Deuterium as a Tracer in Polyketide Biosynthesis: Incorporation of [2-^{13}C,2-$^{2}H_3$]Acetate into Terrein

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Summary [2-^{13}C, 2-$^{2}H_3$]Acetate has been used to investigate the retention of hydrogen from the methyl group of acetate in the biosynthesis of the polyketide, terrein.

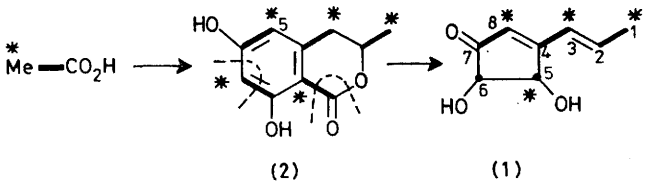
THE application of n.m.r. spectroscopy to tracing the fate of hydrogen in biosynthesis is an attractive goal which has aroused much interest.[1] The detection of deuterium through its coupling to carbon-13 in a ^{13}C n.m.r. spectrum of multiply labelled molecules offers the possibility of establishing the integrity of C–H bonds during the course of a biosynthetic pathway. We have investigated the potential of this approach in polyketide biosynthesis by studying the incorporation of [2-^{13}C,2-$^{2}H_3$]acetate into terrein (**1**). The required precursor was obtained by carboxylation of the Grignard derivative of commercially available [^{13}C,$^{2}H_3$]methyl iodide; [2-^{13}C,1-^{14}C,2-$^{2}H_3$]acetate was prepared using $^{14}CO_2$.

TABLE 1

Incorporation of ^{14}C-labelled acetates into terrein

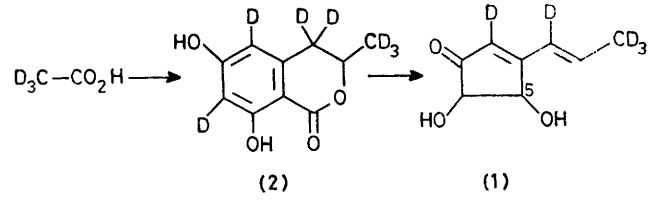
Expt.	Precursor	Incorporation/%	Label at C(2)/%[a]	Ratio of specific activities C(1):C(2)[b]
1	[1-^{14}C] Acetate	0·59	24·1	—
2	[2-^{13}C,1-^{14}C,2-$^{2}H_3$] Acetate	0·44	24·9	—
3	[2-^{14}C] Acetate mixed with [2-^{13}C,1-^{14}C,2-$^{2}H_3$] acetate[c]	0·54	12·1	1·08:1

[a] Obtained by Kuhn–Roth degradation; acetic acid counted as *p*-bromophenacyl derivative. [b] Obtained by comparison of the molar activity of acetic acid (Kuhn–Roth degradation) with that of the derived methylamine (Schmidt degradation; counted as *p*-bromobenzoyl derivative). [c] 65 μCi of each were fed.



SCHEME 1

Terrein (**1**) provides an ideal case study for this investigation. We have shown earlier[2] that the biosynthesis proceeds with the incorporation of three intact C_2 units, the remaining two carbon atoms being derived from separate acetate units (Scheme 1). The dihydroisocoumarin (**2**) is an established intermediate which undergoes an interesting ring contraction as indicated.



SCHEME 2

In principle, 3 of the 4 methyl group-derived carbon atoms of (**1**) could retain deuterium from [2-^{13}C,2-$^{2}H_3$]-acetate, as shown in Scheme 2. The hydrogen atom at C(5) must be derived from the medium and so this carbon atom can be used as an internal standard. It is possible that some loss of deuterium from the other carbon atoms may take place by exchange at one or more of the intermediate stages of biosynthesis. In using deuteriated acetate as a precursor, there is a risk that isotope effects may effect the efficiency of incorporation of acetate into the chain-building units to such an extent that there is a non-uniform labelling of the polyketide chain. We have checked this possibility by incorporation studies with [1-^{14}C,2-^{13}C,2-$^{2}H_3$]acetate. The results of these experiments (Table 1) establish that deuteriated acetate is incorporated in the same way as proteo-acetate within experimental error and that there is no significant 'starter effect.'

The chemical shift data used in interpreting the ^{13}C n.m.r. spectrum of terrein are given in Table 2. As expected, the spectrum of (**1**) enriched with [2-^{13}C,2-$^{2}H_3$]acetate showed intense signals for C(1), C(3), C(5), and C(8), proving that ^{13}C had been incorporated at these positions. Comparison

TABLE 2

^{13}C N.m.r. spectral data for terrein

Carbon	Chemical shifts[a] /p.p.m.	^{13}C–^{13}C coupling[a] in terrein enriched with [2-$^{13}C_2$]acetate	J (^{13}C–H)/Hz
1	18·7[b]	42	121
2	139·5	42	154
3	125·7[b]	56	146
4	168·3	56	—
5	77·2[b]	c	146
6	81·7	c	143
7	202·6	52	—
8	125·1[b]	52	169

[a] Rel. to Me_4Si. Some of these values were inadvertantly transposed in the preparation of a Table in our earlier communication (ref. 2). The correct values are shown here. [b] Enhanced in terrein derived from [2-^{13}C,2-$^{2}H_3$]acetate. [c] Enriched singlet.

of the intensity of the signal from C(6) (unenriched) with that of C(5) (which cannot retain deuterium) indicated that the latter was enriched five-fold relative to natural abundance. The singlets from C(1), C(3), and C(8) were of lower intensity than would be expected on this basis, consistent with the presence of deuterium at these positions.

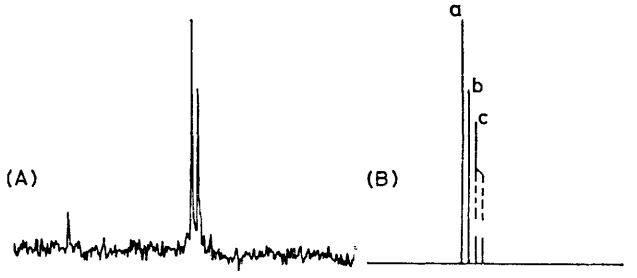


FIGURE 1. Proton-noise-decoupled ^{13}C n.m.r. spectrum of terrein enriched with [2-^{13}C, 2-$^{2}H_3$]acetate. (A) Actual spectrum; (B) line diagram: a, C(3); (b), C(8); (c), two lines of a ^{13}C–D triplet.

The signals from C(3) and C(8) are shown in Figure 1. In addition to the two singlets, it was possible to see two of the three lines of a ^{13}C–D triplet arising from molecules multiply labelled with ^{2}H and ^{13}C at one of these positions. The triplet nature of this signal was confirmed by running the spectrum without proton decoupling so that the ^{13}C–H signals were no longer superimposed on the ^{13}C–D signal (Figure 2). The triplet (J_{C-D} 27·0 Hz) was centred 0·3 p.p.m. upfield from the ^{13}C–H signal for C(8). This is the normal chemical shift difference resulting from deuterium

substitution,[3] and so the triplet was assigned to this carbon rather than C(3). This assignment was supported by the ratio of J_{C-H} : J_{C-D}; the value of 6·3 : 1 for C(8) corresponds within experimental error to the theoretical value of 6·5 : 1, whereas that of 5·4 : 1 for C(**3**) is too low. Thus C(8) of terrein retains one of the hydrogen atoms to which it was attached in the methyl group of acetate. This result eliminates the involvement of the 5-hydroxy derivative of (**2**) during the biosynthesis of terrein.

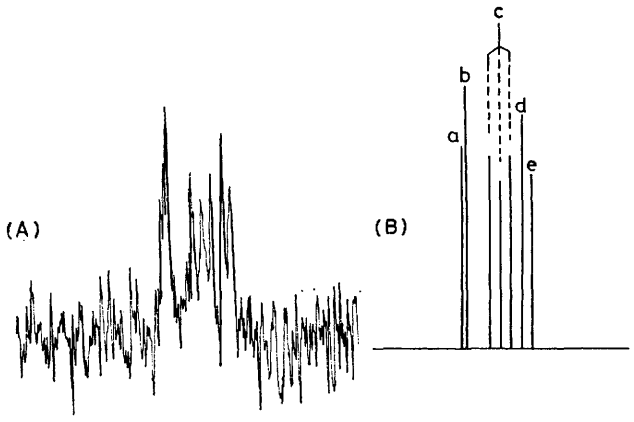


FIGURE 2. Proton-coupled ^{13}C n.m.r. spectrum of terrein enriched with [2-^{13}C, 2-$^{2}H_3$]acetate. (A) Actual spectrum; (B) line diagram: a, C(**3**); b, C(8); c, ^{13}C–D triplet from C(8); d, C(**3**); e, C(8).

The ^{13}C–D triplet corresponding to C(3) (if present) must be much less intense than that from C(8). However, this may be the result of a longer relaxation time (T_1) for C(3) rather than a greater degree of exchange at that position during the biosynthesis. The ^{13}C–D multiplet corresponding to C(**1**) was also too weak to be analysed. We plan in the future to run the spectrum with deuterium decoupling which should greatly simplify analysis of these signals. It will be particularly interesting to see if C(1) shows a singlet in this spectrum, indicating the presence of some $^{13}CD_3$ molecules. This may provide a convenient method of detecting chain starter units in polyketide biosynthesis.

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