^{13}C and ^{2}H N.M.R. Studies on the Biosynthesis of *O*-Methylasparvenone, a Hexaketide Metabolite of *Aspergillus parvulus*

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Summary Incorporations of singly and doubly labelled ^{13}C acetates and [$^{2}H_3$]acetate into *O*-methylasparvenone, a dihydronaphthalene metabolite of *Aspergillus parvulus*, and analysis of the resultant enriched samples by high-field ^{13}C and ^{2}H n.m.r. spectroscopy indicate a hexaketide biosynthesis and a novel acetate assembly pattern; the ^{2}H-labelling pattern and levels of enrichment provide information on the sequence and mechanisms of the reduction, oxidation, and deoxygenation steps on the biosynthetic pathway.

THE use of precursors doubly labelled with both ^{13}C and ^{2}H, combined with the use of ^{13}C n.m.r. spectroscopy to determine the fate of acetate or mevalonate hydrogen during the course of a biosynthetic pathway, has been applied with some success in polyketide and terpenoid studies.[1] The alternative method of using ^{2}H-labelled precursors and direct ^{2}H n.m.r. spectroscopy determination of labelling has also been used successfully, particularly in the terpenoid area, notably by Cane and co-workers,[2] but also in the polyketide field, a notable example being the studies of Sato on griseofulvin biosynthesis.[3] With the availability of high-field n.m.r. spectrometers the direct use of ^{2}H n.m.r. spectroscopy becomes an even more attractive option. We now report the results of incorporation studies using both ^{13}C-labelled acetates and ^{2}H-labelled acetate to investigate the biosynthesis of *O*-methylasparvenone (**1**), a metabolite of *Aspergillus parvulus*, a fungus found in the acid soil of pine or sweetgum forests.[4] Preliminary ^{14}C-labelling studies[5] have suggested a polyketide origin for (**1**).

Before carrying out incorporation studies both the ^{1}H and ^{13}C n.m.r. spectra of *O*-methylasparvenone were rigorously assigned. These studies, which will be described in detail elsewhere, resulted in the assignments summarised in Tables 1 and 2. The [1-^{13}C]-, [2-^{13}C]-, and [1,2-$^{13}C_2$]acetates were efficiently incorporated into (**1**) by shaken cultures of

TABLE 1. ^{13}C Chemical shifts (δ, relative to Me_4Si) of *O*-methylasparvenone (**1**); coupling constants (Hz) of [1,2-$^{13}C_2$]acetate-enriched (**1**); and enrichments observed in [1-^{13}C]acetate- and [2-^{13}C]acetate-enriched (**1**).

Carbon	δ/p.p.m.	$^{1}J(^{13}C-^{13}C)$	Enrichment
1	202·0	42	●[a]
2	34·5	41	*[b,c]
3	31·7	37	●
4	68·2	37	*
4a	145·3	62	●
5	100·5	63	*
6	163·6	70	●
7	119·4	71	*
8	161·8	61	●
8a	109·8	62	*[c]
9	15·4	33	●
10	12·9	34	*
MeO	55·6	—	—

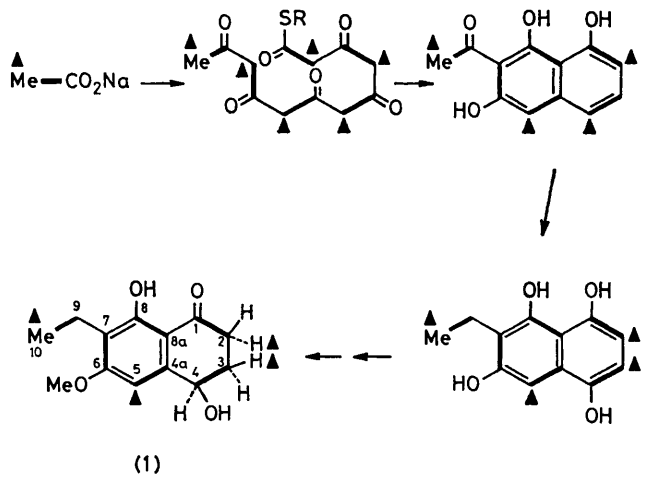
[a] ●, Average enrichment for [1-^{13}C]acetate is 2 atom %. [b] *, Average enrichment for [2-^{13}C]acetate is 14 atom %. [c] ^{2}J ($^{13}C-^{13}C$) of 10 Hz observed.

TABLE 2. ^{1}H Chemical shifts (δ, relative to Me_4Si) of *O*-methylasparvenone (**1**); ^{2}H chemical shifts and relative intensities observed in [$^{2}H_3$]acetate-enriched (**1**).[a]

Hydrogen	δ(^{1}H)[b]	δ(^{2}H)[c]	Relative intensity[d]
2ax	2·52	2·56	0·4
2eq	2·80		
3ax	2·04	2·08	0·2
3eq	2·24		
4ax	4·77		
5	6·61	6·67	1·0
9	2·59		
10	1·03	1·05	1·8
OMe	3·85		

[a] [$^{2}H_3$]Acetate (2g) was distributed among 12 shaken flasks (75 ml medium per 250 ml flask) 36 h after inoculation. After a further 24 h growth, (**1**), (65 mg) was isolated. [b] Measured at 360 MHz. [c] Measured at 55·3 MHz on a Bruker WH 360 spectrometer. [d] Normalised to H-5, which is itself enriched to *ca.* 5 atom % ^{2}H.

A. parvulus and the resultant ^{13}C n.m.r. spectra indicated the enrichments summarised in Table 1. The enrichment levels were essentially identical throughout the molecule and so indicate its formation from a hexaketide with the acetate assembly pattern shown in the Scheme.



(1)

SCHEME. ▲ = ^2H label.

To convert the hexaketide precursor into (**1**) it is necessary to lose acetate-derived oxygen from C-3 and C-9, insert an 'extra' oxygen atom at C-4, and reduce to the dihydronaphthalene oxidation level. It is generally assumed that polyketide oxygen is lost by reduction of the ketone and dehydration of the resulting alcohol as in fatty-acid biosynthesis. The dehydration may be followed by reduction of the double bond to give a saturated system.[6] Information on the timing of these reduction–oxidation processes during biosynthesis of *O*-methylasparvenone has been obtained by incorporation of [$^2H_3$]acetate and determination of the resultant ^2H n.m.r. spectrum. This indicated enrichment of the 10-methyl, 5-, 2-axial, and 3-axial hydrogens, and significantly there was no label on C-4 (Table 2). The only satisfactory explanation for the loss of label from C-4 and its appearance on C-3 is that an N.I.H. shift[7] occurs during hydroxylation of a 1,6,8-trihydroxynaphthalene intermediate to a 1,4,6,8-tetrahydroxynaphthalene as indicated in the Scheme. N.I.H. shifts have been observed during fungal metabolism of a number of aromatic substrates.[8] (The possibility that ^2H appeared on C-3 as a result of ^2H enrichment of the hydrogen-transfer coenzymes by exchange with ^2H from the precursor acetate *via* the medium can be ruled out by the absence of label on C-9). Thus the C-3 polyketide oxygen must be lost before aromatisation; introduction of the 'extra' oxygen on C-4, and reduction to the dihydronaphthalene level must occur after condensation and aromatisation of the precursor polyketide. These results are interesting in relation to recent studies on vermelone [3,4-dihydro-3,8-dihydroxynaphthalen-1(2*H*)-one] and scytalone [3,4-dihydro-8-hydroxynaphthalen-1(2*H*)-one], pentaketide metabolites of *Verticillium dahliae*, which indicate that both reduction and loss of oxygen are post-aromatic processes,[9] and in relation to aflatoxin biosynthesis where the apparent intermediacy of averufin had been questioned on the grounds of the required loss of phenolic oxygen.[10]

While it is recognised that the enriched positions are probably subject to differing probabilities of loss of ^2H through exchange, as their environments alter during the course of the biosynthetic pathway, so making interpretation of differing levels of enrichment subject to uncertainty, the following further observations can be made. (a) The 3-axial position is labelled to only 50% of the level of the 2-axial position. This is entirely consistent with the levels of retention of label commonly observed as a result of N.I.H. shifts. Note that there is no evidence of enrichment of the 2- or 3-equatorial hydrogens so that the ring reduction is an entirely stereospecific process. (b) The 10-methyl is labelled to less than twice the level of H-5. This is particularly surprising as recent ^2H-labelling studies have shown preferential labelling of acetyl coenzyme A-derived 'starter' positions relative to positions derived from malonyl coenzyme A in polyketide metabolites.[11,12] We interpret this observation as a strong indication that the C-9 ketide oxygen is not lost until after aromatisation. This would then allow loss of ^2H-label from C-10 relative to C-5 by exchange from an acetyl side chain and/or *via* reduction and dehydration of the resultant 1'-hydroxyethyl side chain. Some support for this hypothesis comes from the isolation of trace amounts of 9-oxygenated analogues of *O*-methylasparvenone from *A. parvulus* fermentations.[13]

In conclusion, the present studies demonstrate how ^2H n.m.r. labelling studies can provide valuable information on the often inaccessible intermediates and mechanisms of polyketide metabolism. The differing levels of ^2H-labelling and the nature of the intermediates on the biosynthetic pathway are the subject of further investigations which will be reported in due course.

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