Active Site Mapping in a Methyl Group Hydroxylation in Aphidicolin Biosynthesis

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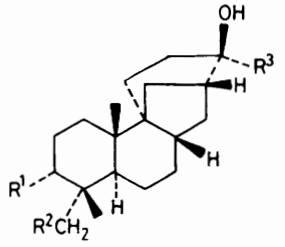
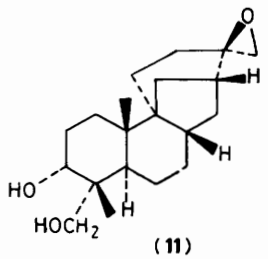
The hydroxylation of 17-methyl-3α, 16β, 18-trihydroxyaphidicolane by *Cephalosporium aphidicola* proceeds preferentially to afford the 17(*R*) homologue of aphidicolin, a result which may be interpreted in terms of the geometry of the active site.

The hydroxylation of a methyl group is an important step in many terpenoid and steroid biosyntheses.[1,2] The stereochemical features of, for example, the oxidation of the steroidal 19-methyl group (the aromatase sequence) have been the subject of intensive study in recent years.[3] The use of substrate analogues in active site mapping may shed light on the constraints of such enzymatic processes.[4] Microbial hydroxylation of an sp^3 carbon may be accompanied by epoxidation of the corresponding sp^2 carbon.[1] Therefore a general strategy for determining the stereochemistry of a methyl group hydroxylation would be comparison of the stereochemistry of epoxidation of a related sp^2 centre with any chiral preference shown in the hydroxylation of an ethyl analogue. The major route in the biosynthesis of the diterpenoid tumour inhibitor aphidicolin (**1**),[5] *via* aphidicolan-16β-ol (**2**), involves an hydroxylation at C-17.[6] This step is an efficient one in which 3α,16β,18-trihydroxyaphidicolane (**3**) is converted into aphidicolin (**1**) in up to 52% yield. We have also proposed a minor biosynthetic route *via* epoxidation of a 16-ene.[6] Hydroxylation of the pro-chiral 17-methyl homologue (**4**) at C-17 may generate either the 17(*R*) or 17(*S*) isomer, (**5**) and (**6**), respectively. A preference for one of these, when compared with the stereochemistry of the epoxidation of the 16-ene, could shed light on the geometry of the active site for hydroxylation of this methyl group.

Authentic samples of the (16*R*,17*R*)- and (16*R*,17*S*)-17-methylaphidicolins (**5**) and (**6**) were prepared by Pfitzner–Moffatt oxidation of the partially protected aphidicolin derivative (**7**) followed by reaction with methyl-lithium. The stereochemistry was assigned on the basis of nuclear Overhauser effects in the bisacetonides (**8**) and (**9**), between the 17-C*H*-O and 17-methyl protons and H-12, respectively. [^{14}C]-17-Methyl-3α,16β,18-trihydroxyaphidicolane (**4**) was prepared by treating the 17-monotoluene-*p*-sulphonate of





(11)

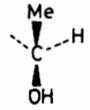
(1) $R^1 = R^2 = OH$, $R^3 = CH_2OH$

(2) $R^1 = R^2 = H$, $R^3 = Me$

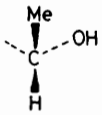
(3) $R^1 = R^2 = OH$, $R^3 = Me$

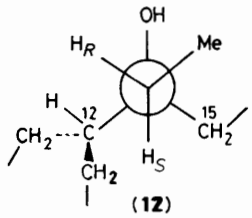
(4) $R^1 = R^2 = OH$, $R^3 = Et$

(5) $R^1 = R^2 = OH$, R^3 =



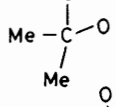
(6) $R^1 = R^2 = OH$, R^3 =





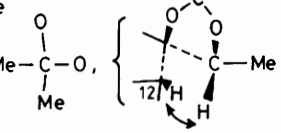
(12)

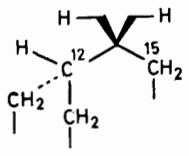
(7) R^1, R^2 =



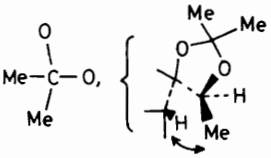
, $R^3 = CH_2OH$

(8) R^1, R^2 =





(9) R^1, R^2 =



(10) $R^1 = R^2 = OH$, $R^3 = CH_2OTs$ (Ts = Tosyl)

aphidicolin (**10**) with lithium [^{14}C]dimethylcuprate, and fed (70 mg, 1.09×10^6 d.p.m.) to *Cephalosporium aphidicola*, (3 l, 8 days after inoculation). When the fermentation was harvested after a further 20 days, the extract was divided into two portions and separately diluted (50 mg) with authentic (16*R*,17*R*)- and (16*R*,17*S*)-methylaphidicolins (**5** and **6**). The diluents were then recovered by careful chromatography. The 17*R*-epimer (**5**) had 230 d.p.m. mg^{-1} (*ca.* 2.1% incorporation), whilst the 17*S*-epimer (**6**) had 43 d.p.m. mg^{-1}, representing only 0.49% incorporation. The fermentation also produced aphidicolin (90 mg) and unchanged substrate (43 mg) was also recovered. Hence there was a preferential hydroxylation of the 17*R*-position.

The minor biosynthetic route to aphidicolin involves the 16β,17-epoxidation of a 16-ene (**13**). Molecular models show that the preferred conformation of the 17-ethylcarbinol (**4**) is (**12**). If this bears a resemblance to the conformation which is undergoing hydroxylation, then it suggests that the hydroxylation is taking place from the (*R*) face, *i.e.* the same face as epoxidation of (**13**). Thus this methyl substitution strategy and comparison with epoxidation sheds some light on the geometry of the delivery of oxygen at the active site involved in a methyl group hydroxylation.

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