Stereochemistry of Some Reactions of Ring D of the Diterpenoid Aphidicolin

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The stereochemistry of a number of reactions at C-16 in the diterpenoid, aphidicolin, has been examined, and, unlike simpler bicyclo[3.2.1]octane derivatives, there is only a limited degree of stereospecificity. The 15-enes can be prepared by dehydration of the epimeric 16-alcohols. Epoxidation and catalytic reduction have been shown to occur from the β-(*exo*)-face of the bicyclo[3.2.1]octene system that constitutes rings C and D of the aphidicol-15-enes. The stereochemistry or aphidicolane-3α,16α,18-triol has been established by X-ray crystallography.

Aphidicolin **1** is a metabolite of *Cephalosporium aphidicola*[1] and it is of interest as a specific inhibitor of DNA polymerase α.[2–5] In the course of our studies on the biotransformation of relatives of intermediates in aphidicolin biosynthesis, we have examined the stereochemistry of some reactions of ring D. A problem which has been encountered in several of the total syntheses[6,7] has been the construction of the aphidicolin (16β-hydroxy) stereochemistry at C-16. Knowledge of the stereochemistry of reactions on this ring is also of value in the preparation of compounds for structure–activity studies. Although rings C and D of aphidicolin are part of a bicyclo[3.2.1]octane system, the spiranic centre at C-9 of aphidicolin generates strong interactions between 1α-H and 14α-H, 5-H and 14β-H, and 1β-H and 11α-H, which might in turn lead to some distortions of rings C and D. Interestingly the position of the 5-H resonance, which appears as a double-doublet, *J* 3.2 and 12 Hz in the δ 2.80 region, reflects variations in the substituents on ring D.

Attack from the '*exo*' face of bicyclo[3.2.1]octan-2-ones and the related exocyclic 2-enes is a dominant feature of their chemistry.[8,9] The corresponding 17-nor 16-ketone **4** can readily be obtained by the periodate cleavage of aphidicolin **1**.[1] Treatment of this ketone with methyllithium in ether–tetrahydrofuran (THF) gave aphidicolan-3α,16α,18-triol **6**. The stereochemistry of this triol was established by X-ray crystallography (see Fig. 1). As expected attack has taken place from the '*exo*' face of the molecule leading to a stereochemistry at C-16 which is epimeric to that of the natural aphidicolin series at this centre.

Reaction of bicyclo[3.2.1]octan-2-ones with methylmagnesium iodide has been shown[8] to give a 2:1 mixture of the '*exo*' and '*endo*' adducts. However Hiranuma *et al.*, in their study on the biological activity of aphidicolin derivatives, have reported[3] that the 3α,18-acetonide **5** of aphidicolin 17-nor 16-ketone gave only the '*endo*' (natural) stereochemistry at C-16 on reaction with methyl magnesium iodide. In our hands the reaction gave a 2:1 mixture of '*exo*' and '*endo*' adducts. The two isomers may be distinguished by their ^{1}H NMR spectra. In deuteriopyridine solution, the 17-H signal of **6** is at δ 1.44 whereas in **3** it is at δ 1.28. A signal at δ 1.94, assigned to 11α-H in **6** moves downfield to δ 2.33 in **3** due to a 1:3-diaxial interaction with the hydroxy group at C-16 and may be diagnostic of the natural stereochemistry at that centre. The authentic '*endo*' derivative **3** may be prepared by reduction of the 17-monotoluene-*p*-sulfonate **2** of aphidicolin with lithium aluminium hydride. Examination of the ^{1}H NMR data reported[3] by Hiranuma *et al.* suggests that they had isolated the '*exo*' derivative and not the '*endo*' derivative which they had claimed.

Reduction of the 17-nor 16-ketone **5** with sodium borohydride in methanol gave a 1:1 mixture of the 16α- and 16β-alcohols which could not be separated. However reduction with sodium in isopropyl alcohol was stereospecific yielding the 16β-alcohol **7**. The multiplicity of the 16-H signal (δ 3.70, ddd, *J* 2.9, 5.9 and 9.7 Hz) indicated that the 16-hydroxy group was an equatorial (β) substituent on the six-membered ring. In the α-epimer the C*H*(OH) signal (δ 3.64) was a narrow mulitplet.

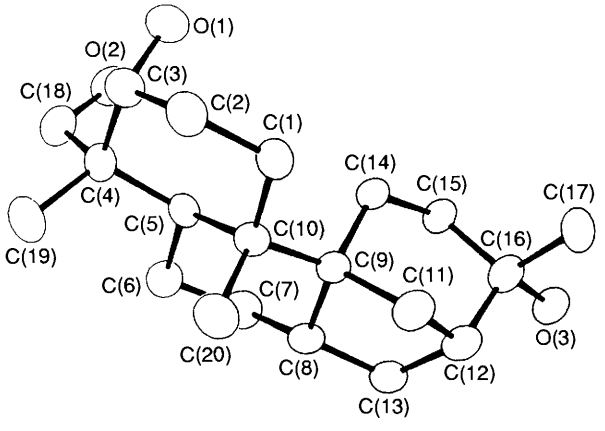
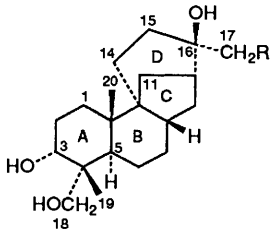


Fig. 1 X-Ray molecular structure for compound **6**

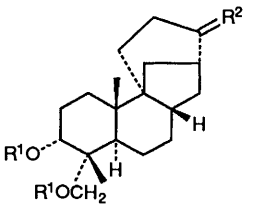
Aphidicol-16-enes may be prepared[10] by the Wittig reaction on the 17-noraphidicol-16-ones. Hydroboronation of the exocyclic methylenes of β-cedrene **19**[11] and α-fenchene **21**[12] is reported to be virtually stereospecific. However hydroboronation of the exocyclic alkene, aphidicol-16-ene-3α,18-diol **8** gave a mixture of the 16-epimeric 17-alcohols **9** and **10** (1:1). Treatment of the mixed toluene-*p*-sulfonates **11** and **12** with lithium aluminium hydride followed by hydrolysis of the acetonide with acid, gave a 1:1 mixture of the 16-epimeric aphidicolane-3α,18-diols **13** and **14**. Although it was not possible to separate the isomers, they may be distinguished by their ^{1}H NMR spectra. In deuteriochloroform solution the 17-H of **14** appears at δ 0.74 whilst in **13** it is at δ 0.91. The 16-*S* isomer **14** has been obtained pure by hydrogenation of a 15-ene (*vide infra*).

We turned our attention to the addition reactions of the Δ^{15}-ene. There is a formal similarity between this portion of the molecule and the sesquiterpene, α-cedrene **20**. Many of the addition reactions of α-cedrene are stereospecific and proceed from the *exo*-face.[13,14] Such additions in the aphidicolin series would generate substituents with a 16-configuration typical of the natural substrate.

Aphidicol-15-ene-3α,17,18-triol **22** has been prepared[3] by dehydration of the 3α,17,18-triacetate of aphidicolin with



1; R = OH
2; R = OTs
3; R = H

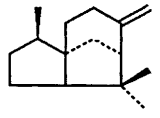


4; R^1 = H, R^2 = O

5; R^1R^1 = CMe_2, R^2 = O

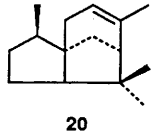
6; R^1 = H, R^2 = α-OH, β-Me

7; R^1R^1 = CMe_2, R^2 = α-H, β-OH



19

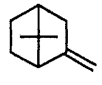
8; R^1 = H, R^2 = CH_2
9; R^1 = H, R^2 = α-H, β-CH_2OH
10; R^1 = H, R^2 = α-CH_2OH, β-H



20

11; R^1R^1 = CMe_2, R^2 = α-H, β-CH_2OTs

12; R^1R^1 = CMe_2, R^2 = α-CH_2OTs, β-H



21

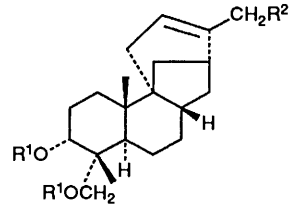
13; R^1 = H, R^2 = α-H, β-Me
14; R^1 = H, R^2 = α-Me, β-H

15; R^1R^1 = CMe_2, R^2 = α-Me, β-OH

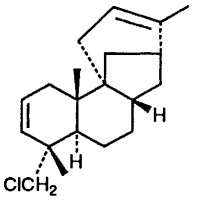
16; R^1 = Ac, R^2 = α-Me, β-OH

17; R^1R^1 = CMe_2, R^2 = α-OH, β-Me

18; R^1 = H, R^2 = 



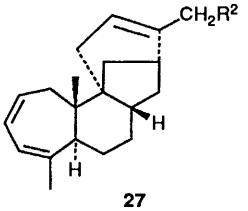
22; R^1 = H, R^2 = OH



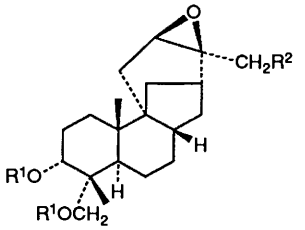
26

23; R^1R^1 = CMe_2, R^2 = H

24; R^1 = Ac, R^2 = H
25; R^1 = R^2 = H



27



28; R^1R^1 = CMe_2, R^2 = H

29; R^1 = R^2 = H
30; R^1 = H, R^2 = OH

methanesulphonyl chloride in pyridine followed by hydrolysis of the triacetate. We utilized this method for the preparation not only of this alkene but also for the dehydration of the 3a,18-acetonide **15** obtained from the 3a,16β,18-triol.[10] However on several occasions the ^{1}H NMR spectrum of the reaction mixture showed that the required product was accompanied by some of the isomeric 16-ene. An alternative dehydration utilized triphenylphosphine–carbon tetrachloride in pyridine. Dehydration of both the 3α,18-acetonide **15** and the 3α,18-diacetate **16** with this reagent gave the corresponding Δ^{15}-enes **23** and **24** (δ 5.03, 15-H; 1.66, 17-H) which were in turn hydrolysed to aphidicol-15-ene-3α,18-diol **25**. This dehydration reaction could also be applied to the 3α,18-acetonide **17** of aphidicolane-3α,16α,18-triol to afford the 15-ene **23**. Hence it is possible to form the 15-ene from both epimeric alcohols at C-16. This feature may be useful in the light of the lack of stereospecificity in some of the addition reactions at C-16.

On one occasion dehydration of the acetonide **17** with triphenylphosphine–carbon tetrachloride led not only to the 15-ene **23** but also to the chlorodiene **26** and the A-homo triene **27**. The structure of the latter followed from its UV spectrum (λ_{max}/nm 247) and ^{1}H NMR spectrum which showed the ring A alkene signals at δ 5.77, 5.88 and 6.01 together with the olefinic *C*-methyl resonance at δ 1.82. This is similar to the data observed for the analogous 17-nor ketone.[14] These by-products presumably arose through hydrolysis of the acetonide and displacement of the corresponding alcohols. A low yield of the 15-ene **22** was obtained on treatment of 16β,17-epoxyaphidicolane-3α,18-diol **18** with lithium diethylamide.

Epoxidation of the acetonide **23** of aphidicol-15-ene-3α,18-diol with *m*-chloroperbenzoic acid (MCPBA) in chloroform gave a single 15,16-epoxide [δ 2.83 (15-H), 1.27 (17-H)]. The stereochemistry of the epoxide **28** was established by reduction with lithium aluminium hydride followed by hydrolysis of the acetonide. This gave the known 3α,16β,18-triol **3**.[10] Thus epoxidation has taken place from the anticipated '*exo*' face of the molecule. Epoxidation of α-cedrene takes place exclusively from the '*exo*' face.[11] Epoxidation of aphidicol-15-ene-3α,18-diol **25** and -3α,17,18-triol **22**, with MCPBA also gave single epoxides which were assigned the 15β,16β-stereochemistry **29** and **30**.

Catalytic reduction of the 15-ene **25** over 10% palladium-on-charcoal also gave a single dihydro compound (δ 0.74, 3 H, doublet, *J* 6.6 Hz, 17-H_3). Decoupling experiments located the 16-H resonance at δ 1.51 and showed that it possessed couplings of 2, 6.5 and 11 Hz. The presence of the large (11 Hz) diaxial coupling showed that the 16-H must be an axial hydrogen and hence hydrogenation of the 15-ene had generated the aphidicolane stereochemistry **14** at C-16.

In conclusion we have shown that the addition reactions of aphidicol-15-enes occur from the β-(*exo*)-face of the molecule. This should be useful in the preparation of compounds with the aphidicolin stereochemistry at C-16 for biological activity studies. A similar result has recently been reported[7] in studies directed at total syntheses in this series.

Experimental

General Details.—M.p.s were determined on a Kofler hot stage apparatus and are uncorrected. IR spectra were recorded for Nujol mulls on a Perkin-Elmer 1710 spectrometer. ^{1}H NMR spectra were obtained at 360 MHz on a Bruker WM 360 spectrometer for solutions in deuteriochloroform, *J* values are given in Hz. Light petroleum refers to the fraction b.p. 60–80 °C. Chromatography was carried out on Merck 9385 silica. Extracts were dried over sodium sulphate.

16-*Epiaphidicolane*-3α,16α,18-*triol.*—Methyllithium (5 cm^3; 1.4 mol dm^{-3} solution in ether) was added to a stirred solution of 3α,18-dihydroxy-17-noraphidicol-16-one (400 mg)[1] in dry THF (5 cm^3) at 0 °C under nitrogen. After 1.5 h, saturated aqueous ammonium chloride was added and the mixture was acidified with dil. hydrochloric acid. The product was recovered

in ethyl acetate and the extract was washed with aqueous sodium hydrogen carbonate, brine and dried. The solvent was evaporated to give the *title compound* **6** (325 mg) which crystallized from ethyl acetate as needles, m.p. 225–227 °C (Found: C, 74.3; H, 10.7. $C_{20}H_{34}O_3$ requires C, 74.5; H, 10.6%), ν_{max}/cm^{-1} 3395, 3350 and 1060; $\delta(CDCl_3)$ 0.70, 0.97 and 1.28 (each 3 H, s, 19-H_3, 20-H_3 and 17-H_3), 3.39 and 3.50 (each 1 H, d, *J* 12, 18-H_2) and 3.69 (1 H, br s, 3-H); $\delta(C_5D_5N)$ 0.77, 1.00 and 1.44 (each 3 H, s), 2.80 (1 H, dd, *J* 3.2 and 12, 5-H), 3.63 and 3.80 (each 1 H, d, *J* 11, 18-H_2) and 3.92 (1 H, s, 3-H).

Reaction of 3α,18-*Isopropylidenedioxy*-17-*noraphidicol*-16-*one with Methyl Magnesium Iodide*.—Methyl magnesium iodide (0.13 cm^3 of a 3 mol dm^{-3} solution) was added to a stirred solution of the ketone (90 mg) in THF (5 cm^3) at room temperature. After 1.5 h, aqueous ammonium chloride was added and the mixture was extracted with ethyl acetate. The extract was washed with water, brine and dried. The solvent was evaporated to give a gum which was taken up in methanol (50 cm^3) containing water (10 cm^3) and toluene-*p*-sulfonic acid (5 mg). After 2 d at room temp., the solvent was evaporated and the product was recovered in ethyl acetate. The extract was washed with aqueous sodium hydrogen carbonate, brine and dried. The solvent was evaporated to give a mixture (2:1) of aphidicolane-3α,16α,18- and -3α,16β,18-triols $\delta(C_5D_5N)$ (compound **6**) 1.44 (17-H_3) and 2.80 (5-H); (compound **3**) 1.28 (17-H_3) and 2.87 (5-H).

Reduction of the Ketone **5** *with Sodium in Isopropyl Alcohol*.—A solution of 3α,18-isopropylidenedioxy-17-noraphidicol-16-one **5**[1] (400 mg) in isopropyl alcohol (2 cm^3) was added rapidly to a suspension of sodium (250 mg) in toluene (6 cm^3). The mixture was heated under reflux for 3 h and then cooled and poured into water. The products were recovered in ethyl acetate. The extract was washed with dil. hydrochloric acid, aqueous sodium hydrogen carbonate and water, and dried. The solvent was evaporated and the residue crystallized from ethyl acetate–light petroleum to afford 3α,18-*isopropylidenedioxy*-17-*noraphidicolan*-16β-*ol* **7** (370 mg) as needles, m.p. 133–135 °C (Found: C, 74.5; H, 10.45. $C_{22}H_{36}O_3 \cdot 0.5H_2O$ requires C, 73.9; H, 10.4%), ν_{max}/cm^{-1} 3440; δ 0.73 (3 H, s, 19-H), 0.98 (3 H, s, 20-H), 1.42 (6 H, s, acetonide Me), 2.57 (1 H, dd, *J* 13 and 3.2, 5-H), 3.23 and 3.64 (each 1 H, d, *J* 12, 18-H), 3.63 (1 H, m, 3-H) and 3.70 (1 H, ddd, *J* 2.9, 5.9 and 9.7, 16-H). The 16β-*acetate*, prepared with acetic anhydride in pyridine, crystallized from ethyl acetate–methanol as plates, m.p. 127–129 °C (Found: C, 73.75; H, 9.85. $C_{24}H_{38}O_4$ requires C, 73.8; H, 9.8%), ν_{max}/cm^{-1} 1737; δ 0.64 (3 H, s, 19-H), 0.89 (3 H, s, 20-H), 1.32 (6 H, s, acetonide Me), 2.48 (1 H, dd, *J* 3 and 10, 5-H), 3.14 and 3.54 (each 1 H, d, *J* 12, 18-H), 3.54 (1 H, m, 3-H) and 4.64 (1 H, m, 16-H).

Reduction of the Ketone **5** *with Sodium Borohydride*.—The ketone (300 mg) in methanol (30 cm^3) was treated with sodium borohydride (150 mg) at 0 °C for 2 h. The excess of reagent was destroyed with acetic acid and the methanol was evaporated. The residue was extracted with ethyl acetate, the extract was washed with aqueous sodium hydrogen carbonate, brine and dried. The solvent was evaporated to give a gum which was shown by ^1H NMR spectroscopy to be a 1:1 mixture of 16-epimers. It could not be separated by chromatography on silica.

Hydroboronation of Aphidicol-16-*ene*-3α,18-*diol* **8**.—Borane (2.5 cm^3 of a 1 mol dm^{-3} solution in THF) was added to the alkene (600 mg) in THF (10 cm^3) and the mixture was stirred at room temp. for 3 h. Water (2 cm^3) followed by 30% aqueous hydrogen peroxide (8 cm^3) and sodium hydroxide (2 mol dm^{-3}; 8 cm^3) were then added and the mixture was stirred at room temperature overnight. Aqueous sodium sulfite was added and the mixture was 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 mixture of aphidicolane-3α,17,18-triol and 16-epi-aphidicolane-3α,17,18-triol (570 mg), m.p. 186–198 °C (Found: C, 74.0; H, 10.9. $C_{20}H_{34}O_3$ requires C, 74.5; H, 10.6%), ν_{max}/cm^{-1} 3280 $\delta(C_5D_5N)$ 0.725, 0.73 (3 H, s, 19-H_3), 0.93, 0.935 (3 H, s, 20-H_3), 2.59 and 2.73 (1 H, dd, *J* 13 and 3, 5-H), 3.55 (2 H, m, 17-H_2), 3.72 (2 H, m, 18-H) and 3.86 (1 H, m, 3-H). The isomer ratio was 1:1. The mixture could not be separated by chromatography on silica.

Conversion to Aphidicolane-3α,18-*diol and its* 16-*Epimer*.—The above mixture of triols (420 mg) in acetone (20 cm^3) and toluene-*p*-sulfonic acid (5 mg) was heated gently for 10 min. Pyridine (1 cm^3) was added and the solution was dried over sodium sulfate. The solvent was evaporated and the residue was dissolved in pyridine (10 cm^3). Toluene-*p*-sulfonyl chloride (554 mg) was added and the mixture was stirred overnight. The mixture was poured into dil. hydrochloric acid and the product was recovered in ethyl acetate. The extract was washed with aqueous sodium hydrogen carbonate and water, and dried. The solvent was evaporated to give a mixture of toluene-*p*-sulfonates (730 mg). The mixture of toluene-*p*-sulfonates (590 mg) in THF (10 cm^3) was treated with lithium hydride (450 mg) in THF (50 cm^3) under reflux for 30 min. The mixture was cooled, treated with ethyl acetate, water and dil. hydrochloric acid, and stirred at room temperature for 30 min. The THF was evaporated and the residue was extracted with ethyl acetate. The extract was washed with aqueous sodium hydrogen carbonate, brine and dried. The solvent was evaporated and the residue was taken up in methanol (200 cm^3). Water (25 cm^3) and toluene-*p*-sulfonic acid (60 mg) were added and the mixture was left at room temperature for 48 h. The methanol was evaporated and the residue was extracted with ethyl acetate. The extract was washed with aqueous sodium hydrogen carbonate, brine and dried. The solvent was evaporated to give a solid which was chromatographed on silica. Elution with ethyl acetate–light petroleum (3:7) gave aphidicolane-3α,18-diol **14** and 16-epi-aphidicolane-3α,18-diol **13** (305 mg), m.p. 128–155 °C (Found: C, 78.2; H, 11.2. Calc. for $C_{20}H_{34}O_2$, C, 78.4; H, 11.2%), ν_{max}/cm^{-1} 3275; δ (compound **14**) 0.69 (3 H, s, 19-H), 0.74 (4 H, d, *J* 6.6, 17-H), 0.95 (3 H, s, 20-H), 2.40 (1 H, dd, *J* 3.2 and 12.6, 5-H), 3.4 and 3.45 (overlapping signals, 18-H), 3.65 (overlapping signals, 3-H); δ(16-epimer **13**), 0.70 (3 H, s, 19-H), 0.91 (3 H, d, *J* 7, 17-H), 0.96 (3 H, s, 20-H), 2.31 (1 H, dd, *J* 3 and 12.5, 5-H), 3.4 and 3.45 (overlapping signals, 18-H) and 3.65 (overlapping signals, 3-H). Ratio of natural:16-epimer, 45:55.

Preparation of Aphidicol-15-*ene*-3α,17,18-*triol* **22**.—Methanesulfonyl chloride (2.5 cm^3) was added to 3α,17,18-triacetoxy-aphidicolan-16β-ol (1.52 g)[1] in pyridine (50 cm^3) and the mixture left to stand at room temperature for 24 h. The mixture was poured into dil. hydrochloric acid and ice and the product extracted with ethyl acetate. The extract was washed with dil. hydrochloric acid, aqueous sodium hydrogen carbonate and brine and dried over sodium sulfate. Evaporation of the solvent gave a gum (1.41 g) δ(90 MHz; $CDCl_3$) 1.0, 1.5 (each 3 H, s, 19- and 20-H), 1.95 (9 H, s, OAc), 3.7 and 4.0 (each 1 H, d, *J* 10, 18-H), 4.45 (2 H, s, 17-H), 4.8 (1 H, br s, 5-H) and 5.4 (1 H, br s, 15-H). The gum was dissolved in methanol (200 cm^3) and aqueous potassium hydroxide (1 mol dm^{-3}; 40 cm^3) was added. The mixture was kept at room temperature for 2 d. The methanol was removed under reduced pressure and the residue extracted with ethyl acetate. The extract was washed with dil. hydrochloric acid, aqueous sodium hydrogen carbonate and brine, and dried over sodium sulfate. The solvent was evapor-

ated and the residue chromatographed on silica. Elution with ethyl acetate–light petroleum (4:1) gave aphidicol-15-ene-3α,17,18-triol (450 mg) which crystallized from ethyl acetate as needles, m.p. 196–198 °C (lit.,[3] 199–201 °C) (Found: C, 74.5; H, 9.9. Calc. for $C_{20}H_{32}O_3$. C, 74.95; H, 10.05%), ν_{max}/cm^{-1} 3300, 1045 and 1020; δ(CD_3OD) 0.70 (3 H, s, 19-H_3), 1.06 (3 H, s, 20-H_3), 3.28 and 3.48 (each 1 H, d, *J* 11, 18-H_2), 3.62 (1 H, t, *J* 3, 3-H), 3.91 (2 H, s, 17-H_2) and 5.28 (1 H, m, 15-H).

3α,18-*Isopropylidenedioxyaphidicolan*-16-*ol* **15**.—Aphidicolane-3α,16,18-triol (600 mg) in acetone (50 cm^3) containing toluene-*p*-sulfonic acid (5 mg) was heated under reflux for 15 min. The solvent was evaporated and the product recovered in ethyl acetate and washed with aqueous sodium hydrogen carbonate and brine. The extract was dried and the solvent evaporated to give a gum which was chromatographed on silica in ethyl acetate–light petroleum (1:1) to give the *title compound* (580 mg), m.p. 188–190 °C (Found: C, 76.2; H, 10.5. $C_{23}H_{38}O_3$ requires C, 76.2; H, 10.6%), ν_{max}/cm^{-1} 3485 and 1090; δ($CDCl_3$) 0.73 (3 H, s, 19-H_3), 0.98 (3 H, s, 20-H_3), 1.13 (3 H, s, 17-H_3), 1.41 (6 H, s, acetonide Me), 3.23 and 3.64 (each 1 H, d, *J* 12, 18-H_2) and 3.63 (1 H, br s, 3-H).

3α,18-*Isopropylidenedioxyaphidicol*-15-*ene* **23**.—(*a*) Methanesulfonyl chloride (2.5 cm^3) was added to a solution of 3α,18-isopropylidenedioxyaphidicolan-16-ol (1.5 g) in dry pyridine (20 cm^3). The mixture was left at room temperature for 48 h. 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 over sodium sulfate. The solvent was evaporated to give a residue which was purified by chromatography on silica in ethyl acetate–light petroleum (1:4) to give a solid (1.25 g). ^1H NMR spectroscopy indicated that this was a 3:1 mixture of the 15-ene (δ 5.03, 15-H) and the 16-ene (δ 4.45, m, 17-H_2). Repeated recrystallization from ethyl acetate gave 3α,18-*isopropylidenedioxyaphidicol*-15-*ene* (300 mg), m.p. 118–120 °C (Found: C, 79.8; H, 10.6. $C_{23}H_{36}O_2$ requires C, 80.2; H, 10.5%) ν_{max}/cm^{-1} 1095 and 1195; δ($CDCl_3$) 0.72 (3 H, s, 19-H_3), 1.06 (3 H, s, 20-H_3), 1.42 and 1.45 (each 3 H, s, acetonide Me), 1.66 (3 H, s, 17-H_3), 3.25 and 3.62 (each 1 H, d, *J* 12, 18-H_2), 3.65 (1 H, t, *J* 2.9, 3-H) and 5.03 (1 H, m, 15-H).

(*b*) The 16-alcohol (225 mg) was dissolved in acetonitrile (2 cm^3) and carbon tetrachloride (1 cm^3). Triphenylphosphine (480 mg) and pyridine (0.2 cm^3) were added and the solution was stirred under nitrogen at 75 °C for 12 h. The mixture was cooled and the solvents were evaporated under reduced pressure. The residue was chromatographed on silica to give the above alkene (150 mg).

Aphidicol-15-*ene*-3α,18-*diol* **25**.—The above acetonide **23** (300 mg) was dissolved in methanol (40 cm^3). Water (5 cm^3) and toluene-*p*-sulfonic acid (15 mg) were added and the mixture was stirred at room temperature for 3 d. The methanol was evaporated under reduced pressure and the product recovered in ethyl acetate. The extract was washed with aqueous sodium hydrogen carbonate, brine and dried over sodium sulfate. The solvent was evaporated to give a gum which crystallized from ethyl acetate to afford *aphidicol*-15-*ene*-3α,18-*diol* (245 mg), m.p. 161–162 °C (Found: C, 78.8; H, 10.6. $C_{20}H_{32}O_2$ requires C, 78.9; H, 10.6%); ν_{max}/cm^{-1} 3240, 1060 and 1040; δ($CDCl_3$) 0.70 (3 H, s, 19-H_3), 1.04 (3 H, s, 20-H_3), 1.65 (3 H, s, 17-H_3), 3.36 and 3.50 (each 1 H, d, *J* 11, 18-H_2), 3.69 (1 H, m, 3-H) and 5.01 (1 H, br s, 15-H).

3α,18-*Diacetoxyaphidicolane*-16β-*ol* **16**.—Aphidicolane-3α,-16β,18-triol (300 mg) in pyridine (2 cm^3) and acetic anhydride (2 cm^3) was left at room temp. for 24 h. Water (5 cm^3) was added to the mixture at 0 °C and the solution was left for 4 h. It was then poured into dil. hydrochloric acid and the product was recovered in ethyl acetate. The extract was washed with aqueous sodium hydrogen carbonate, brine and dried over sodium sulfate. The solvent was evaporated and the residue was chromatographed on silica in ethyl acetate–light petroleum (1:1) to give 3α,18-*Diacetoxyaphidicolan*-16β-*ol* (280 mg) which crystallized from ethyl acetate–light petroleum as prisms, m.p. 155–156 °C (Found: C, 71.2; H, 9.55. $C_{24}H_{38}O_5$ requires C, 70.9; H, 9.4%), ν_{max}/cm^{-1} 3520, 1743, 1730 and 1265; δ($CDCl_3$) 0.95 and 0.97 (each 3 H, s, 19- and 20-H_3), 1.10 (3 H, s, 17-H_3), 1.96 and 1.97 (each 3 H, s, OAc), 3.73 and 3.98 (each 1 H, d, *J* 10, 18-H_2) and 4.79 (1 H, m, 3-H).

3α,18-*Diacetoxyaphidicol*-15-*ene* **24**.—The above diacetate (240 mg) in acetonitrile (2 cm^3) and carbon tetrachloride (1 cm^3) was treated with triphenylphosphine (0.51 g) at 75 °C for 4 h. The solvents were evaporated and the residue was chromatographed on silica. Elution with ethyl acetate–light petroleum (5:95) gave 3α,18-*diacetoxyaphidicol*-15-*ene* (135 mg) which crystallized from ethyl acetate as needles, m.p. 205–208 °C (Found: C, 74.0; H, 9.4. $C_{24}H_{36}O_4$ requires C, 74.2; H, 9.3%), ν_{max}/cm^{-1} 1750, 1745 and 1255; δ($CDCl_3$) 0.99 and 1.04 (each 3 H, s, 19- and 20-H_3), 1.64 (3 H, br s, 17-H), 1.98 and 2.01 (each 3 H, s, OAc), 3.75 and 4.01 (each 1 H, d, *J* 10, 18-H_2), 4.84 (1 H, m, 3-H) and 5.00 (1 H, m, 15-H).

Hydrolysis of 3α,18-*Diacetoxyaphidicol*-15-*ene* **24**.—The diacetate (105 mg) in methanol (80 cm^3) was treated with aqueous sodium hydroxide (2 mol dm^{-3}) (10 cm^3) at room temp. overnight. The solvents were evaporated and the residue was 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 3α,18-dihydroxyaphidicol-15-ene identical (TLC and ^1H NMR) to the material described above.

Reaction of 3α,18-*Isopropylidenedioxyaphidicolan*-16α-*ol with Triphenylphosphine and Carbon Tetrachloride*.— Aphidicolane-3α,16α,18-triol **6** (2.04 g) was heated gently in acetone (150 cm^3) containing toluene-*p*-sulfonic acid (20 mg) for 30 min (TLC control). The acetone was evaporated and the residue was taken up in ethyl acetate and washed with aqueous sodium hydrogen carbonate, dried and evaporated. The resultant acetonide was used immediately. Triphenylphosphine (3.25 g) and carbon tetrachloride (6 cm^3) were added to a solution of the acetonide (2.2 g) in acetonitrile (20 cm^3) and the mixture was stirred at 75 °C (under nitrogen for 3 h. The solvents were removed by evaporation under reduced pressure and the residue was then chromatographed on silica in ethyl acetate–light petroleum (1:9). 3(4 → 18)*abeo-Aphidicol*-2,18(4),15-*triene* (50 mg) was obtained as a gum (Found: M^+, 268. $C_{20}H_{28}$ requires *M*, 268), ν_{max}/cm^{-1} 1650 and 1600; λ_{max}/nm 247 (ε 3200); δ($CDCl_3$) 1.03 (3 H, s, 20-H_3), 1.65 (3 H, br s, 17-H_3), 1.82 (3 H, br s, 19-H), 5.01 (1 H, m, 15-H), 5.77 (1 H, m, 18-H), 5.88 (1 H, br d, *J* 10, 2-H) and 6.01 (1 H, ddd, *J* 10, 8 and 5, 3-H). On irradiation at δ 1.65 the signal at δ 5.01 became a triplet *J* 3.5 and signals at 2.28 and 2.40 became double-doublets *J* 3.5 and 18 (14-H_2). Further elution gave 18-*chloroaphidicol*-2,15-*diene* (45 mg) which crystallized from methanol as prisms, m.p. 138–140 °C (Found: C, 78.0; H, 10.0. $C_{20}H_{29}Cl$ requires C, 78.8; H, 9.5%); ν_{max}/cm^{-1} 715; δ($CDCl_3$) 0.97 (3 H, s, 19-H_3) 1.05 (3 H, d, *J* 1.2, 20-H_3), 1.65 (3 H, d, *J* 1.5 of t 2.3, 17-H_3), 3.30 and 3.41 (each 1 H, d, *J* 11, 18-H_2), 4.99 (1 H, m, 15-H), 5.35 (1 H, ddd, *J* 10, 3 and 1, 3-H) and 5.74 (1 H, ddd, *J* 10, 6 and 2, 2-H). Further elution gave 3α,18-isopropylidenedioxyaphidicol-15-ene (0.8 g) identical (NMR) to the sample described previously. Elution with ethyl acetate–light petroleum (1:1) gave aphidicol-

Table 1 Crystal data and structure refinement details

Formula	$C_{20}H_{34}O_3$
M	322.2
Crystal size (mm)	0.3 × 0.3 × 0.3
Crystal system	orthorhombic
Space group	$P2_12_12_1$
a, *b*, *c* (Å)	11.874(3), 11.980(4), 12.255(2)
V (Å^3)	1743.2
Z, D_c (g cm^{-3}), *F*(000)	4, 1.23, 712
μ Mo-Kα cm^{-1}	0.9
Total unique reflections	1772
Significant reflections	1123
Abs. corr.	none
Hydrogen atoms	fixed calculated
R	0.057
R'	0.055

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

	x	*y*	*z*
C(1)	364(4)	1564(4)	4004(4)
C(2)	−675(4)	2056(4)	3445(5)
C(3)	−1477(4)	1158(4)	3077(4)
C(4)	−1872(4)	380(4)	3998(4)
C(5)	−832(4)	−58(4)	4651(4)
C(6)	−1151(4)	−846(4)	5601(4)
C(7)	−125(5)	−1446(5)	6088(4)
C(8)	738(4)	−565(4)	6417(4)
C(9)	1092(4)	151(4)	5429(4)
C(10)	58(4)	829(4)	5011(4)
C(11)	2072(4)	809(4)	5957(4)
C(12)	2761(4)	−139(5)	6474(4)
C(13)	1839(4)	−896(5)	6985(4)
C(14)	1655(4)	−526(4)	4495(4)
C(15)	2605(4)	−1323(4)	4840(4)
C(16)	3433(4)	−774(4)	5618(4)
C(17)	4287(4)	−37(5)	5018(5)
C(18)	−2520(4)	−617(5)	3501(4)
C(19)	−2749(4)	1003(5)	4693(5)
C(20)	−382(5)	1641(5)	5911(5)
O(1)	−969(3)	489(3)	2235(3)
O(2)	−1861(3)	−1410(3)	2949(3)
O(3)	4146(3)	−1604(3)	6131(3)

Table 3 Intramolecular distances (Å) and angles (°) with estimated standard deviations in parentheses for compound **6**

	Distance (Å)		Distance (Å)
C(1)–C(2)	1.529(6)	C(1)–C(10)	1.559(6)
C(2)–C(3)	1506(6)	C(3)–C(4)	1.537(6)
C(3)–O(1)	1.439(5)	C(4)–C(5)	1.562(6)
C(4)–C(18)	1.547(6)	C(4)–C(19)	1.538(6)
C(5)–C(6)	1.546(6)	C(5)–C(10)	1.561(6)
C(6)–C(7)	1.535(6)	C(7)–C(8)	1.526(6)
C(8)–C(9)	1.543(5)	C(8)–C(13)	1.533(6)
C(9)–C(10)	1.559(5)	C(9)–C(11)	1.547(6)
C(9)–C(14)	1.554(5)	C(10)–C(20)	1.561(6)
C(11)–C(12)	1.536(6)	C(12)–C(13)	1.553(6)
C(12)–C(16)	1.522(6)	C(14)–C(15)	1.537(6)
C(15)–C(16)	1.518(6)	C(16)–C(17)	1.533(6)
C(16)–O(3)	1.449(5)	C(18)–O(2)	1.404(5)
	Angle (°)		**Angle (°)**
C(2)–C(1)–C(10)	112.6(3)	C(1)–C(2)–C(3)	111.6(3)
C(2)–C(3)–C(4)	114.0(4)	C(2)–C(3)–O(1)	110.3(4)
C(4)–C(3)–O(1)	108.5(3)	C(3)–C(4)–C(5)	109.8(3)
C(3)–C(4)–C(18)	109.3(4)	C(3)–C(4)–C(19)	108.6(4)
C(5)–C(4)–C(18)	109.6(3)	C(5)–C(4)–C(19)	114.5(4)
C(18)–C(4)–C(19)	104.8(4)	C(4)–C(5)–C(6)	113.4(3)
C(4)–C(5)–C(10)	116.8(3)	C(6)–C(5)–C(10)	111.6(3)
C(5)–C(6)–C(7)	112.6(3)	C(6)–C(7)–C(8)	108.2(4)
C(7)–C(8)–C(9)	111.1(3)	C(7)–C(8)–C(13)	120.9(4)
C(9)–C(8)–C(13)	105.6(3)	C(8)–C(9)–C(10)	109.5(3)
C(8)–C(9)–C(11)	99.1(3)	C(8)–C(9)–C(14)	113.9(3)
C(10)–C(9)–C(11)	117.7(3)	C(10)–C(9)–C(14)	111.7(3)
C(11)–C(9)–C(14)	104.5(3)	C(1)–C(10)–C(5)	108.6(3)
C(1)–C(10)–C(9)	111.7(3)	C(1)–C(10)–C(20)	106.6(3)
C(5)–C(10)–C(9)	105.7(3)	C(5)–C(10)–C(20)	113.4(3)
C(9)–C(10)–C(20)	110.9(3)	C(9)–C(11)–C(12)	101.3(3)
C(11)–C(12)–C(13)	102.8(3)	C(11)–C(12)–C(16)	111.4(4)
C(13)–C(12)–C(16)	110.9(4)	C(8)–C(13)–C(12)	105.5(3)
C(9)–C(14)–C(15)	115.9(3)	C(14)–C(15)–C(16)	112.2(3)
C(12)–C(16)–C(15)	108.1(4)	C(12)–C(16)–C(17)	113.0(4)
C(12)–C(16)–O(3)	110.5(4)	C(15)–C(16)–C(17)	112.1(4)
C(15)–C(16)–O(3)	110.7(4)	C(17)–C(16)–O(3)	102.5(3)
C(4)–C(18)–O(2)	115.7(3)		

15-ene-3α,18-diol (0.45 g) identical (NMR) to the sample described previously.

Reaction of 16β,17β-*Epoxyaphidicolane*-3α,18-*diol with Lithium Diethylamide*.—Butyllithium in hexane (7.15 cm^3; 1.42 mol dm^{-3}) was added to a stirred solution of diethylamine (1.05 cm^3) in THF (50 cm^3) at 25 °C under nitrogen. The mixture was stirred for 30 min and then cooled in an ice-bath and a solution of 16β,17-epoxyaphidicolane-3α,18-diol **18** (650 mg) in THF (10 cm^3) was added. The mixture was stirred at room temperature overnight and then poured into ice–water. The products were recovered in ethyl acetate. The extract was washed with brine and dried. The solvent was evaporated and the residue was chromatographed on silica. Elution with ethyl acetate–light petroleum (3:7) gave aphidicol-15-en-3α,17,18-triol (80 mg) which crystallized from ethyl acetate as needles, m.p. 196–198 °C, identical (NMR) to the material described above.

3α,18-*Isopropylidenedioxy*-15β,16β-*epoxyaphidicolane* **28**.—3α,18-Isopropylidenedioxyaphidicol-15-ene (710 mg) in chloroform (30 cm^3) was treated with MCPBA (1 g) at 0 °C for 30 min. The solution was washed with aqueous sodium sulfite, aqueous sodium hydrogen carbonate and brine, and dried over sodium sulfate. The solvent was evaporated to afford the *title compound* **28** (700 mg) which crystallized from methanol as cubes, m.p. 161–162 °C (Found: C, 76.2; H, 10.1. $C_{23}H_{36}O_3$ requires C, 76.6; H, 10.1%), ν_{max}/cm^{-1} 1260, 1200 and 1085; δ($CDCl_3$) 0.68 (3 H, s, 19-H_3), 0.94 (3 H, s, 20-H_3), 1.27 (3 H, s, 17-H_3), 1.41 and 1.42 (each 3 H, s, acetonide Me), 2.83 (1 H, d, *J* 4.1, 15-H), 3.22 and 3.58 (each 1 H, d, *J* 12, 18-H_2) and 3.62 (1 H, t, *J* 2.6, 3-H).

Reduction of the 15β,16β-*Epoxide with Lithium Aluminium Hydride*.—3α,18-Isopropylidenedioxy-15β,16β-epoxyaphidicolane (150 mg) was added to a stirred suspension of lithium aluminium hydride (100 mg) in THF (20 cm^3) under nitrogen and heated under reflux for 30 min. The mixture was cooled to 0 °C and the excess of reagent was destroyed by the addition of ethyl acetate and water. The mixture was then acidified with dil. hydrochloric acid and stirred at room temperature for 30 min. The mixture was extracted with ethyl acetate and the extracts were washed with aqueous sodium hydrogen carbonate and brine. Evaporation of the solvent gave a gum which was dissolved in methanol (60 cm^3). Water (10 cm^3) and toluene-*p*-sulfonic acid (15 mg) were added and the mixture was stirred at room temperature for 2 d. The solvents were evaporated and the product was recovered in ethyl acetate. The extract was washed with aqueous sodium hydrogen carbonate, brine and dried. The solvent was evaporated to give aphidicolane-3α,16β,18-triol (90 mg) identical (NMR) to authentic material.[4]

15β,16β-*Epoxyaphidicolane*-3α,18-*diol* **29**.—Aphidicol-15-

ene-3α,18-diol (450 mg) in chloroform (40 cm^3) was treated with MCPBA (500 mg) at 0 °C for 20 min. The solution was washed with aqueous sodium sulfite, aqueous sodium hydrogen carbonate, brine and dried. The solvent was evaporated to give a gum which was chromatographed on silica in ethyl acetate–light petroleum (1:1) to give 15β,16β-*epoxyaphidicolane*-3α,18-*diol* **29** (235 mg) as prisms, m.p. 199–200 °C (Found: C, 75.1; H, 10.2. $C_{20}H_{32}O_3$ requires C, 75.0; H, 10.1%), ν_{max}/cm^{-1} 3430, 1060 and 1045; δ 0.67 (3 H, s, 19-H_3), 0.94 (3 H, s, 20-H_3) 1.28 (3 H, s, 17-H_3), 2.85 (1 H, d, *J* 4, 15-H), 3.35 and 3.45 (each 1 H, d, *J* 11, 18-H_2) and 3.67 (1 H, t, *J* 3, 3-H).

15β,16β-*Epoxyaphidicolane*-3α,17,18-*triol* **30**.—Aphidicol-15-ene-3α,17,18-triol (60 mg) in chloroform (15 cm^3) was treated with MCPBA (100 mg) at room temp. for 30 min. The solution was diluted with ethyl acetate, washed with aqueous sodium sulfite, aqueous sodium hydrogen carbonate and brine, and dried. The solvent was evaporated to give 15β,16β-*epoxyaphidicolane*-3α,17,18-*triol* **30** (40 mg) which crystallized from ethyl acetate as prisms, m.p. 220–221 °C (Found: C, 70.7; H, 9.75. $C_{20}H_{32}O_4$ requires C, 71.4; H, 9.6%); ν_{max}/cm^{-1} 3480, 1040 and 1030; δ(C_5D_5N) 0.73 (3 H, s, 19-H_3), 0.91 (3 H, s, 20-H_3), 3.23 (1 H, d, *J* 4, 15-H), 3.58 and 3.76 (each 1 H, d, *J* 11, 18-H_2), 3.75 and 4.12 (each 1 H, d, *J* 12.5, 17-H_2) and 3.89 (1 H, m, 3-H).

Aphidicolane-3α,18-*diol* **14**.—3α,18-Isopropylidenedioxy-aphidicol-15-ene **23** (500 mg) was dissolved in ethyl acetate (25 cm^3) containing a suspension of 10% palladium-on-charcoal (100 mg). This was stirred vigorously under hydrogen. After 30 min the uptake of hydrogen ceased. The mixture was filtered through Celite and the solvent was evaporated to give a gum which was taken up in methanol (80 cm^3). Water (20 cm^3) and toluene-*p*-sulfonic acid (50 mg) were added and the solution was left to stand at room temperature for 2 d. The solvent was evaporated and the residue was recovered in ethyl acetate. The extract was washed with aqueous sodium hydrogen carbonate and brine, and dried. The solvent was evaporated to give *aphidicolane*-3α,18-*diol* **14** (425 mg) which crystallized from ethyl acetate as needles, m.p. 191–195 °C (Found: C, 77.7; H, 11.6. $C_{20}H_{34}O_2$ requires C, 78.3; H, 11.1%); ν_{max}/cm^{-1} 3315, 1050 and 1030; δ 0.69 (3 H, s, 19-H), 0.74 (3 H, d, *J* 6.6, 17-H), 0.95 (3 H, s, 20-H), 2.41 (1 H, dd, *J* 3.1 and 12.5, 5-H), 3.35 and 3.48 (each 1 H, d, *J* 11.1, 18-H) and 3.65 (1 H, br s, 3-H).

Crystal Structure Determination.—A summary of the crystal data and structure refinement details are given in Table 1. Data were collected from a crystal mounted on an Enraf–Nonius CAD4 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 $2 < \theta < 25°$ 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.[15] 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 with a common B_{iso} = 6.0 $Å^2$. All calculations were done on a PDP 11/34 using the Enraf–Nonius SDP-Plus program package. Tables of fractional atomic co-ordinates and bond lengths and angles are given in Tables 2 and 3. The remaining crystallographic tables have been deposited with the Cambridge Crystallographic Data Centre.*

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

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