Deuterium as a Tracer in Polyketide Biosynthesis: a New Method for the Detection of Chain Starter Units

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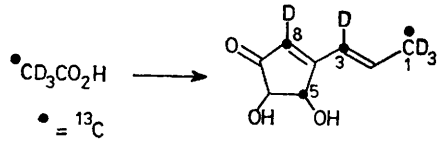
Summary An intact incorporation of a $^{13}CD_3$ group from [2-^{13}C,2-$^{2}H_3$] acetate into the C(1) methyl group of terrein is established from the deuterium-decoupled ^{13}C n.m.r. spectrum of the metabolite; a varying degree of exchange of label along the polyketide chain is detected.

WE recently reported preliminary results[1] of the incorporation of [2-^{13}C,2-$^{2}H_3$]acetate into terrein (**1**). Although there are three potential sites of deuteriation in this metabolite [C(1), C(3), and C(8)], positive evidence for the presence of deuterium was only obtained for C(3), for which a clearly defined ^{13}C-^{2}H triplet 0·3 p.p.m. upfield of the normal ^{13}C signal was observed.

We drew attention to the potential of this approach for identifying chain starter methyl groups in polyketide biosynthesis: it could be a useful alternative to the standard method of studying the differential incorporation of ^{14}C or ^{13}C labelled malonate into the chain starter and chain building units, a method which often gives inconclusive results because the precursor is interconverted with acetate faster than it is incorporated into the metabolite.

Unfortunately, the signals arising from the starter methyl carbon C(1) in the labelled terrein were too complex to be analysed because of the multiplicity which results from deuterium coupling. However, as with C(3) and C(8), the presence of deuterium could be inferred from the weakness of the ^{13}C singlet (arising from molecules labelled with protium at this position) compared with that of C(5) which



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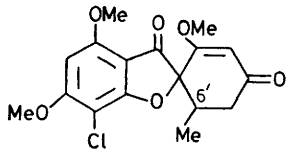

(2)

is enriched with ^{13}C but does not carry deuterium. We have now had the opportunity of running the spectrum with deuterium decoupling which greatly simplifies the analysis of the signals from C(1). A singlet at 17·95 p.p.m. [relative to Me_4Si; 0·81 p.p.m. upfield of the normal chemical shift value for C(1)] is assigned to molecules tri-substituted with deuterium at this position (the normal chemical shift difference arising from isotopic substitution is 0·3 p.p.m. per deuterium). This result confirms that C(1) is a chain starter unit and establishes the viability of the approach. There is also a doublet (J 123 Hz) centred at 18·22 p.p.m., 0·55 p.p.m. upfield of the value for C(1) corresponding to molecules labelled with CHD_2 at this position. The

detection of a CHD_2 doublet and an enriched CH_3 singlet (in the proton noise decoupled spectrum) shows that there is considerable exchange of hydrogen from the methyl group in the course of the biosynthesis.

It is interesting to compare the results of this approach with earlier work[2] in which the incorporation of [2-$^2H_3$]-acetate into griseofulvin (**2**) was monitored by ^2H n.m.r. spectroscopy. It was shown that the presumed chain starter unit (6′-Me) retained approximately 2/3 of the deuterium label from acetate. However, the labelling pattern of individual molecules cannot be determined using ^2H n.m.r. spectroscopy, (in contrast to our indirect ^{13}C–^2H approach) and so it was not possible to establish that the 6′-methyl group is indeed a chain starter unit. The ^{13}C–^2H n.m.r. method is therefore superior for this purpose.

In the deuterium decoupled spectrum of terrein, singlets were observed at 125·3 and 124·8 p.p.m. for C(3) and C(8), respectively (as well as the ^{13}C–H doublets), which establish the retention of some deuterium at both these positions. The intensity of the singlet from C(3) was significantly lower than that from C(8) confirming the evidence obtained from the preliminary spectra that a greater degree of exchange had taken place at C(3). It would be of interest to study the retention of deuterium in a wider range of polyketide metabolites to see if a pattern emerges which might provide information concerning the nature of the early biosynthetic steps.

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[2] Y. Sato, T. Oda, and H. Saito, *Tetrahedron Letters*, 1976, 2695.