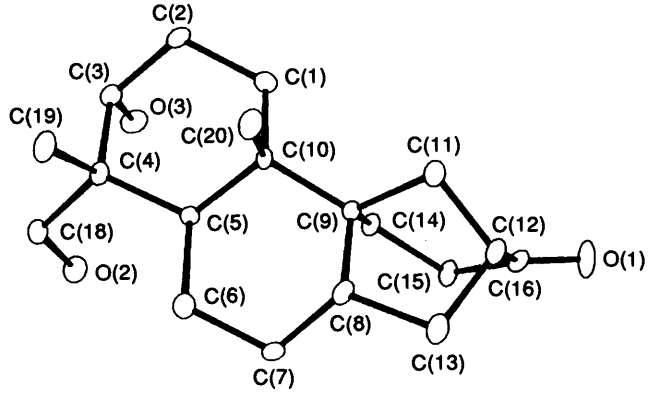


Fig. 2 X-Ray molecular structure of compound **13**

at δ_H 9.55 assigned to the 18-CHO. A minor metabolite arising from the incubation of the epoxide **4** was assigned the structure **12**. The 12(13→16) rearrangement has been observed[9] in the acid-catalysed cleavage of aphidicolane 16β,17-epoxides. Its occurrence in this metabolite was established by ^{1}H NMR NOE experiments. Irradiation at the 20-H signal (δ_H 0.92) produced enhancements of 12% at δ_H 1.91 and 9% at 1.71. These signals which were therefore assigned to the C(11) protons, were also enhanced by irradiation of the C(12)-*H* signal (δ_H 4.26) (6 and 3% respectively). The location of the aldehyde at C-18 was established by the observation of an NOE enhancement (8%) of the 5-H signal on irradiation of the aldehydic C–H (δ_H 9.90). It is possible that the rearrangement occurred in the medium and is not enzyme catalysed.

Compounds with C-18 at higher oxidation levels than alcohol are not normal metabolites of *C. aphidicola*. The further transformation of aphidicolin by this organism involves hydroxylation at C-6β and C-11β.[10] It was therefore possible that the 3α,18-oxetane was acting as a substrate for the methyl group hydroxylase. Molecular models suggested that this readily formed oxetane ring did not distort the conformation of ring A to such a gross extent as to make this unreasonable. An X-ray crystallographic structure of the 3α,18-oxetane **6** (see Fig. 1) was obtained and compared to that of aphidicolin 17-nor-16-ketone **13** (see Fig. 2). The overall similarity of ring A in these molecules is shown in the two projections in Figs. 3(*a*) and (*b*) and Figs. 4(*a*) and (*b*). Since the oxetane ring locks C-18 preventing free rotation, this provided an opportunity for determining the stereochemical relationship between the substrate and the hydroxylase at this centre.

The 16β,17-epoxy 3α,18-oxetane **4** was labelled at C-18 with

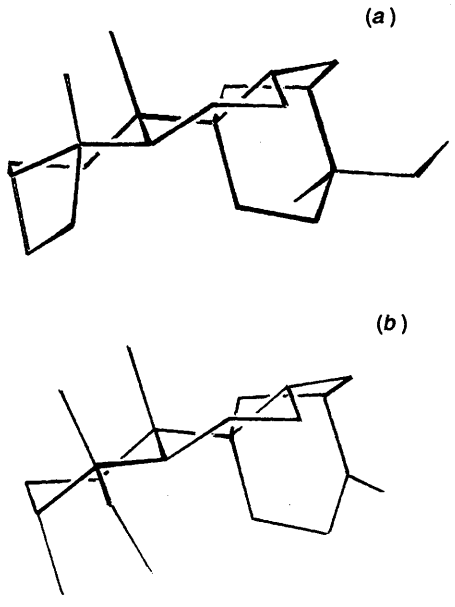


Fig. 3 Projection of compounds **6** and **13** viewed from C-18

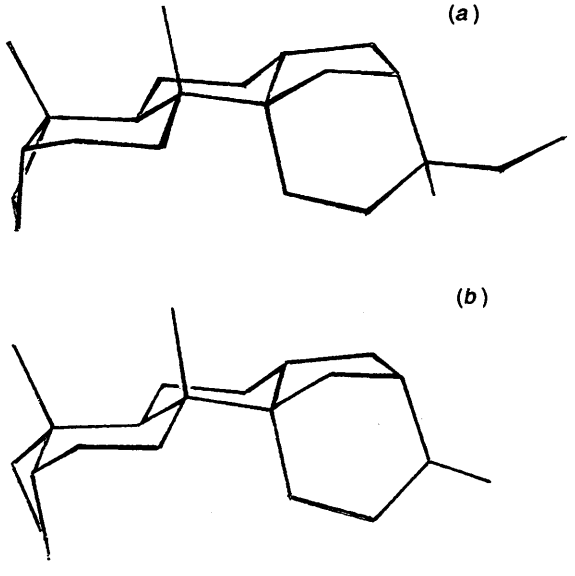
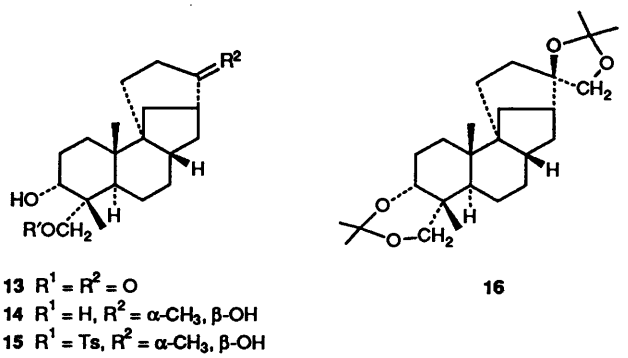


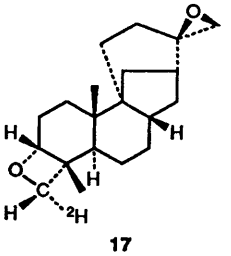
Fig. 4 Projection of compounds **6** and **13** viewed from C-1

deuterium by reducing the aldehyde **11** with sodium borodeuteride in methanol–tetrahydrofuran[11] to afford [18-^{2}H]-aphidicolin. The stereochemistry of the label was established as follows. The ^{1}H NMR spectrum of aphidicolin bis acetonide **16** contains two AB doublets (δ_H 3.25 and 3.63) which have been assigned[1] to the 18-H. Irradiation of the acetonide methyl (δ_H 1.40) produced NOE enhancements at the 3-H signal (δ_H 3.61, 7%) and the 18-H signal at δ_H 3.25 (4%). Molecular models show that the latter must therefore correspond to the *pro-R*-hydrogen. In the deuteriated material the signal at δ_H 3.63 was a singlet and the upfield signal bore the substantial amount of the label (only 20% ^{1}H). Therefore the reduction had occurred predominantly from the *Re* face of the aldehyde to generate the (18*R*)-isomer. Subsequent treatment with toluene-*p*-sulfonyl chloride and then base ($NaHCO_3$, DMSO) gave the labelled epoxide **17**. The 18-H proton resonances of the 3α,18-oxetane appear at δ_H 3.87 and 4.31. An NOE enhancement (2%) between the 3-H (δ 4.68) and the *pro-R*-hydrogen at C-18 established that the signal at δ 4.31 corresponded to this proton and that at δ 3.87 to the *pro-S*-proton. The ^{1}H NMR spectrum of the labelled material showed that it contained 80% of the (18*S*)-



13 $R^1 = R^2 = O$
14 $R^1 = H$, $R^2 = \alpha$-CH_3, β-OH
15 $R^1 = Ts$, $R^2 = \alpha$-CH_3, β-OH

16



17

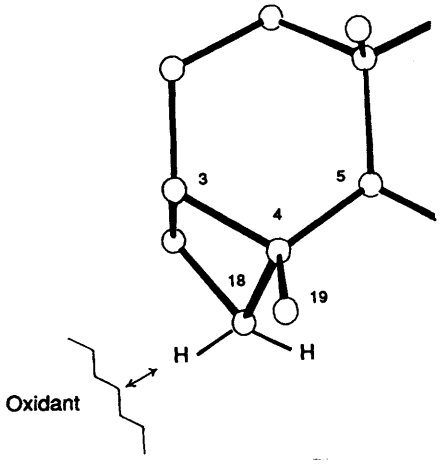


Fig. 5 Relationship between oxidant and substrate for hydroxylation at C-18

labelled species **17** and 20% of its epimer. As expected inversion of configuration had taken place on formation of the 3α,18-oxetane. Incubation of this material with *C. aphidicola* gave the aldehyde **11** in which the aldehydic proton (δ_H 9.55) contained only 30% C–H, *i.e.* a protium rather than a deuterium atom had been removed. The hydroxylation of a methylene is known to proceed with retention of configuration.[2] Hence the hydroxylation had proceeded from the *Re* face of the oxetane ring at C-18 thus defining the topological relationship between the oxidant and the substrate at this centre (see Fig. 5).

If the assumptions are made, firstly, that the enzyme system which is responsible for this hydroxylation is also that which hydroxylates aphidicolan-16β-ol at C-18, and secondly, that the artificial and natural substrates both bind in the same way to this system, *i.e.* the additional oxygen atom does not perturb the binding, then this reveals the general face from which the hydroxy oxygen is delivered. In this context it is worth noting that this strain of *C. aphidicola* does not oxidize aphidicolin beyond the hydroxymethyl level at C-18. Furthermore, there is not an 18-deoxy series of metabolites which might provide an alternative pathway if the initial C–H bond breaking step had a high isotope effect. This method of constraining the freely rotating methyl group and examining the stereochemistry of hydroxylation of the methyleneoxy ether is obviously open to extension to other biosyntheses such as those of the gibberellins and the steroid hormones where active-site directed inhibitors of methyl group hydroxylation are important.

Experimental

General Experimental Details.—^{1}H NMR spectra were determined at 360 MHz on a Bruker WM 360 spectrometer for solutions in deuteriochloroform except where otherwise stated; *J* values are given in Hz. IR spectra were determined as Nujol mulls. Solutions were dried over sodium sulfate. Light petroleum refers to the fraction b.p. 60–80 °C. Silica for chromatography was Merck 9385.

*Reaction of Aphidicolin with Toluene-*p*-sulfonyl Chloride.*—(*a*) Aphidicolin **1** (3.5 g) in dry pyridine (125 cm^3) in a sealed flask was kept at −5 °C and toluene-*p*-sulfonyl chloride (2.6 g) was added in portions over 6 h and then the mixture was left overnight at −5 °C. The mixture was then poured into dil. hydrochloric acid and the product was recovered in ethyl acetate. The extract was washed with dil. hydrochloric acid, aqueous sodium hydrogen carbonate and brine and dried. The solvent was evaporated to give a gum which was chromatographed on silica. Elution with ethyl acetate–light petroleum (2:3) gave 3α,16β-*dihydroxy*-17,18-*toluene*-p-*sulfonyloxyaphidicolane* **8** (790 mg) which crystallized from methanol as cubes, m.p. 149–150 °C (Found: C, 63.1; H, 7.1. $C_{34}H_{46}O_8S_2$ requires C, 63.1; H, 7.2%); ν_{max}/cm^{-1} 3500 and 1600; δ_H 0.90 and 0.92 (each 3 H, s, 19- and 20-H), 2.45 (6 H, s, Ar-Me), 3.66 (1 H, br s, 3-H), 3.78 and 4.01 (each 1 H, d, *J* 8.6, 18-H), 3.80 and 3.87 (each 1 H, d, *J* 9.7, 17-H) and 7.35 and 7.77 (each 4 H, d, *J* 7.5, Ar-H).

Further elution gave 3α,16β,18-*trihydroxy*-17-*toluene*-p-*sulfonyloxyaphidicolane* **7** (3.4 g) which crystallized from ethyl acetate as needles, m.p. 140–142 °C (Found: C, 63.8; H, 8.2. $C_{27}H_{40}O_6S$ requires C, 63.5; H, 8.3%); ν_{max}/cm^{-1} 3250br and 1600; $\delta_H(C_5D_5N)$ 0.77 (3 H, s, 19-H), 0.97 (3 H, s, 20-H), 2.21 (3 H, s, Ar-Me), 3.63 and 3.79 (each 1 H, d, *J* 10.8, 18-H), 3.91 (1 H, br s, 3-H), 4.19 and 4.31 (each 1 H, d, *J* 9.7, 17-H) and 7.25 and 8.00 (each 2 H, d, *J* 7.5, Ar-H).

(*b*) Toluene-*p*-sulfonyl chloride (4.24 g) was added to aphidicolin (2.75 g) in pyridine (100 cm^3) and the mixture was kept at room temperature for 48 h. The mixture was poured into dil. hydrochloric acid and the product was extracted with ethyl acetate. The extract was washed with dil. hydrochloric acid, aqueous sodium hydrogen carbonate, brine and dried. The solvent was evaporated and the residue was chromatographed on silica. Elution with ethyl acetate–light petroleum (3:7) gave the 17,18-ditoluene-*p*-sulfonate **8** (4.15 g) identical (TLC, m.p. and IR) to the material described above.

Preparation of 3α,18-*Dihydroxy*-16β,17-*epoxyaphidicolane* **10**.—The monotoluene-*p*-sulfonate **7** (4.2 g) was added to a stirred suspension of sodium hydrogen carbonate (10 g) in dimethyl sulfoxide (DMSO) (25 cm^3) at 90 °C. After 1 h, the mixture was poured into water and extracted with ethyl acetate. The extract was washed with water, brine and dried. The solvent was evaporated to give 3α,18-dihydroxy-16β,17-epoxyaphidicolane **10** (2.76 g), m.p. 156–157 °C (lit.,[8] 155–158 °C) identified by its ^{1}H NMR spectrum.

Preparation of 3α,18;16β,17-*Diepoxyaphidicolane* **4**.—The 17,18-ditoluene-*p*-sulfonate **8** (400 mg) was added to a stirred suspension of sodium hydrogen carbonate (2 g) in DMSO (10 cm^3) at 75 °C. The stirred mixture was maintained at 75 °C for 24 h. It was then poured into water and the product was extracted with ethyl acetate. The extract was washed with water and brine and dried. The solvent was evaporated to give 3α,18;16β,17-diepoxyaphidicolane **4** (140 mg) which crystallized

from ethyl acetate–light petroleum as prisms, m.p. 219–221 °C (Found: C, 79.3; H, 10.1. $C_{20}H_{30}O_2$ requires C, 79.5; H, 9.9%); ν_{max}/cm^{-1} 960; δ_H 0.83 (3 H, s, 19-H), 1.05 (3 H, s, 20-H), 2.62 and 2.63 (2 H, AB q, J 4.7, 17-H), 3.87 and 4.30 (each 1 H, d, J 5, 18-H) and 4.68 (1 H, br s, 3-H).

Preparation of 3α,16-*Dihydroxy*-18-*toluene*-p-*sulfonyloxyaphidicolane* **15**.—Toluene-*p*-sulfonyl chloride (0.64 g) was added to 3,16,18-trihydroxyaphidicolane[7] **14** (0.55 g) in pyridine (25 cm^3) and the mixture was left at room temperature for 2 d. It was then poured into dil. hydrochloric acid and the products were extracted with ethyl acetate. The extract was washed with dil. hydrochloric acid, aqueous sodium hydrogen carbonate, brine and dried. The solvent was evaporated to give a residue which was chromatographed on silica. Elution with ethyl acetate–light petroleum (3:2) gave 3α,16β-*dihydroxy*-18-*toluene*-p-*sulfonyloxyaphidicolane* **15** (0.63 g) which crystallized from ethyl acetate–light petroleum as needles, m.p. 156–158 °C (Found: C, 67.8; H, 8.3. $C_{27}H_{40}O_5S$ requires C, 68.1; H, 8.4%); ν_{max}/cm^{-1} 3560, 3420 and 1600; δ_H 0.91 and 0.92 (each 3 H, s, 19- and 20-H), 1.11 (3 H, s, 17-H), 2.45 (3 H, s, Ar-Me), 3.66 (1 H, t, J 2.5, 3-H), 3.79 and 4.03 (each 1 H, d, J 8.5, 18-H) 7.35 and 7.78 (each 2 H, d, J 9, Ar-H).

Preparation of 3α,18-*Epoxy*-16β-*hydroxyaphidicolane* **3**.—The above toluene-*p*-sulfonate **15** (520 mg) was added to a stirred suspension of sodium hydrogen carbonate (2.5 g) in DMSO (20 cm^3) at 80 °C. The mixture was maintained at 80 °C for 7 h and then poured into water. The product was recovered in ethyl acetate. The extract was washed with water, brine and dried. The solvent was evaporated to give a gum which was chromatographed on silica. Elution with ethyl acetate–light petroleum (2:3) gave 3α,18-*epoxy*-16β-*hydroxyaphidicolane* **3** (360 mg) which crystallized from ethyl acetate as needles, m.p. 77–80 °C (Found: C, 74.95; H, 10.5. $C_{20}H_{32}O_2{\cdot}H_2O$ requires C, 74.5; H, 10.6%); ν_{max}/cm^{-1} 3480, 1040 and 955; δ_H 0.80 (3 H, s, 19-H), 1.05 (3 H, s, 20-H), 1.15 (3 H, s, 17-H), 3.87 and 4.31 (each 1 H, d, J 5, 18-H) and 4.68 (1 H, d, J 4, 3-H).

Preparation of 3α,18-*Epoxy*-17-*noraphidicolan*-16-*one* **5**.—Toluene-*p*-sulfonyl chloride (190 mg) was added to a solution of 3α,18-dihydroxy-17-noraphidicolan-16-one[6] **13** (150 mg) in dry pyridine (10 cm^3) and the mixture was left at room temperature for 24 h. The mixture was poured into ice-cold dil. hydrochloric acid and the product was recovered in ethyl acetate. The extract was washed with dil. hydrochloric acid, aqueous sodium hydrogen carbonate and brine and dried. The solvent was evaporated to give a gum (205 mg) which was dissolved in DMSO (7 cm^3). Sodium hydrogen carbonate (1.5 g) was added and the mixture was stirred at 75 °C for 26 h. The mixture was poured into water and the products were extracted with ethyl acetate. The extract was washed with water and brine and dried. The solvent was evaporated to give a gum which was chromatographed on silica. Elution with ethyl acetate–light petroleum (1:4) gave 3α,18-*epoxy*-17-*noraphidicolan*-16-*one* **5** (80 mg) which crystallized from ethyl acetate–light petroleum as flakes, m.p. 95–97 °C (Found: C, 78.9; H, 9.8. $C_{19}H_{28}O_2$ requires C, 79.1; H, 9.8%); ν_{max}/cm^{-1} 1720 and 960; δ_H 0.88 (3 H, s, 19-H), 1.07 (3 H, s, 20-H), 3.88 and 4.32 (each 1 H, d, J 5, 18-H) and 4.70 (1 H, br s, $W_{\frac{1}{2}}$ 7, 3-H).

Preparation of 16,17-*Dihydroxy*-3α,18-*epoxyaphidicolane* **6**.—3α,16β,17-Trihydroxy-18-toluene-*p*-sulfonoxyaphidicolane **9** (90 mg) was added to a stirred slurry of sodium hydrogen carbonate (1 g) in DMSO (10 cm^3) at 75 °C. After 48 h, the mixture was poured into water (50 cm^3) and the product extracted with ethyl acetate. The extract was washed with water and brine and dried. The solvent was evaporated and the residue was chromatographed on silica. Elution with ethyl acetate–light petroleum (3:2) gave 16β,17-*dihydroxy*-3α,18-*epoxyaphidicolane* **6** (35 mg) which crystallized from ethyl acetate–light petroleum as needles, m.p. 159–161 °C (Found: C, 75.0; H, 9.9. $C_{20}H_{32}O_3$ requires C, 74.95; H, 10.05%). This analysis was carried out on a sample which was dried at 100 °C *in vacuo* whereas the X-ray crystal structure, which revealed the presence of 0.5 H_2O, was carried out on a sample that was dried at room temperature. ν_{max}/cm^{-1} 3380, 1040 and 960; δ_H 0.81 (3 H, s, 19-H), 1.04 (3 H, s, 20-H), 3.36 and 3.46 (each 1 H, d, J 11, 17-H), 3.87 and 4.30 (each 1 H, d, J 5, 18-H) and 4.69 (1 H, d, J 3.5, 3-H).

Reduction of 3α,16β,17-*Trihydroxyaphidicolan*-18-*al* **11**.—Sodium borodeuteride (420 mg) was added to tetrahydrofuran (THF) (150 cm^3, freshly distilled from lithium aluminium hydride) under an atmosphere of nitrogen. The mixture was stirred and heated to 50 °C for 20 min to aid dissolution and then cooled to room temperature. Methanol (1.05 cm^3) in dry THF (10 cm^3) was added dropwise to the solution over 30 min.

Table 1 Crystal data and structure refinement details for the X-ray structures

	Compound **6**[a]	Compound **13**
Formula	$C_{20}H_{32}O_3{\cdot}0.5H_2O$	$C_{19}H_{30}O_3$
M	329.5	306.5
Crystal size (mm)	0.6 × 0.1 × 0.1	0.2 × 0.2 × 0.2
Crystal system	monoclinic	orthorhombic
Space group	C_2	$P2_12_12_1$
a, b, c (Å)	22.717(6), 6.627(8), 24.035(7)	7.106(1), 11.430(2), 19.875(2)
β°	101.48(2)	
V (Å^3)	3546.0	1614.3
Z, D_c (g cm^{-3}), F(000)	8, 1.23, 1448	4, 1.26, 672
μ (Mo-Kα)/cm^{-1}	0.7	0.9
Total unique reflections	3449	1993
Significant reflections	1664	1263
Abs. corr.	none	none
Hydrogen atoms	fixed calculated	fixed calculated
R	0.089	0.062
R′	0.090	0.075
T/K	295	295
Max. final shift/esd	0.01	0.01
Max. min residual electron densities/eÅ^{-3}	0.5	0.3

[a] The asymmetric unit of the crystal structure of **6** contains two independent molecules of essentially identical conformation and a water molecule. The absolute configurations were taken from the known chemical origin of the compounds.

Table 2 Fractional atomic coordinates ($\times 10^4$) with estimated standard deviations in parentheses for compound **6**

	x	*y*	*z*
Molecule 1			
O(1)	9 104(4)	5 948(16)	8 734(4)
O(2)	5 763(3)	4 883(0)	7 724(3)
O(3)	4 753(4)	3 750(15)	8 175(4)
C(1)	7 962(5)	3 625(20)	8 058(5)
C(2)	8 628(5)	3 345(22)	8 042(5)
C(3)	9 049(5)	3 804(21)	8 580(5)
C(4)	8 861(5)	3 294(22)	9 158(5)
C(5)	8 171(5)	3 436(20)	9 114(5)
C(6)	7 991(5)	2 666(20)	9 662(5)
C(7)	7 315(5)	2 891(20)	9 665(5)
C(8)	6 980(5)	1 835(21)	9 123(5)
C(9)	7 113(4)	2 844(18)	8 578(4)
C(10)	7 796(5)	2 487(18)	8 559(4)
C(11)	6 626(5)	1 816(20)	8 138(5)
C(12)	6 057(4)	2 105(17)	8 380(4)
C(13)	6 285(5)	1 634(20)	9 029(5)
C(14)	6 958(4)	5 117(19)	8 541(4)
C(15)	6 325(5)	5 718(20)	8 626(5)
C(16)	5 839(5)	4 326(21)	8 302(5)
C(17)	5 257(5)	4 665(22)	8 506(5)
C(18)	9 140(6)	5 414(23)	9 329(6)
C(19)	9 184(6)	1 498(25)	9 484(6)
C(20)	7 935(5)	231(22)	8 509(5)
Molecule 2			
O(1a)	6 011(4)	11 267(16)	5 427(4)
O(2a)	9 274(3)	10 166(13)	6 992(3)
O(3a)	10 165(3)	7 179(13)	6 974(3)
C(1a)	7 017(5)	10 423(19)	6 520(5)
C(2a)	6 353(5)	10 927(21)	6 470(5)
C(3a)	5 960(5)	10 152(23)	5 950(5)
C(4a)	6 116(4)	8 149(17)	5 658(4)
C(5a)	6 814(4)	7 789(17)	5 778(4)
C(6a)	6 960(5)	5 758(21)	5 523(5)
C(7a)	7 632(5)	5 408(19)	5 600(4)
C(8a)	7 917(4)	5 701(18)	6 219(4)
C(9a)	7 822(4)	7 834(18)	6 421(4)
C(10a)	7 134(4)	8 254(18)	6 395(4)
C(11a)	8 245(5)	7 735(19)	7 026(5)
C(12a)	8 818(4)	6 878(18)	6 857(4)
C(13a)	8 598(5)	5 201(20)	6 433(5)
C(14a)	8 108(4)	9 525(19)	6 112(4)
C(15a)	8 753(5)	9 258(18)	6 067(4)
C(16a)	9 163(4)	8 485(18)	6 610(4)
C(17a)	9 747(5)	7 791(19)	6 495(5)
C(18a)	5 947(5)	9 408(21)	5 111(5)
C(19a)	5 732(5)	6 384(23)	5 697(5)
C(20a)	6 911(5)	6 924(20)	6 833(5)
Water molecule			
O(4)	5 124(5)	8 212(20)	7 162(5)

The mixture was cooled to 0 °C and then the 18-aldehyde **11** (345 mg) in dry THF (10 cm^3) was added. The mixture was stirred for 30 min at 0 °C and then for a further 1 h at room temperature. Dil. hydrochloric acid (10 cm^3) was cautiously added. After 10 min the THF was evaporated under reduced pressure and the residue was extracted with ethyl acetate. The extract was washed with aqueous sodium hydrogen carbonate and brine and dried. The solvent was evaporated to give [18-^{2}H]-aphidicolin **1** (310 mg), m.p. 226–230 °C (lit.,[6] 227–233 °C). A portion (55 mg) was heated with acetone (40 cm^3) containing toluene-*p*-sulfonic acid (5 mg) for 20 min. The toluene-*p*-sulfonic acid was neutralized with sodium hydrogen carbonate and the bis acetonide **16** was recovered. It possessed ^{1}H NMR signals at δ_H 0.72 (3 H, s, 19-H), 0.98 (3 H, s, 20-H), 1.34 (3 H, s), 1.41 [9 H, s, $O_2C(Me)_2$], 2.59 (1 H, dd, *J* 3 and 13, 5-H), 3.54 and 3.75 (each 1 H, d, *J* 8.3, 17-H), 3.61 (0.8 H, s, 18-H) and 3.63 (1 H, m, 3-H). The (18*S*)-epimer was present to the extent of 20% (δ_H 3.21, s, 18-H). The material was treated with toluene-*p*-sulfonic acid (20 mg) in aqueous methanol (50 cm^3) overnight to regenerate [18-^{2}H]aphidicolin.

Preparation of (18S)-[18-^{2}H]-3α,18;16β,17-*Diepoxyaphidicolane* **17**.—Toluene-*p*-sulfonyl chloride (435 mg) was added to 18-^{2}H-aphidicolin (310 mg) in pyridine (20 cm^3) and the mixture was kept at room temperature for 48 h. The mixture was poured into dil. hydrochloric acid and the product extracted with ethyl acetate. The extract was washed with aqueous sodium hydrogen carbonate and brine and dried. The solvent was evaporated to give a gum (505 mg) which was dissolved in DMSO (10 cm^3). Sodium hydrogen carbonate (2 g) was added and the mixture was stirred at 75 °C for 24 h. The mixture was poured into water and the product was extracted with ethyl acetate. The extract was washed with water and brine and dried. The solvent was evaporated to give a residue which was chromatographed on silica. Elution with ethyl acetate–light petroleum (1:4) gave (18*S*)-[18-^{2}H]-3α,18;16β,17-diepoxyaphidicolane **17**, m.p. 216–218 °C, δ_H 0.85 (3 H, s, 19-H), 1.05 (3 H, s, 20-H), 2.6 (2 H, br s, 17-H), 4.25 (0.8 H, s, 18-H) and 4.7 (1 H, m, 3-H) containing 20% of the (18*R*)-epimer (δ_H 3.85, 18-H).

Incubation Experiments.—Cephalosporium aphidicola (IMI 68689) was grown on a medium comprising (per dm^3): glucose (50 g), potassium dihydrogen phosphate (5 g), magnesium sulfate (2 g), potassium chloride (1 g), glycine (2 g) and trace elements solution (2 cm^3). The trace elements solution contained (per dm^3): cobalt nitrate (1 g), iron(II) sulfate (1 g), copper sulfate (0.15 g), zinc sulfate (1.61 g), manganese sulfate (0.1 g) and ammonium molybdate (0.1 g). Chlorocholine chloride (CCC) (70 mg dm^{-3}) was added 4 d after inoculation.

(*a*) *Incubation of* 3α,18-*epoxy*-16β-*hydroxyaphidicolane* **3**. The oxetane **3** (350 mg) in DMSO (70 cm^3) containing Tween 80 (5 drops) was evenly distributed between ten Thompson bottles (7.5 dm^3) of *C. aphidicola* 8 d after inoculation. The culture was filtered on the 28th day and the filtrate was extracted with ethyl acetate. Analysis by TLC revealed a polar component that was not present in the control fermentation. The extract was dried and the solvent evaporated. The residue was chromatographed on silica. Elution with ethyl acetate–light petroleum (4:1) gave 3α,16β,17-trihydroxyaphidicolan-18-al **11** (105 mg) as a gum, *m/z* (CI, NH_3) 354 ($M^+ + NH_4$), 336 (M^+) and 324 (354 − CH_2O); ν_{max}/cm^{-1} 3415 and 1720; δ_H 1.01 and 1.05 (each 3 H, s, 19- and 20-H), 2.71 (1 H, dd, *J* 3.1 and 12, 5-H), 3.38 and 3.45 (each 1 H, d, *J* 11, 17-H), 3.77 (1 H, br s, w_{v2} 7, 3-H) and 9.55 (1 H, s, 18-H).

(*b*) *Incubation of* 3α,18;16β,17-*diepoxyaphidicolane* **4**. The oxetane **4** (905 mg) in DMSO (48 cm^3) and Tween 80 (5 drops) was evenly distributed between twelve Thompson bottles (9 dm^3) of *C. aphidicola*, 11 d after inoculation. The fermentation was grown for 29 d. The culture was filtered and the filtrate was extracted with ethyl acetate. Analysis by TLC revealed the presence of two polar compounds that were not present in the control fermentation. The extract was dried and the solvent was evaporated. The residue was chromatographed on silica. Elution with ethyl acetate–light petroleum (4:1) gave 3α,12α,17-*trihydroxy*-12(13→16)-abeo-*aphidicolan*-18-*al* **12** (38 mg) which crystallized from methanol–ethyl acetate as needles, m.p. 143–145 °C (Found: C, 69.5; H, 9.7. $C_{20}H_{32}O_4 \cdot 0.5H_2O$ requires C, 69.5; H, 9.6%); ν_{max}/cm^{-1} 3515, 3380 and 1716; $\delta_H(C_5D_5N)$ 0.92 (3 H, s, 20-H), 1.07 (3 H, s, 19-H), 2.96 (1 H, dd, *J* 2.8 and 12, 5-H), 3.71 and 3.80 (each 1 H, d, *J* 10, 17-H), 3.96 (1 H, br s, w_{112} 6, 3-H), 4.26 (1 H, d, *J* 8.8, 12-H) and 9.90 (1 H, s, 18-H). Irradiation at δ_H 9.9 gave an NOE of 8% at δ_H 2.96; irradiation at δ_H 4.26 gave an NOE of 6% at 1.91 and 3% at 1.71; irradiation

Table 3 Intramolecular distances (Å) and angles (°) with estimated standard deviations in parentheses, for the two independent moleucles (1) and (2) of compound **6**

	(1)	(2)		(1)	(2)
(*a*) Bonds					
O(1)–C(3)	1.47(2)	1.48(2)	O(1)–C(18)	1.46(2)	1.44(2)
O(2)–C(16)	1.414(14)	1.433(14)	O(3)–C(17)	1.397(14)	1.399(12)
C(1)–C(2)	1.53(2)	1.52(2)	C(1)–C(10)	1.53(2)	1.50(2)
C(2)–C(3)	1.48(2)	1.48(2)	C(3)–C(4)	1.57(2)	1.57(2)
C(4)–C(5)	1.55(2)	1.571(14)	C(4)–C(18)	1.56(2)	1.54(2)
C(4)–C(19)	1.53(2)	1.47(2)	C(5)–C(6)	1.54(2)	1.54(2)
C(5)–C(10)	1.564(15)	1.547(14)	C(6)–C(7)	1.55(2)	1.52(2)
C(7)–C(8)	1.54(2)	1.512(14)	C(8)–C(9)	1.55(2)	1.52(2)
C(8)–C(13)	1.56(2)	1.565(14)	C(9)–C(10)	1.580(15)	1.577(15)
C(9)–C(11)	1.529(14)	1.577(14)	C(9)–C(14)	1.55(2)	1.56(2)
C(10)–C(20)	1.54(2)	1.53(2)	C(11)–C(12)	1.53(2)	1.55(2)
C(12)–C(13)	1.576(15)	1.52(2)	C(12)–C(16)	1.55(2)	1.51(2)
C(14)–C(15)	1.54(2)	1.501(15)	C(15)–C(16)	1.53(2)	1.533(14)
C(16)–C(17)	1.52(2)	1.48(2)			
(*b*) Angles					
C(3)–O(1)–C(18)	90(1)	90(1)	C(2)–C(1)–C(10)	111.1(9)	114(1)
C(1)–C(2)–C(3)	115(1)	114(1)	O(1)–C(3)–C(2)	115(1)	114(1)
O(1)–C(3)–C(4)	91(1)	88.8(9)	C(2)–C(3)–C(4)	119(1)	121(1)
C(3)–C(4)–C(5)	112.0(9)	110.8(8)	C(3)–C(4)–C(18)	83(1)	83.3(9)
C(3)–C(4)–C(19)	116(1)	117(1)	C(5)–C(4)–C(18)	108(1)	108.1(9)
C(5)–C(4)–C(19)	117(1)	117(1)	C(18)–C(4)–C(19)	116(1)	115.7(9)
C(4)–C(5)–C(6)	110.9(9)	110.4(8)	C(4)–C(5)–C(10)	114(1)	114.3(9)
C(6)–C(5)–C(10)	113.8(9)	117.2(9)	C(5)–C(6)–C(7)	113.9(9)	112.1(9)
C(6)–C(7)–C(8)	106(1)	108.9(9)	C(7)–C(8)–C(9)	112(1)	111.9(9)
C(7)–C(8)–C(13)	118(1)	119.9(9)	C(9)–C(8)–C(13)	106.1(8)	106.5(8)
C(8)–C(9)–C(10)	108.5(8)	110.7(9)	C(8)–C(9)–C(11)	99.0(9)	99.0(8)
C(8)–C(9)–C(14)	113(1)	114.6(9)	C(10)–C(9)–C(11)	119.5(9)	117.4(9)
C(10)–C(9)–C(14)	111.0(9)	111.0(9)	C(11)–C(9)–C(14)	105.7(8)	103.6(8)
C(1)–C(10)–C(5)	107.1(9)	108.3(9)	C(1)–C(10)–C(9)	110.3(9)	112.0(9)
C(1)–C(10)–C(20)	109(1)	108(1)	C(5)–C(10)–C(9)	106.6(9)	106.0(9)
C(5)–C(10)–C(20)	111.8(9)	112.7(9)	C(9)–C(10)–C(20)	111.5(9)	109.4(9)
C(9)–C(11)–C(12)	103.3(9)	99.3(8)	C(11)–C(12)–C(13)	102.4(8)	105.2(8)
C(11)–C(12)–C(16)	110(1)	112(1)	C(13)–C(12)–C(16)	109.9(9)	112.2(9)
C(8)–C(13)–C(12)	104.5(9)	103.7(9)	C(9)–C(14)–C(15)	117(1)	117(1)
C(14)–C(15)–C(16)	112(1)	114.5(9)	O(2)–C(16)–C(12)	109.8(9)	109.6(9)
C(2)–C(16)–C(15)	106(1)	106.6(9)	O(2)–C(16)–C(17)	109.6(9)	108.1(8)
C(12)–C(16)–C(15)	109.4(9)	107.2(8)	C(12)–C(16)–C(17)	112(1)	114(1)
C(15)–C(16)–C(17)	109(1)	111.1(9)	O(3)–C(17)–C(16)	115(1)	115.0(9)
C(1)–C(18)–C(4)	91.1(9)	91.9(9)			

Table 4 Fractional atomic coordinates ($\times 10^4$) with estimated standard deviations in parentheses for compound **13**

	x	*y*	*z*
O(1)	7874(7)	3803(4)	5584(2)
O(2)	1734(6)	4387(3)	1920(2)
O(3)	5117(5)	5460(3)	1796(2)
C(1)	6195(8)	6608(5)	3099(3)
C(2)	5785(8)	7234(5)	2430(2)
C(3)	4345(8)	6587(5)	2007(3)
C(4)	2492(7)	6370(4)	2391(2)
C(5)	2921(6)	5784(4)	3083(2)
C(6)	1099(8)	5430(5)	3470(3)
C(7)	1536(8)	4729(5)	4110(3)
C(8)	2938(8)	5430(5)	4528(2)
C(9)	4803(7)	5615(4)	4168(2)
C(10)	4402(7)	6431(4)	3436(2)
C(11)	5974(9)	6132(5)	4743(3)
C(12)	5552(9)	5256(5)	5327(3)
C(13)	3441(9)	4962(5)	5238(3)
C(14)	5838(8)	4493(5)	3965(2)
C(15)	6308(9)	3593(4)	4537(3)
C(16)	6709(9)	4187(5)	5199(2)
C(18)	1190(8)	5597(5)	1961(3)
C(19)	1428(9)	7552(5)	2421(3)
C(20)	3758(9)	7645(5)	3771(3)

at δ_H 1.07 gave an NOE of 6% at δ_H 3.96; irradiation at δ_H 0.92 gave an NOE of 12% at δ_H 1.91 and 9% at δ_H 1.71. Further elution gave 3α,16β,17-trihydroxyaphidicolan-18-al **11** (310 mg) identical (^{1}H NMR) to the sample described in (*a*).

(*c*) *Incubation of* (18S)-[18-^{2}H]-3α,18;16β,17-*diepoxyaphidicolane*. The oxetane **17** (with 20% of its 18-epimer) (80 mg) was dissolved in DMSO (7 cm^3) and mixed with CCC (40 mg) in ethanol (1 cm^3) and Tween 80 (1 drop). The solution was added to a Thompson bottle (750 cm^3) of *C. aphidicola* 5 d after inoculation. The culture was filtered on the 25th day of growth and the filtrate was extracted with ethyl acetate. The solvent was evaporated and the residue chromatographed on silica as above to give [18-^{2}H]3α,16β,17-trihydroxyaphidicolan-18-al (19 mg). Its ^{1}H NMR spectrum was identical with that of the unlabelled material except that the aldehyde resonance (δ_H 9.55) contained only 30% C–H; $\delta(^2H)$ (55.26 MHz) 9.61 (s, 18-^{2}H).

Crystal Structure Determinations.—A summary of the crystal data and structure refinement details are given in Table 1. In each case data were collected from a crystal mounted on an Enraf–Nonius CAD 4 diffractometer operating in the θ–2θ mode with $\Delta\theta = (0.8 + 0.35 \tan\theta)°$ and a maximum scan time of 1 min and with monochromated Mo-Kα radiation (λ = 0.710 69 Å). Unique reflections were measured for $2 < \theta < 25°$

Table 5 Intramolecular distances (Å) and angles (°) with estimated standard deviations in parentheses for compound **13**

(*a*) Bonds			
O(1)–C(16)	1.210(4)	O(2)–C(18)	1.438(5)
O(3)–C(3)	1.462(4)	C(1)–C(2)	1.538(5)
C(1)–C(10)	1.555(5)	C(2)–C(3)	1.517(5)
C(3)–C(4)	1.543(5)	C(4)–C(5)	1.560(4)
C(4)–C(18)	1.539(5)	C(4)–C(19)	1.550(5)
C(5)–C(6)	1.559(4)	C(5)–C(10)	1.570(4)
C(6)–C(7)	1.536(5)	C(7)–C(8)	1.524(5)
C(8)–C(9)	1.521(5)	C(8)–C(13)	1.551(5)
C(9)–C(10)	1.590(4)	C(9)–C(11)	1.533(5)
C(9)–C(14)	1.532(5)	C(10)–C(20)	1.533(5)
C(11)–C(12)	1.562(5)	C(12)–C(13)	1.547(6)
C(12)–C(16)	1.495(5)	C(14)–C(15)	1.569(5)
C(15)–C(16)	1.508(5)		
(*b*) Angles			
C(2)–C(1)–C(10)	112.8(3)	C(1)–C(2)–C(3)	112.4(3)
O(3)–C(3)–C(2)	109.6(3)	O(3)–C(3)–C(4)	108.7(3)
C(2)–C(3)–C(4)	112.3(3)	C(3)–C(4)–C(5)	109.8(3)
C(3)–C(4)–C(18)	109.3(3)	C(3)–C(4)–C(19)	107.2(3)
C(5)–C(4)–C(18)	111.2(3)	C(5)–C(4)–C(19)	115.8(3)
C(18)–C(4)–C(19)	103.2(3)	C(4)–C(5)–C(6)	112.6(3)
C(4)–C(5)–C(10)	115.7(3)	C(6)–C(5)–C(10)	113.4(3)
C(5)–C(6)–C(7)	112.1(3)	C(6)–C(7)–C(8)	108.1(3)
C(7)–C(8)–C(9)	112.7(3)	C(7)–C(8)–C(13)	117.7(3)
C(9)–C(8)–C(13)	106.0(3)	C(8)–C(9)–C(10)	107.3(3)
C(8)–C(9)–C(11)	100.1(3)	C(8)–C(9)–C(13)	115.1(3)
C(10)–C(9)–C(11)	117.4(3)	C(10)–C(9)–C(14)	111.7(3)
C(11)–C(9)–C(14)	104.9(3)	C(1)–C(10)–C(5)	106.9(2)
C(1)–C(10)–C(9)	111.8(3)	C(1)–C(10)–C(20)	107.3(3)
C(5)–C(10)–C(9)	107.2(2)	C(5)–C(10)–C(20)	113.6(3)
C(9)–C(10)–C(20)	110.1(3)	C(9)–C(11)–C(12)	101.8(3)
C(11)–C(12)–C(13)	103.9(3)	C(11)–C(12)–C(16)	106.9(3)
C(13)–C(12)–C(16)	109.6(3)	C(8)–C(13)–C(12)	104.7(3)
C(9)–C(14)–C(15)	117.4(3)	C(14)–C(15)–C(16)	112.1(3)
O(1)–C(16)–C(12)	124.4(3)	O(1)–C(16)–C(15)	121.2(4)
C(12)–C(16)–C(15)	114.4(3)	O(2)–C(18)–C(4)	115.0(3)

and those reflections with $|F^2| > \sigma(F^2)$ were used in the refinement where $\sigma(F^2) = \{\sigma^2(I) + (0.04\ I)^2\}^{\frac{1}{2}}/L_p$. Structures were solved by direct methods using MULTAN.[12] Refinement was by full matrix least squares with non-hydrogen atoms anisotropic and weights of $w = 1/\sigma^2(F)$. Hydrogen atoms were included at calculated positions and held fixed. All calculations were done on a PDP 11/34 using the Enraf–Nonius SDP-Plus program package. Tables of fractional atomic coordinates and bond lengths and angles are given in Tables 2 and 3 (compound **6**), and Tables 4 and 5 (compound **13**). Hydrogen atom coordinates and thermal parameters are available from the Cambridge Crystallographic Data Centre.*

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* For full details of the CCDC Deposition Scheme see 'Instructions for Authors,' *J. Chem. Soc., Perkin Trans. 1*, 1992, Issue 1.

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