Oxidation of Aphidicolin and Its Conversion into 19-Noraphidicolan-16β-ol

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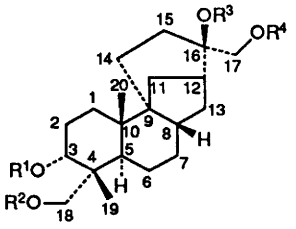
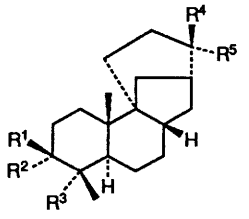
The preparation of the 3α,18-monoacetonide of aphidicolin and its selective oxidation at C-17, is described. Catalytic oxidation of aphidicolin affords 16β-hydroxy-3-oxo-19-noraphidicolan-17-oic acid. The conversion of this into 19-noraphidicolan-16β-ol and its biotransformation by the fungus, *Cephalosporium aphidicola*, to a 19-noraphidicolin, is reported.

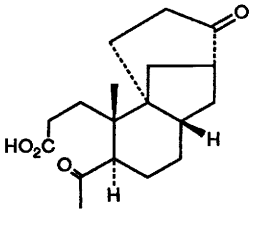
The diterpenoid fungal metabolite, aphidicolin **1**,[1] is a specific inhibitor[2] of DNA polymerase α and, consequently, it has received attention as an antiviral and tumour-inhibitory agent.[3] Aphidicolin possesses two primary, a secondary and a tertiary alcohol disposed as a 1,2- and a 1,3-glycol. In the course of biosynthetic studies[4] on this molecule, it has been necessary to develop some selective oxidations of these hydroxy groups. These studies form the subject of this paper.



1 R^1–R^4 = H
2 $R^1 = R^3$ = H, $R^2 = R^4$ = Bu^tMe_2Si
3 $R^1 = R^2 = R^3$ = H, R^4 = Ts
4 $R^1 = R^3$ = H, $R^2 = R^4$ = Ts



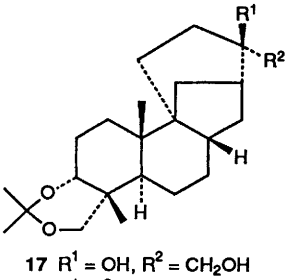
5 R^1, R^2 = O, $R^3 = R^5 = CH_2OH$, R^4 = OH
6 R^1 = H, R^2 = OH, R^3 = CHO, R^4 = OH, $R^5 = CO_2H$
7 R^1,R^2 = O, R^3 = CHO, R^4 = OH, $R^5 = CO_2H$
8 R^1,R^2 = O, $R^3 = CH_2OH$, R^4 = OH, $R^5 = CO_2H$
9 R^1 = H, R^2 = OH, $R^3 = CH_2OH$, R^4, R^5 = O
10 R^1 = H, R^2 = OH, $R^3 = CH_2OH$, R^4 = OH, R^5 = CHO
11 R^1 = H, R^2 = OH, $R^3 = R^5$ = CHO, R^4 = OH
12 R^1 = H, R^2 = OH, $R^3 = CH_2OH$, R^4 = OH, R^5 = Me
13 R^1 = H, R^2 = OH, R^3 = CHO, R^4 = OH, R^5 = Me
14 R^1 = H, R^2 = OH, R^3 = CHO, R^4 = OH, $R^5 = CO_2Me$
15 R^1 = H, R^2 = OH, $R^3 = CH_2OH$, R^4 = OH, $R^5 = CO_2Me$



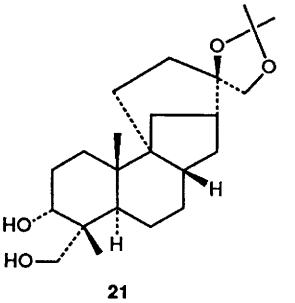
16

Prior work[5] had shown that the primary alcohols of aphidicolin could be protected as the 17,18-bis(*tert*-butyldimethylsilyloxy) derivative **2**. Oxidation with pyridinium chlorochromate and removal of the protecting groups afforded the 3-ketone **5**. The same compound has been obtained[6] by the microbial oxidation of aphidicolin with *Chaetomium funiculum* and *Streptomyces griseus*. On the other hand oxidation[7] of the unprotected aphidicolin **1** with pyridinium dichromate gave a mixture of 17-carboxylic acids **6**–**8** in which oxidation had also taken place at C-3 and/or C-18. More vigorous oxidation with chromic acid[1] afforded the diketo acid **16** in which not only had the C(16)–C(17) bond been cleaved but ring A had also been opened. The selective cleavage of the C(16)–C(17) bond can be achieved[1] by oxidation with periodic acid which affords the 17-nor-16-ketone **9**.

The hydroxy groups of aphidicolin differ in the ease with which they form derivatives. Thus, at low temperatures aphidicolin forms a 17-mono-toluene-*p*-sulfonate **3** whilst on more prolonged treatment with toluene-*p*-sulfonyl chloride in pyridine at room temperature, it gives a 17,18-ditoluene-*p*-sulfonate **4**. However the relatively mild Pfitzner–Moffatt oxidation[8] with dicyclohexylcarbodiimide (DCC) in dimethyl sulfoxide (DMSO)–trifluoroacetic acid, which also relies on esterification for the oxidation, showed only a limited regiospecificity and gave a mixture of the 17-aldehyde **10** and the 17,18-dialdehyde **11**. The location of the aldehyde group at C(17) in **10** followed from its oxidation with periodic acid which gave the known[1] 3α,18-dihydroxy-17-noraphidicolan-16-one **9**. Since the 17-aldehyde was required for further study, attempts were made to obtain a better yield. The selective protection of the glycols using acetonides was attempted. Although it was possible to prepare and separate the 3α,18- and 16β,17-monoacetonides, **17** and **21**, the yields were not satisfactory. The mono-acetonides



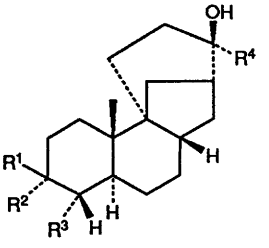
17 R^1 = OH, $R^2 = CH_2OH$
18 R^1, R^2 = O
19 R^1 = OH, $R^2 = CH_2OTs$
20 R^1 = OH, R^2 = CHO



21

were distinguished by oxidation of the 3α,18-monoacetonide **17** with chromium trioxide in pyridine which gave the known[1] 3α,18-monoacetonide of 3α,18-dihydroxy-17-noraphidicolan-16-one **18**. A simple method for preparing the 3α,18-monoacetonide involved firstly preparing the 17-monotoluene-*p*-sulfonate **3** and thence the 3α,18-acetonide **19**. Hydrolysis of the toluene-*p*-sulfonate with potassium hydroxide in DMSO then gave the 3α,18-monoacetonide of aphidicolin **17**. This, in turn, was oxidized under the Pfitzner–Moffatt conditions to give the 17-aldehyde **20**. Oxidation of aphidicolane-3α,16β,18-triol **12** under Pfitzner–Moffatt conditions gave the 18-aldehyde **13** in rather poor yield.

Catalytic oxidation may show contrasting regiospecificity.[9,10] Catalytic oxidation of aphidicolin **1** with oxygen and finely divided platinum gave a mixture of 17-carboxylic acids which were separated as their methyl esters. The major product was the 19-nor compound **22**. Its IR spectrum contained

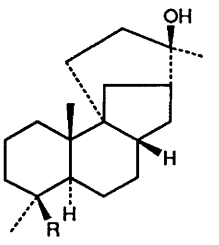


22 R^1, R^2 = O, R^3 = Me, R^4 = CO_2Me
23 R^1 = OH, R^2 = H, R^3 = Me, R^4 = CH_2OH
24 R^1 = H, R^2 = OH, R^3 = R^4 = CH_2OH

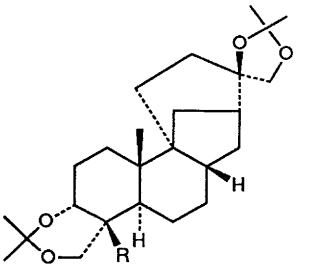
carbonyl (1699 and 1720 cm^{-1}) and hydroxy absorptions (3475 cm^{-1}). In the ^1H NMR spectrum the 18-H_3 signal (δ 0.99) now appeared as a doublet (*J* 6.5 Hz). The minor products were assigned the structures **14** and **15**. The location of the primary hydroxy group at C(18) in **15** followed from the position of the CH_2OH resonances (δ 3.38 and 3.47, *J* 11 Hz) which fall within the range found for the 18-CH_2OH in the 17-nor-ketone **9**, the 16-deoxy and 16-methylene analogues.

Reduction of the 19-nor compound **22** with lithium aluminium hydride gave a triol **23**, the stereochemistry of which was assigned on the basis of its ^1H NMR spectrum. A selective population transfer decoupling experiment based on irradiating the methyl signal at δ 1.21 (d, *J* 6.5 Hz) led to the identification of the 4-H resonance at δ 1.52. Analysis of this resonance and that of the secondary alcohol [δ 3.24 (*J* 10 Hz) d (*J* 4.8 Hz)], revealed $J_{3,4}$ 10 and $J_{4,5}$ 11.5 Hz. Thus there are diaxial relationships between the protons at C-3, C-4 and C-5. An NOE experiment based on irradiating the 20-H signal (δ 0.89) led to an 8% enhancement of the 4-H signal (δ 1.52). Hence this hydrogen atom is β and the stereochemistry of the triol **23** is as shown. Epimerization of the C-4 methyl group has taken place presumably during the loss of C-18. Hence these compounds are regarded as 19-noraphidicolanes. This also confirms the assignment made by Rozassa[11] to a metabolite of aphidicolin produced by *Trichothecium roseum*.

The triol was converted into its ditoluene-*p*-sulfonate which was then reduced with lithium aluminium hydride to afford 19-noraphidicolan-16-ol **25**. This compound is the 19-nor analogue of a precursor **26**[12] of aphidicolin **1** in *Cephalosporium aphidicola*. Its biotransformation by *C. aphidicola* was therefore examined. We have shown that chlorocholine chloride (CCC) can inhibit the biosynthesis of aphidicolin by *C. aphidicola*. Although the inhibition is not complete at concentrations that are not also lethal to the organism, nevertheless, the reduction in the amount of aphidicolin that is formed, is sufficient to facilitate the detection of the bio-transformation products of



25 R = H
26 R = Me



27 R = H
28 R = Me

exogenous substrates. 19-Nor-aphidicolan-16β-ol **25** was incubated with *C. aphidicola* for 29 days. The metabolites were isolated and chromatographed. The aphidicolin fractions were carefully monitored by conversion into their bis-acetonides. ^1H NMR and mass spectroscopy showed that the 'last' aphidicolin fraction comprised the bis-acetonides **27** and **28** of aphidicolin **1** and 19-noraphidicolin **24** in a 1:1 ratio. In the mass spectrum of the bisacetonide of aphidicolin **28** the most prominent peak is M − CH_3 ($C_{26}H_{42}O_4$ − CH_3, *m/z* 403). There was a corresponding peak at *m/z* 389 ($C_{25}H_{40}O_4$ − CH_3). In the ^1H NMR spectrum there were distinct resonances [*e.g.* δ 0.87 (20-H), 2.37 (5-H), 3.87 (2 H, m, 18-H_2)] which could be assigned to a 19-noraphidicolin bisacetonide.

In conclusion, we have shown that although there is only limited selectivity in the oxidation of the C-17 primary alcohol over that at C-18, this may be enhanced by the careful choice of protecting groups.

Experimental

General Procedure.—^1H NMR spectra were determined at 80 and 360 MHz on Bruker WP 80 and WM 360 spectrometers for solutions in deuteriochloroform except where otherwise stated; *J*-values 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.

Pfitzner–Moffatt Oxidation of Aphidicolin.—Aphidicolin **1** (1 g) was dissolved in dimethyl sulfoxide (DMSO) (4 cm^3) and benzene (10 cm^3). Pyridine (0.36 cm^3), trifluoroacetic acid (0.11 cm^3) and DCC (1.8 g) were added to this solution and the mixture was then stirred overnight. The solvents were removed under reduced pressure and the residue was dissolved in ethyl acetate (50 cm^3). The solution was stirred for 15 min and then filtered through Celite. The filtrate 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 (1:1) gave 3α,16β-dihydroxyaphidicolane-17,18-dial **11** (300 mg) as a gum (M^+, 305.214. Calc. for $C_{20}H_{30}O_4$ − CHO, 305.212); ν_{max}/cm^{-1} 3350 and 1720; δ-(90 MHz) 0.92 and 0.97 (each 3 H, s, 19-, 20-H), 3.7 (1 H, m, 3-H) and 9.49 and 9.54 (each 1 H, s, CHO).

Further elution gave 3α,16β,18-trihydroxyaphidicolan-17-al **10** (170 mg) as a gum (M^+, 307.228. Calc. for $C_{20}H_{32}O_4$ − CHO 307.227); ν_{max}/cm^{-1} 3350 and 1720; δ(60 MHz) 0.86 and 1.02 (each 3 H, s, 19-, 20-H), 3.6 (2 H, m, 18-H), 3.8 (1 H, m, 3-H) and 9.6 (1 H, s, CHO).

Periodate Oxidation of the Aldehyde **10**.—A solution of the aldehyde **10** (110 mg) in pyridine (5 cm^3) and water (1.5 cm^3) was treated with aqueous periodic acid (50%; 0.4 cm^3) for 15 min at room temperature. The solvents were evaporated and the

residue taken up in ethyl acetate. The extract was washed with dil. hydrochloric acid, aqueous sodium hydrogen carbonate and brine and dried. The solvent was evaporated and the residue was chromatographed on silica. Elution with ethyl acetate gave 3α,18-dihydroxy-17-noraphidicolan-16-one **9** (63 mg), m.p. 155 °C (lit., 155–156 °C) identified by its IR spectrum.

Partial Acetonide Formation with Aphidicolin.—Conc. sulfuric acid (1 drop) and acetone (0.1 cm^3) were added to a solution of aphidicolin (200 mg) in tetrahydrofuran (THF) (25 cm^3) at room temperature. The reaction was monitored by TLC. After 30 min two products were observed and further acetone (0.06 cm^3) was added. After 3 h the starting material had disappeared and a third product was becoming apparent. The acid was quenched by the addition of aqueous sodium hydrogen carbonate and the solvents were evaporated. The residue was extracted with ethyl acetate and the extract was washed with brine, dried, and evaporated. The resultant gum was chromatographed on silica with toluene–ethyl acetate (9:1) as eluent to give the bisacetonide of aphidicolin **28** (108 mg), m.p. 144–145 °C (lit.,[1] 145–146 °C) identified by its ^1H NMR spectrum.

Elution with toluene–ethyl acetate (1:1) gave 16β,17-isopropylidenedioxyaphidicolane-3α,18-diol **21** (12 mg), m.p. 197–201 °C; *m/z* 378 (1%, M^+) and 363 (45, $M - CH_3$); δ 0.70 (3 H, s, 19-H), 0.98 (3 H, s, 20-H), 1.34 and 1.40 (each 3 H, s, O_2CMe_2), 3.37 (2 H, s, 18-H), 3.51 and 3.74 (each 1 H, d, *J* 8, 17-H) and 3.66 (1 H, m, 3-H).

Further elution gave 3α,18-*isopropylidenedioxyaphidicolane*-16β,17-*diol* **17** (49 mg), m.p. 161–163 °C (Found: C, 73.4; H, 10.3. $C_{23}H_{38}O_4$ requires C, 73.0; H, 10.1%); ν_{max}/cm^{-1} 3390; δ(80 MHz) 0.72 (3 H, s, 19-H), 0.99 (3 H, s, 20-H), 3.03 and 3.80 (2 H, d, *J* 12, 18-H), 3.41 (2 H, s, 17-H) and 3.60 (1 H, m, 3-H).

Oxidation of the Acetonide **17**.—Dry chromium trioxide (160 mg) was added to a solution of dry pyridine (0.24 cm^3) in dry dichloromethane (4 cm^3) under argon. The deep red solution was stirred at room temperature for 15 min and then the acetonide **17** (100 mg) in dichloromethane (0.5 cm^3) was added. After 10 min (TLC control) the reaction mixture was filtered through Celite which was repeatedly washed with ether. The combined filtrates were washed with aqueous sodium hydrogen carbonate and brine and dried. The solvents were evaporated to give a gum which was chromatographed on silica. Elution with toluene–ethyl acetate (9:1) gave 3α,18-isopropylidenedioxy-17-noraphidicolan-16-one **18** (80 mg), m.p. 147–149 °C (lit.,[1] 143–146 °C), identified by its IR and NMR spectra.

3α,18-*Isopropylidenedioxy*-17-(p-*tolylsulfonyloxy*)*aphidicolan*-16β-*ol* **19**—The 17-monotoluene-*p*-sulfonate **3** of aphidicolin (500 mg) was heated under reflux in acetone (25 cm^3) containing toluene-*p*-sulfonic acid (5 mg) for 30 min. The reaction mixture was neutralised with aqueous sodium hydrogen carbonate and washed with brine, dried and evaporated to give a gum which was chromatographed on silica with toluene–ethyl acetate (1:1) as eluent to give the aphidicolane **17** (390 mg) as needles, m.p. 161–163 °C, identical with the sample described above.

Pfitzner–Moffatt Oxidation of Aphidicolane **17**.—The above acetonide **17** (380 mg) in dry benzene (5 cm^3) and dry DMSO (5 cm^3) was treated with pyridine (0.08 cm^3), trifluoroacetic acid (0.04 cm^3) and dicyclohexylcarbodiimide (0.65 g) and the mixture stirred overnight in a tightly stoppered flask. The mixture was filtered through Celite which was then repeatedly washed with ether. The filtrates were washed with brine, dried, and evaporated and the residue was chromatographed on silica with ethyl acetate–light petroleum (1:1) as eluent to give 16β-*hydroxy*-3α,18-*isopropylidenedioxyaphidicolan*-17-*al* **20** (295 mg), m.p. 197–201 °C (Found: C, 72.5; H, 9.8. $C_{23}H_{36}O_4$ requires C, 73.3; H, 9.6%); ν_{max}/cm^{-1} 3510 and 1725; δ(80 MHz) 0.74 (3 H, s, 19-H), 1.0 (3 H, s, 20-H), 3.24 and 3.64 (each 1 H, d, *J* 10, 18-H), 3.62 (1 H, m, 3-H) and 9.60 (1 H, s, 17-H).

Pfitzner–Moffatt Oxidation of Aphidicolane-3α,16β,18-*triol* **12**.—Pyridine (120 mg), trifluoroacetic acid (85 mg) and DCC (0.93 g) were added to a solution of the triol **12** (480 mg) in anhydrous DMSO (3 cm^3) and benzene (5 cm^3) and the mixture stirred at room temperature for 30 h. Although TLC indicated that most of the starting material was unchanged, the solvents were evaporated and the residue was taken up in ethyl acetate (30 cm^3). Water (5 cm^3) was added and the mixture was stirred for 30 min before being filtered through Celite. The filtrate was washed with dil. hydrochloric acid, aqueous sodium hydrogen carbonate and brine, dried and evaporated. The residue was chromatographed on silica with ethyl acetate–light petroleum (2:3) as eluent to give 3α,16β-dihydroxyaphidicolan-18-al **13** (55 mg) which crystallized from ethyl acetate–light petroleum as needles, m.p. 172 °C (Found: C, 75.1; H, 10.2. $C_{20}H_{32}O_3$ requires C, 75.0; H, 10.0%); ν_{max}/cm^{-1} 3495, 3440 and 1710; δ 1.00 and 1.05 (each 3 H, s, 19- and 20-H), 1.15 (3 H, s, 17-H), 3.78 (1 H, t, *J* 2.7, 3-H) and 9.57 (1 H, s, 18-H). Further elution gave the unchanged triol (180 mg).

Aerial Oxidation of Aphidicolin **1**.—Adams catalyst (3.0 g) in methanol (50 cm^3) was reduced under hydrogen and then washed with water. The black solid was suspended in water (50 cm^3) and added to a suspension of aphidicolin (3.5 g, finely powdered) in water (500 cm^3). A stream of air was bubbled through the vigorously stirred mixture at 90 °C for 24 h. The mixture was filtered through Celite whilst hot and then allowed to cool. It was acidified to pH 2 with dil. hydrochloric acid and then extracted with ethyl acetate. This extract was separated into acidic and neutral fractions with aqueous sodium hydrogen carbonate. The acidic fraction contained most of the material (3.2 g). This was dissolved in methanol (200 cm^3) and treated with an excess of diazomethane in ether. The excess of diazomethane was destroyed with acetic acid and the solvents

prisms, m.p. 189–191 °C (Found: C, 68.8; H, 9.4. $C_{21}H_{34}O_5$ requires C, 68.8; H, 9.4%); ν_{max}/cm^{-1} 3520, 3395, 3275 and 1736; δ 0.71 (3 H, s, 19-H), 0.95 (3 H, s, 20-H), 3.38 and 3.47 (each 1 H, d, *J* 11, 18-H), 3.68 (1 H, br s, 3-H) and 3.75 (3 H, s, OMe).

Reduction of the Keto Ester **22**.—The above keto ester **22** (1.35 g) in THF (10 cm^3) was added to a stirred suspension of lithium aluminium hydride (0.8 g) in dry THF (100 cm^3) under nitrogen and heated under reflux for 2.5 h. The mixture was cooled to 0 °C and the excess of reagent was destroyed by the addition of ethyl acetate followed by the dropwise addition of water. The mixture was acidified with dil. hydrochloric acid and stirred at room temperature for 30 min. The THF was removed under reduced pressure and the residue was extracted with ethyl acetate. The extract was washed with aqueous sodium hydrogen carbonate and brine, dried and evaporated to give 19-*nor-aphidicolane*-3β,16β,17-*triol* **23** (1.01 g) which crystallized from ethyl acetate–methanol as cubes, m.p. 159–161 °C (Found: C, 70.4; H, 10.6. $C_{19}H_{32}O_3 \cdot H_2O$ requires C, 69.9; H, 10.4%); ν_{max}/cm^{-1} 3335 and 1030; $\delta(C_5D_5N)$ 0.89 (3 H, s, 20-H), 1.21 (3 H, d, *J* 6.3, 18-H), 3.24 (1 H, t of d, *J* 10 and 4.8, 3-H) and 3.72 and 3.80 (each 1 H, d, *J* 12, 17-H).

19-*Noraphidicolan*-16β-*ol* **25**.—Toluene-*p*-sulfonyl chloride (1.61 g) was added in portions over a period of 3 d to a solution of 19-noraphidicolane-3β,16β,17-triol **23** (870 mg). After 4 d the mixture was poured into dil. hydrochloric acid and the product was extracted with ethyl acetate. The extract was washed with aqueous sodium hydrogen carbonate and brine, dried and evaporated. The residue was chromatographed on silica with ethyl acetate–light petroleum (3:7) as eluent to give 3β,17-di(p-tolylsulfonyloxy)-19-noraphidicolan-16β-ol (790 mg) as a gum; *m/z* (FAB) 599 (M − OH) 427 (599 − TsOH) and 255 (427 − TsOH); ν_{max}/cm^{-1} 3400, 1600 and 1175; δ 0.70 (3 H, d, *J* 6.3, 18-H), 0.83 (3 H, s, 20-H), 2.40 and 2.41 (each 3 H, s, ArMe), 3.78 and 3.84 (each 1 H, d, *J* 10, 17-H), 4.04 (1 H, t, d, *J* 10, 5.6, 3-H) and 7.29, 7.32, 7.75 and 7.76 (each 2 H, d, *J* 10, ArH).

The above toluene-*p*-sulfonate (665 mg) in THF (5 cm^3) was added to a stirred suspension of lithium aluminium hydride (0.8 g) in dry THF (100 cm^3) under nitrogen and heated under reflux for 2 h. The mixture was cooled to 0 °C and the excess of reagent was destroyed by the addition of ethyl acetate followed by the dropwise addition of water. The mixture was acidified with dil. hydrochloric acid and stirred for 30 min. The THF was removed under reduced pressure and the residue was extracted with ethyl acetate. The extract was washed with aqueous sodium hydrogen carbonate and brine, dried and evaporated. The residue was chromatographed on silica with ethyl acetate–light petroleum (3:17) as eluent to give 19-noraphidicolan-16β-ol **25** (245 mg) which crystallized from ethyl acetate as needles, m.p. 128–130 °C (Found: C, 82.3; H, 11.6. $C_{19}H_{32}O$ requires C, 82.6; H, 11.7%); ν_{max}/cm^{-1} 3370 and 1115; δ_H 0.79 (3 H, d, *J* 6.3, 18-H), 0.88 (3 H, s, 20-H) and 1.13 (3 H, s, 17-H); δ_C 13.4 (C-20), 21.2 (C-18), 22.0 (C-2), 24.6 (C-14), 26.7 (C-6), 27.0 (C-7), 28.1 (C-17), 31.7 (C-4), 32.2 (C-1), 32.8, 33.3 and 34.2 (C-15, -13, -11), 36.6 (C-3), 39.6 (C-10), 39.8 (C-8), 45.5 (C-5), 46.7 (C-12), 46.8 (C-9) and 73.0 (C-16).

Incubation of 19-*Noraphidicolan*-16β-*ol* **25** *with* Cephalosporium aphidicola.—The fungus was grown on a medium as described previously.[13] The substrate **25** (145 mg) and CCC (110 mg) were dissolved in ethanol (6 cm^3) and DMSO (15 cm^3) containing Tween 80 (2 drops). This solution was evenly distributed between three Thompson bottles (2.25 dm^3) of *C. aphidicola* on the sixth day after inoculation. On the 35th day, the culture was filtered and the broth was extracted with ethyl acetate. The metabolites were chromatographed on silica. Elution with ethyl acetate–light petroleum (1:4) afforded the untransformed substrate (31 mg). Further elution with ethyl acetate gave three 'aphidicolin' fractions: A (78 mg), 5 (52 mg) and C (12 mg). Each fraction was separately treated with acetone and toluene-*p*-sulfonic acid to afford the bis-acetonides. Analysis by ^1H NMR showed that the products from fractions A and B were pure aphidicolin bis-acetonide **28**. Fraction C acetonides were examined by MS and ^1H NMR; *m/z* 403 ($C_{26}H_{42}O_4 - CH_3$; compound **28**) and 389 ($C_{25}H_{40}O_4 - CH_3$; compound **27**); δ_H 0.87 (3 H, s, 20-H), 1.25, 1.34, 1.40 and 1.43 (each 3 H, s, O_2CMe_2), 2.37 (1 H, m, 5-H), 3.54 and 3.76 (each 1 H, d, *J* 8.3, 17-H), 3.87 (2 H, m, 18-H) and 4.05 (1 H, m, 3-H) for compound **27** together with resonances attributable to aphidicolin bis-acetonide **28**.[1] The signals were in the ratio 1:1 suggesting the presence of 3α,18;16β,17-bisisopropylidenedioxy-19-noraphidicolane **27** (*ca.* 6 mg).

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References

1 W. Dalziel, B. Hesp, K. M. Stevenson and J. A. J. Jarvis, *J. Chem. Soc., Perkin Trans. 1*, 1973, 2841.
2 S. Ikegami, T. Taguchi, M. Ohashi, M. Oguro, H. Nagano and Y. Mano, *Nature (London)*, 1978, **275**, 458.
3 For a review, see S. Spadari, F. Focher, F. Sala, G. Ciarrocchi, G. Koch, A. Falaschi and G. Pedrali-Noy, *Arzneim.-Forsch.*, 1985, **35**, 1108.
4 M. J. Ackland, J. Gordon, J. R. Hanson, B. L. Yeoh and A. H. Ratcliffe, *J. Chem. Soc., Perkin Trans. 1*, 1988, 1477 and refs. therein.
5 A. H. Ratcliffe, unpublished work.
6 J. Ipsen, J. Fuska, A. Fuskova and J. P. Rosazza, *J. Org. Chem.*, 1982, **47**, 3278.
7 S. Hiranuma, T. Shimizu, H. Yoshioka, K. Ono, H. Nakone and T. Takahashi, *Chem. Pharm. Bull.*, 1987, **35**, 1641.
8 K. E. Pfitzner and J. G. Moffatt, *J. Am. Chem. Soc.*, 1965, **87**, 5661.
9 K. Heyns and L. Blazajewicz, *Tetrahedron*, 1960, **9**, 67.
10 R. P. A. Sneeden and R. B. Turner, *J. Am. Chem. Soc.*, 1954, **77**, 190.
11 J. Ipsen and J. P. Rosazza, *J. Nat. Prod.*, 1984, **47**, 497.
12 M. J. Ackland, J. R. Hanson, B. L. Yeoh and A. H. Ratcliffe, *J. Chem. Soc., Perkin Trans. 1*, 1985, 2705.
13 M. J. Ackland, J. R. Hanson and A. H. Ratcliffe, *J. Chem. Soc., Perkin Trans. 1*, 1984, 2751.

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