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AN OXYGEN-18 STUDY OF THE BIOSYNTHESIS OF THE DITERPENOID, APHIDICOLIN IN *CEPHALOSPORIUM APHIDICOLA**

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Abstract—Deuterium labelling has been used to demonstrate that the loss of the 17-CH_2OH forms the initial fragmentation in the mass spectrum of aphidicolin. The results have been used to establish the site (C-16) of incorporation of ^{18}O from $H_2{}^{18}O$ in the biosynthesis of aphidicolin by the fungus, *Cephalosporium aphidicola*

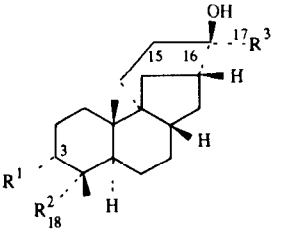
INTRODUCTION

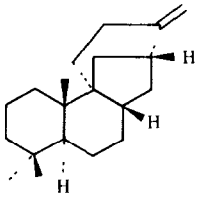
The majority of terpenoid biosynthetic cyclizations are cationic in character and are completed through the discharge of the cation either by the elimination of a proton or by addition of a hydroxyl ion [1]. The origin of the C-16 oxygen atom of the tetracyclic diterpenoid aphidicolin (**1**) [2] presents an interesting problem in this context. Apart from the chemical and biosynthetic interest engendered by its structure, aphidicolin has recently attracted attention as a specific inhibitor of DNA polymerase α. We have shown that although the hydrocarbon, aphidicol-16-ene (**7**) is specifically incorporated into aphidicolin (**1**) to the extent of 0.09% by *Cephalosporium aphidicola*, the corresponding aphidicolan-16β-ol (**2**) is a much more efficient precursor (7 9% incorporation) [3]. This could suggest that the major biosynthetic route to aphidicolin (**1**) involves completion of the rearrangement (see Scheme) that leads to the formation of rings C and D [4] by hydration at C-16 It may be significant in this context that the 16-hydroxyl group is *trans* to the bond which has migrated. We have, therefore, examined the biosynthesis of aphidicolin (**1**) by *C. aphidicola* in the presence of $H_2{}^{18}O$

RESULTS AND DISCUSSION

A pre-requisite to this study involved the identification of some ions in the mass spectrum of aphidicolin. Under electron-impact mass spectrometry, aphidicolin normally gives a base peak at *m/z* 307 $[M-CH_2OH]^+$ and no detectable molecular ion. However, under negative fast atom bombardment conditions, aphidicolin gives an $[M-H]^-$ ion at *m/z* 337 whilst under chemical ionization conditions (ammonia), it gives an $[M+NH_4]^+$ ion at *m/z* 356. The loss of CH_2OH might arise from either C-17 or C-18 or both Hence these centres were selectively deuteriated.

The selective oxidation of aphidicolin (**1**) was achieved using trifluoracetic acid, dicyclohexylcarbodiimide, dimethylsulphoxide and pyridine [5]. This gave a mixture of mono-(**3**) and dialdehydes (**4**) which were separated by chromatography The location of the aldehyde at C-17 in the mono-aldehyde (**3**) was established by oxidation with periodic acid to give 3α,18-dihydroxy-17-noraphidicolan-16-one (**8**) [2] which was identical with an authentic sample Reduction of the separate aldehydes with sodium [$^2H_4$]borohydride gave [17-^{2}H] aphidicolin (**5**) and [17,18-$^2H_2$]aphidicolin (**6**). The negative fast atom bombardment mass spectra of these samples





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1 R^1 = OH , R^2 = R^3 = CH_2OH

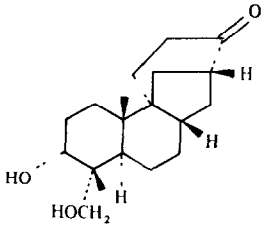
2 R^1 = H , R^2 = R^3 = Me

3 R^1 = OH , R^2 = CH_2OH , R^3 = CHO

4 R^1 = OH , R^2 = R^3 = CHO

5 R^1 = OH , R^2 = CH_2OH , R^3 = CH^2HOH

6 R^1 = OH , R^2 = R^3 = CH^2HOH



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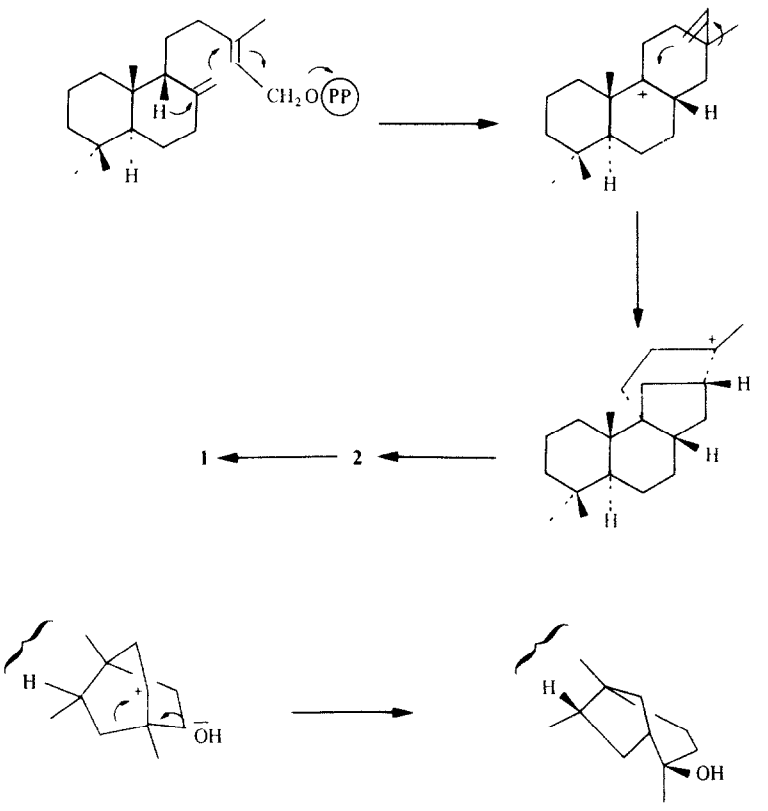
*Part 37 in the series, 'Studies in Terpenoid Biosynthesis' For part 36 see Hanson, J R , O'Leary, M A , Wadsworth, H J and Yeoh, B. L (1988) *Phytochemistry* **27**, 387.



Formation of rings C and D of aphidicolin

showed $[M-H]^-$ ions at *m/z* 338 and 339 respectively. On the other hand, the electron impact mass spectra showed ions at *m/z* 307 and 308 from **5** and **6** respectively Hence, it is the C-17 CH_2OH which is exclusively lost first in the mass spectral fragmentation Further low intensity ions occur at *m/z* 289 and 271 corresponding to the loss of a further one and two molecules of water respectively

The fungus, *C aphidicola* was grown on 20% $H_2{}^{18}O$ for 28 days The aphidicolin was isolated and examined mass spectrometrically under chemical ionization (ammonia) and electron impact conditions The C I mass spectrum revealed the incorporation of a single atom of ^{18}O per molecule in the ion at *m/z* 358 $[M+NH_4]^+$ The fragment ion $[M-CH_2OH]^+$ at *m/z* 307 in the electron-impact mass spectrum also showed the incorporation of a single atom of ^{18}O per molecule (ion at *m/z* 309, c 20% that at 307) However, when the labelled aphidicolin was oxidized with aqueous periodic acid to afford 3α,18-dihydroxy-17-noraphidicolan-16-one (**8**) and then the latter examined by both electron impact and chemical ionization mass spectrometry, there was no ^{18}O label present These acidic conditions during the oxidation would be expected [6] to exchange an ^{18}O label from a carbonyl group Hence, the retention of the label in the $[M-CH_2OH]^+$ ion and its loss in the nor-ketone, established the location of the ^{18}O at C-16 The other oxygen atoms of aphidicolin (**1**) must, therefore, arise by normal oxidative processes

In conclusion, we have established that the C-16 hydroxyl group of aphidicolin arises by hydration rather than by aerial oxidation. Bearing in mind the incorporation of aphidicolan-16-β-ol, it is probable that this involves hydration of the carbocation which is formed during the cyclization Since the incorporation of aphidicol-16-ene (**7**) was only just over 1% that of aphidicolan-16β-ol, our results would not give any information on a minor pathway involving, for example, epoxidation and hydrolysis of the epoxide

EXPERIMENTAL

General experimental details have been described previously [3] Mass spectra were determined through the courtesy of ICI Pharmaceuticals Division

Pfitzner–Moffatt oxidation of aphidicolin Aphidicolin (**1**) (1 0 g) was dissolved in a mixture of DMSO (4 ml) and C_6H_6 (10 ml) C_5H_5N (0 36 ml), trifluoroacetic acid (0 11 ml) and *N,N*-dicyclohexylcarbodiimide (1 8 g) were added and the reaction mixture was stirred overnight The solvents were removed *in vacuo* and the residue was dissolved in EtOAc (50 ml) After stirring for 15 min, the soln was filtered through Celite The filtrate was washed with dil HCl, $NaHCO_3$, NaCl and dried The solvent was evapd and the residue was chromatographed on silica in EtOAc–petrol to give 3α,16-dihydroxyaphidicolan-17,18-dial (**4**) (300 mg) as an oil, (Found MS 305 214 $[M-CHO]^+$, $C_{19}H_{29}O_3$ requires 305 212), IR ν_{max} cm^{-1} 3350, 1720, ^1H NMR ($CDCl_3$, ^2H_2O wash) δ0 92, 0 97 (each 3H, s, H-19 and H-20), 3 70 (1H, *br* s, H-3), 9 49 and 9 54 (each 1H, s, H-17 and H-18)

Further elution gave 3α,16β,18-trihydroxyaphidicolan-17-al (**3**) (170 mg) as an oil, (Found MS 307.228 $[M-CHO]^+$, $C_{19}H_{31}O_3$ requires 307 227), IR ν_{max} cm^{-1} 3350, 1720, ^{1}H NMR ($CDCl_3$, ^2H_2O wash); δ0 70 (3H, *s*, H-20), 0 98 (3H, *s*, H-19), 3 20 and 3 60 (each 1H, *d*, *J* = 13 Hz, H-18), 3 67 (1H, *br s*, H-3) and 9 68 (1H, *s*, H-17)

Periodate oxidation of 3α,16β,18-*trihydroxyaphidicolan*-17-*al* (**3**) 50% Aq periodic acid (0 4 ml) was added to a soln of the aldehyde (**3**) (110 mg) in C_5H_5N (5 ml) and H_2O (1 5 ml) After 15 min at room temp, the solvents were removed *in vacuo* and the residue was partitioned between EtOAc and dil HCl The organic extract was washed with $NaHCO_3$, NaCl and dried The solvent was evapd and the residue was chromatographed on silica to afford 3α,18-dihydroxy-17-noraphidicolan-16-one (**8**) (63 mg) which crystallized from EtOAc as needles, mp 155° (lit [2] 155–156°) identified by its IR spectrum

Sodium borodeuteride reductions (a) NaB^2H_4 (41 mg) was added to a soln of 3α,16β,18-trihydroxyaphidicolan-17-al (**3**) (160 mg) in EtOH (5 ml) at room temp After 3 hr, the solvent was evapd and the residue was partitioned between EtOAc and dil HCl The organic extracts were washed with $NaHCO_3$, NaCl and dried The solvent was evapd and the residue was chromatographed on silica to afford [17-^{2}H]aphidicolin (**5**) (80 mg), mp 231–3° (lit [2] 227–233°), ^{1}H NMR (C_5D_5N) δ0 77 (3H, *s*, H-20), 1 01 (3H, *s*, H-19), 3.56 and 3 74 (each 1H, *d*, *J* = 12 Hz, H-18), 3 68 and 3 74 (each 0 5 H, *br s*, 17-CH^2H OH), 3 86 (1H, *m*, H-3); MS (−ve FAB) *m/z* 338 (90) [M−H], EI *m/z* 307 (100) $[M-CH^2H\ OH]^+$ (b) 3α,16β-Dihydroxyaphidicolan-17,18-dial (**4**) (160 mg) was reduced similarly with NaB^2H_4 (40 mg) in EtOH (5 ml) to afford [17,18-$^2H_2$]aphidicolin (**6**) (85 mg), mp 226–232° ^{1}H NMR (C_5D_5N) δ0 77 (3H, *s*, H-20), 1 01 (3H, *s*, H-19), 3 56 and 3 74 (each 0 5 H, *br s*, 18-CH^2H OH), 3 68 and 3 74 (each 0 5 H, *br s*, CH^2HOH), 3 90 (1H, *m*, H-3), MS (−ve FAB) *m/z* 339 (50) [M−H]$^-$, EI *m/z* 308 (100) $[M-CH^2HOH]^+$

Incubation of C. aphidicola *with* $H_2{}^{18}O$ A culture medium (10 ml) of 20% $H_2{}^{18}O$ containing glucose (0 5 g), KH_2PO_4 (0 05 g), $MgSO_4$ (0 02 g), KCl (0 01 g), glycine (0 02 g) and trace elements soln (0 02 ml) [4] was sterilized by filtration through a 0 2 μm pore filter and transferred to a pre-sterilized 25 ml conical flask The sterile medium was inoculated with 0 5 ml of a mycelial slurry of *C. aphidicola* and incubated for 28 days at 25° The mycelial mat was then lifted off the culture medium with tweezers The culture medium was thoroughly extracted with EtOAc The extract was dried, the solvent was evapd and the residue was chromatographed on silica to afford aphidicolin (4 mg) (identified by TLC) It was recrystallized from EtOAc–petrol and examined mass spectrometrically (see Discussion)

Periodic acid oxidation of the labelled aphidicolin The [^{18}O]aphidicolin (2 mg) in pyridine (0 4 ml) and H_2O (0 1 ml) was treated with a few drops of 50% periodic acid at 0° for 30 min The soln was poured into dil HCl and the product was recovered in $CHCl_3$ The extract was washed with $NaHCO_3$, dried and the solvent was evapd The residue was crystallized from EtOAc–petrol to afford 3α,18-dihydroxy-17-noraphidicolan-16-one (1 mg) which was identified by TLC It was examined mass spectrometrically

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