The Late Stages of the Biosynthesis of the Diterpenoid Aphidicolin in *Cephalosporium aphidicola*

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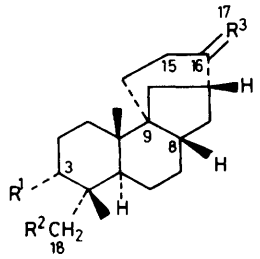
Labelling studies (^{14}C and ^{18}O) have shown that the major biosynthetic pathway leading to the diterpenoid aphidicolin involves the aphidicolan-16β-ols whilst the results of ^{2}H-labelling studies are consistent with a minor pathway involving epoxidation of an aphidicol-16-ene and hydrolysis of the epoxide.

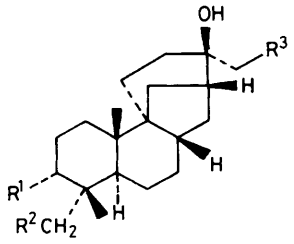
The tetracyclic diterpenoid fungal metabolite aphidicolin (**8**) has attracted interest[1] as a specific inhibitor of DNA polymerase α. In our previous biosynthetic work[2] we have defined the constituent isoprene units using the acetate and mevalonate labelling pattern and we have shown that a hydrogen shift from C-9 to C-8 occurs during the biosynthesis of this variant of the tetracyclic diterpenoid carbon skeleton. More recently we have shown[3] that aphidicol-16-ene (**1**) and aphidicolan-16β-ol (**5**) are incorporated to the extent of 0.09 and 7.9%, respectively. Since both aphidicol-16-ene and aphidicolan-16β-ol are formed by the fungus,[1,3] these results suggested that there might be a major pathway involving the 16β-alcohols and a minor route through the 16-enes. Both intermediates could arise through a common carbocation generated during the cyclization.

In support of this we have now shown that 18-hydroxy-[17-^{14}C]-aphidicol-16-ene (**2**) and 3α,18-dihydroxy[17-^{14}C]-aphidicol-16-ene (**3**)† are specifically incorporated into aphidicolin (**8**) to the extent of 0.86 and 16.4%, repectively by *Cephalosporium aphidicola*. On the other hand 16β,18-dihydroxy[17-^{14}C]aphidicolane (**6**) and 3α,16β,18-trihydroxy-[17-^{14}C]aphidicolane (**7**) are incorporated to the extent of 20.5 and 52.6% respectively.

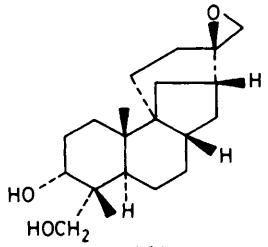
In accordance with the major pathway being that in which the 16β-alcohols are formed by hydration of a carbocation, aphidicolin (**8**) biosynthesized in the presence of 20% $H_2{}^{18}O$ has been shown by mass spectrometry to incorporate an oxygen-18 label at C-16. The chemical ionization (ammonia) mass spectrum showed an ion at 356 a.m.u., $(M + NH_4)^+$, and an additional ion at 358 a.m.u. in the spectrum of the labelled product. Deuterium labelling showed that the significant 307 a.m.u. ion in the electron-impact mass spectrum of aphidicolin represents an $M-CH_2OH$ fragment derived from the loss of C-17. This 307 a.m.u. ion also retained the oxygen-18 label. The 17-nor-16-ketone (**4**), prepared by oxidation with periodic acid, had lost the label; thus the oxygen-18 must have been at C-16. Because the 16-ene pathway is a relatively minor route, this result does not necessarily provide any information on that pathway.

† The preparation of the labelled intermediates will be described in our full paper.





(1) $R^1 = R^2 = H$, $R^3 = CH_2$
(2) $R^1 = H$, $R^2 = OH$, $R^3 = CH_2$
(3) $R^1 = R^2 = OH$, $R^3 = CH_2$
(4) $R^1 = R^2 = OH$, $R^3 = O$
(5) $R^1 = R^2 = R^3 = H$
(6) $R^1 = H$, $R^2 = OH$, $R^3 = H$
(7) $R^1 = R^2 = OH$, $R^3 = H$
(8) $R^1 = R^2 = R^3 = OH$



(9)

The conversion of the aphidicol-16-enes into the 16,17-glycols could proceed by hydration followed by hydroxylation. Alternatively the route may involve epoxidation and hydrolysis. Both pathways have precedent in terpenoid and steroid biosynthesis.[4] If a 17-deuterio-16-ene is used as a substrate, the hydration/hydroxylation route would, in contrast to the epoxidation/hydrolysis pathway, involve some loss of label. 3α,18-Dihydroxy[15-^{2}H,17,17-$^2H_2$]aphidicol-16-ene (**3**) was fed to *C. aphidicola*. ^{2}H N.m.r. spectroscopy of the resultant aphidicolin (**8**) showed that the ratio of the 15-^{2}H and 17-^{2}H signals remained constant, in support of an epoxidation and hydrolysis pathway. 3α,18-Dihydroxy-16β,17-epoxyaphidicolane (**9**) was incorporated into aphidicolin (**8**) by *C. aphidicola* to the extent of 42% although a control experiment showed that this was accompanied by some non-enzymic hydrolysis (*ca.* 30%). Nevertheless, these experiments between them establish the role of an epoxide in the minor pathway and an efficient hydration/hydroxylation route from the initial cyclization as the major pathway.

We thank ICI Pharmaceuticals Division for support for CASE studentships for M. J. A. and J. F. G.

Received, 16th June 1987; Com. 834

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