# Biosynthesis of Fomajorin D: Incorporations of [1-$^{13}$C]-, [2-$^{13}$C]-, and [1,2-$^{13}C_2$]-Acetates

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The $^{13}$C n.m.r. resonances of fomajorin D, a metabolite of *Fomes annosus* (Fr.) Cooke, have been assigned thus confirming the proposed structure (**1**); the labelling patterns of fomajorin D derived from [1-$^{13}$C]-, [2-$^{13}$C]-, and [1,2-$^{13}C_2$]-acetates are in accord with its biosynthesis from farnesyl pyrophosphate in which cyclisation to a protoilludyl cation or its equivalent followed by oxidative cleavage of the appropriate bond would yield the metabolite.

Fomajorin D (**1**)† $C_{15}H_{16}O_3$ and fomajorin S (**2**) $C_{15}H_{14}O_5$ are members of a group of metabolites of the Basidiomycete, *Fomes annosus* (Fr.) Cooke (syn. *Heterobasidion annosum*) and are the first reported naturally occurring fungal isocoumarins without substituents on the lactone ring.[1] Biosynthetic studies[2–4] have shown that naturally occurring isocoumarins (usually found in *Fungi Imperfecti* and *Ascomycetes*) are derived from polyketide. We herein report an example of the biosynthesis of an isocoumarin (fomajorin D) derived from mevalonate.

Cane and Nachbar[5] showed that the sesquiterpene fomannosin (**5**), a co-metabolite of fomajorin D, is biosynthesised from mevalonate *via* a protoilludyl cation (**6**) or its equivalent. A similar biosynthetic pathway for illudin sesquiterpenoids has been demonstrated recently.[6] These studies and the observed presence of fomannosin (**5**), protoilludenol (**3**), and the indan type alcohol (**4**) in the closely related Basidiomycete, *Fomitoposis insularis*,[7] suggested that fomajorin D arises from the same biosynthetic intermediate, the protoilludyl cation (**6**). We now describe incorporation experiments with [1-$^{13}$C]-, [2-$^{13}$C]-, and [1,2-$^{13}C_2$]-acetate which prove this hypothesis for fomajorin D (**1**) and hence for fomajorin S (**2**).

As a prelude to the application of $^{13}$C labelling studies to the biosynthesis, the $^{13}$C n.m.r. signals from fomajorin D (**1**) were assigned. The resonances were identified utilising their multiplicity in the single frequency off-resonance decoupling spectrum (S.F.O.R.D.), by applying the *J*-modulated spin echo technique[8] in which the quaternary and methylene carbons appear as positive peaks whereas the methine and methyl carbons appear as negative peaks, and by comparison with well-known chemical shift data.[4,9,10] The $^{13}$C n.m.r. spectrum of fomajorin D (**1**) (Table 1) confirms fully the proposed structure.

In the enrichment experiments the labelled [1-$^{13}$C]-, [2-$^{13}$C]-, and [1,2-$^{13}$C]-acetates diluted threefold with unlabelled acetate and containing [2-$^{14}$C]acetate tracer were administered to 27 day old intact surface cultures of *Fomes annosus* (Fr.) Cooke (Strain 608) grown on Raulins medium. After a further six days growth the cultures were harvested and the culture filtrate was extracted with chloroform. The enriched fomajorin D (**1**) was isolated by column chromatography on silica gel and repurified by preparative layer chromatography. The incorporation of $^{14}$C acetate in the [1,2-$^{13}C_2$]acetate feed was 2.3%. Fomajorin S (**2**) was not produced by this culture strain.

Six enhanced signals were detected in the $^{13}$C n.m.r. spectrum of fomajorin D (**1**) derived from the sodium [1-$^{13}$C]-acetate feed, whereas nine enhanced signals were observed

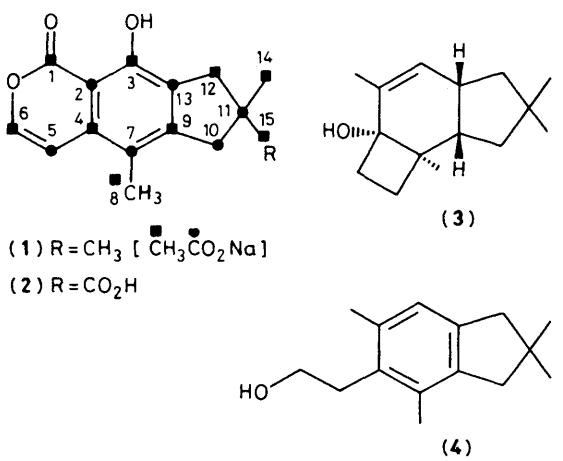
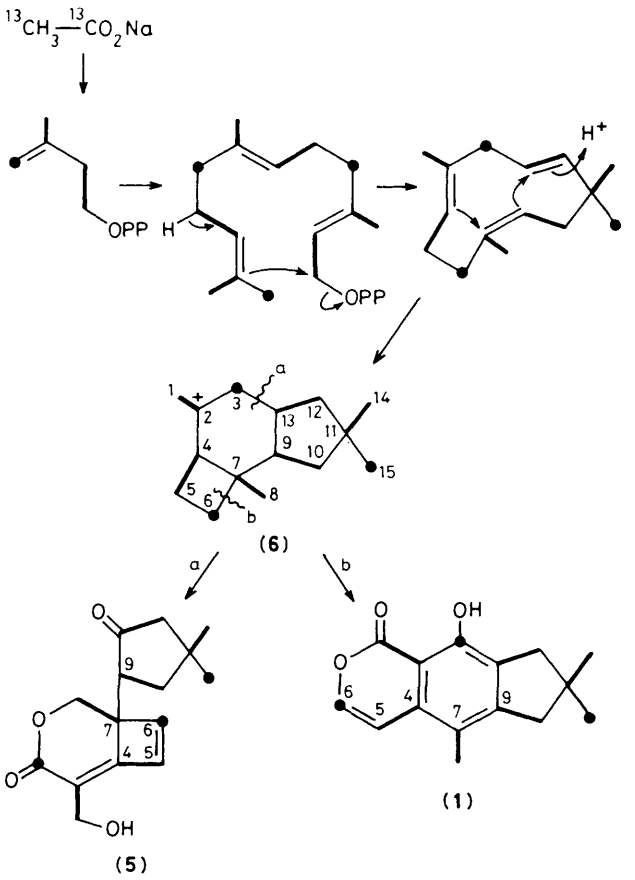



**Figure 1**




**Scheme 1.** The thick lines denote pairs of coupled atoms and ● denotes uncoupled atoms in the [1,2-$^{13}C_2$]acetate labelling experiment. PP = pyrophosphate.

† Fomannosin numbering (not systematic).

**Table 1.** $^{13}C$ N.m.r. data for fomajorin D (**1**).[a]

| Carbon | δ/p.p.m. | | $^{13}C$–$^{13}C$ coupling constants/Hz[b] |
|---|---|---|---|
| 1 | 166.9[c] | s[d] | 69.2 |
| 2 | 105.9[e] | s | f |
| 3 | 155.9[c] | s | s |
| 4 | 133.1[c] | s | 54.9 |
| 5 | 105.4[e] | d | 54.4 |
| 6 | 142.4[c] | d | s |
| 7 | 119.1[e] | s | 46.0 |
| 8 | 14.2[c] | q | 46.3 |
| 9 | 154.1[c] | s | 41.8 |
| 10 | 48.2[e] | t | 41.2 |
| 11 | 39.5[e] | s | 35.1 |
| 12 | 44.0[c] | t | 41.2 |
| 13 | 129.2[e] | s | 41.2 |
| 14 | 29.1[c] | q | 35.1 |
| 15 | 29.1[c] | q | s |

[a] Determination in $CDCl_3$ on a Bruker WM 400 (100.62 MHz). [b] Coupling constants in (**1**) labelled with [1,2-$^{13}C_2$]acetate. [c] Enchanced in intensity after incorporation of [2-$^{13}C$]acetate. [d] Multiplicity in S.F.O.R.D. spectrum: s = singlet, d = doublet, t = triplet, q = quartet. [e] Enchanced in intensity after incorporation of [1-$^{13}C$]acetate. [f] Could not be evaluated because of overlap with C-5.

with the [2-$^{13}C$]acetate feed. This result, shown in Figure 1 and in Table 1, is compatible with a biosynthetic pathway involving a protoilludyl cation (**6**).

The proton noise decoupled $^{13}C$ n.m.r. spectrum of fomajorin D isolated from cultures containing [1,2-$^{13}C_2$]acetate exhibited six pairs of spin coupled doublets appearing as satellites about the natural abundance singlets, as well as three enriched singlets. The latter are due to C-3, C-6, and C-15 and correspond to the C-2 of mevalonate. The observed $^{13}C$ n.m.r. coupling pattern supports a pathway in which mevalonate is converted into an intermediate related to humulene *via* intramolecular cyclisation of farnesyl pyrophosphate. Further cyclisation would afford the protoilludyl cation (**6**) which on oxidative cleavage at bond a would give fomannosin (**5**) and at bond b fomajorin (**1**) (Scheme 1).

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