A ^{2}H N.M.R. Study of the Rearrangement Step in Aphidicolin Biosynthesis

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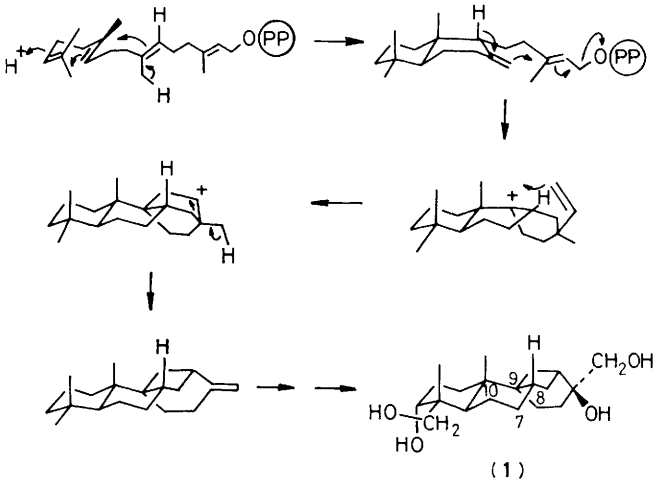
The observation of a ^{2}H–^{13}C coupling in the ^{2}H n.m.r. spectrum of aphidicolin biosynthesized from [4-$^2H_2$,3-^{13}C]mevalonic acid, has established the migration of a 9β-hydrogen atom to C-8 during the biosynthesis.

The antiviral and tumour-inhibitory fungal metabolite, aphidicolin (**1**),[1] has recently attracted considerable attention not only as a synthetic objective[2] but also because of the recognition of its biological activity as an inhibitor of DNA replication.[3] The presence of an 8β-hydrogen atom suggested[1,4] a novel biosynthesis involving a chair–boat rather than a chair–chair cyclization of geranylgeranyl pyrophosphate to form a bicyclic intermediate possessing a 10β-methyl group and a 9β-hydrogen atom. Subsequent formation of ring-C, migration of the 9β-hydrogen atom to C-8, cyclization of the 'pimaradienyl' vinyl group onto the C-9 carbocation, and rearrangement could afford the tetracyclic aphidicolene system (see Scheme 1).

An earlier biosynthetic study[4] of aphidicolin established some features of the carbon-13 labelling pattern from [1,2-$^{13}C_2$]acetate and the location of one (at C-12) of the three *pro*-(4*R*)-[4-^{3}H]mevalonate labels which were incorporated into aphidicolin. However an alternative means of generating the C-9 carbocation involving hydrogen shifts from C-7β to C-8β and from C-9α to C-7α which would have the merit of utilizing the common labdane intermediates, cannot be excluded on the basis of the earlier work. In particular, the position of the key 4-mevalonoid hydrogen atom involved in the rearrangement was not determined. In this communication we present evidence concerning the hydrogen migration step.

The complete ^{13}C n.m.r. spectrum of aphidicolin was assigned by comparison with a series of derivatives.† The



(1)

Scheme 1

optimum time for feeding the labelled substrates to *Cephalosporium aphidicola* (8 days from inoculation on surface culture) and for harvesting the metabolites (20 days further growth), was determined. [1-^{13}C]Acetate, [2-^{13}C]acetate, and [1,2-$^{13}C_2$]-acetate were each fed in separate experiments to *Cephalosporium aphidicola*. The labelling patterns of the resultant aphidicolin are shown in Figure 1. The enrichment and coupling patterns including the induced coupling (J 32 Hz) between C-12 and C-13 arising from the multiple labelling of

† These assignments differ from the previous work. The detailed basis of our assignments will be given in our full paper.

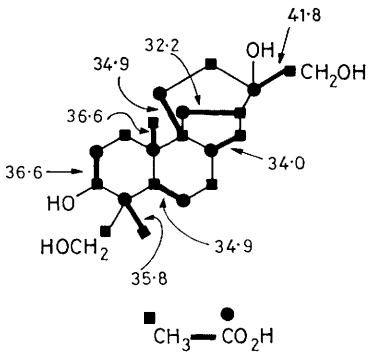


Figure 1. Coupling and enrichment patterns from ^{13}C-acetates J/Hz.

the precursor molecules from [2-^{13}C]acetate, support the proposed biosynthetic scheme. In particular, these patterns define the isoprene units in aphidicolin.

The putative hydrogen migration from C-9 to C-8 was examined by feeding [4-$^{2}H_2$]- and [4-$^{2}H_2$,3-^{13}C]-mevalonic acids. The acetate labelling studies showed that C-8 originates from C-3 of mevalonic acid whilst C-9 originates from C-4. If a 4-^{2}H-mevalonoid hydrogen atom migrates from C-9 to C-8 within the isoprene unit, then in the doubly-labelled species, a new ^{2}H–^{13}C coupling will be generated. Since deuterium has a spin of 1, this heteronuclear coupling is more easily observed in the ^{2}H n.m.r. spectrum.

[4-$^{2}H_2$]- and [4-$^{2}H_2$,3-^{13}C]-Mevalonic acids were prepared and fed to *C. aphidicola*. The aphidicolin (**1**) was isolated and converted into its more soluble tetra(trimethylsilyl)-derivative. The ^{2}H n.m.r. spectrum of the singly-labelled species showed three overlapping signals at δ 1.90, 2.03, and 2.10. In the doubly-labelled material the peak at δ 1.90 was replaced by a doublet (J 20 Hz). That the deuterium was indeed located at C-8 was shown by examination of the carbon-13 n.m.r. spectrum which showed that the signal at δ 39.8 p.p.m., assigned to C-8, bore satellites from the ^{13}C-^{2}H material (J 18.5 $\pm$ 1.5 Hz) showing an upfield shift of δ 0.23 p.p.m. Hence the postulated hydrogen shift has occurred within an isoprene unit from C-9 to C-8.

These results suggest that those cyclic diterpenoids in which the initial cyclization involves a bicyclic labdane type of intermediate, should be divided into two groups based on the different (chair–chair or chair–boat) conformations of geranylgeranyl pyrophosphate in this initial step in the biosynthesis.

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