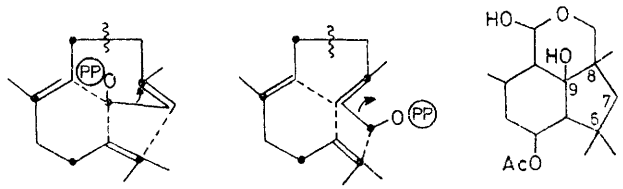
Use of Induced ^{13}C–^{13}C Coupling in Terpenoid Biosynthesis

By A. PETER W. BRADSHAW, JAMES R. HANSON,* and MICHAEL SIVERNS
(*School of Molecular Sciences, University of Sussex, Brighton, Sussex* BN1 9QJ)

Summary The coupling pattern of the sesquiterpenoid, dihydrobotrydial (**3**), biosynthetically multiply labelled from [1-^{13}C]acetate, has been used to define the folding of farnesyl pyrophosphate as (**2**) during its biosynthesis.

DIHYDROBOTRYDIAL (**3**) is a sesquiterpenoid metabolite of the plant pathogen, *Botrytis cinerea*.[1] The coupling and enrichment pattern of material which was biosynthesized from [1,2-$^{13}C_2$]acetate and [4,5-$^{13}C_2$]mevalonate implied[2] that it was formed from either 2-*cis* (**1**) or 2-*trans*-farnesyl pyrophosphate (**2**) in the manner shown. We favoured the 2-*trans* folding (**2**) because of the number of [5-$^{3}H_2$]mevalonate labels which were incorporated. However because of the relative inaccessibility of C-7, which is flanked by two quaternary centres, this was not accompanied by the appropriate degradation to locate these labels. We now report an alternative solution to this problem.



(1) (2) (3)

• label from $CH_3^{\bullet}CO_2H$

When [1,2-$^{13}C_2$]acetate has been used to study polyketide biosynthesis, small couplings have occasionally been noted[3] between acetate units as well as within acetate units. We have recently observed[4] this in the case of the sesquiterpenoid trichothecin and traced its origin to the inhibition of endogenous acetate synthesis from pyruvate and citrate by the exogenous acetate. If sufficient [1,2-$^{13}C_2$]acetate is then fed in one batch at the time of maximum metabolite production, a number of molecules will be biosynthesized containing more than one labelled unit and hence the additional couplings will be observed. The normal protocol to overcome this effect is to feed the [1,2-$^{13}C_2$]acetate diluted with unlabelled material in a number of small batches over the period of metabolite production. However these inter-unit couplings can be utilized in terpenoid biosynthesis to reveal rearrangements as in the case of the sesquiterpenoid dihydrobotrydial.

If [1-^{13}C]acetate is used as the substrate, folding (**1**) would generate coupling between C-8 and C-9, whereas folding (**2**) would generate couplings between C-6 and C-7 and between C-7 and C-8. In the event, when sodium [1-^{13}C]acetate was fed to the fungus at the time of maximum metabolite production, coupling was observed in the product between C-6 and C-7 (J 33·6 Hz) and between C-7 and C-8 (J 35·1 Hz). There was sufficient incorporation from the [1-^{13}C] acetate for five lines to be observed from C-7 implying that molecules had been biosynthesized with exogenous acetate in both the starter and terminal isoprene units. This result is in accord with the incorporation of 2-*trans*-farnesyl pyrophosphate as in (**2**). 1,2-Shifts of the type shown by dihydrobotrydial are quite common in terpenoid biosynthesis and hence the induction of ^{13}C–^{13}C couplings in this way might be useful in demonstrating these rearrangements.

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[4] B. Dockerill and J. R. Hanson, *Phytochemistry*, 1977, in the press.