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DETECTION OF DEUTERIDE SHIFTS IN THE BIOSYNTHESIS OF THE FUNGAL TRIPRENYLPHENOL, ASCOCHLORIN, BY ^{13}C NUCLEAR MAGNETIC RESONANCE SPECTROSCOPY FOLLOWING INCORPORATION OF [3-^{13}C, 4-$^{2}H_2$]-MEVALONIC ACID

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On the basis of the number of intact [1,2-^{13}C]-acetate residues incorporated into the fungal triprenylphenol, ascochlorin (1), Tanabe et al. have suggested[1] that cyclisation of the farnesyl residue occurs via the terminal epoxide intermediate (2) (scheme). Following ring formation, two 1,2-hydride (C-2 → C-1 and C-6 → C-5) and a 1,2-methyl shift (C-1 → C-6) were proposed during formation of the cyclohexanone moeity of (1).

We have recently reported[2] the usefulness of deuterium as a tracer, in conjunction with Fourier Transform ^{13}C n.m.r. spectroscopy, in following deuteride shifts to ^{13}C enriched centres in terpenoid biosynthesis, following incorporation of suitably $^{2}H/^{13}C$ specifically labelled mevalonoid precursors. The method relies on the observation that ^{2}H directly bonded to a ^{13}C nucleus causes the ^{13}C signal in the proton noise decoupled ^{13}C n.m.r. spectrum to collapse, due to the longer relaxation time (T_1) of ^{13}C and the absence of a nuclear Overhauser effect (cf. ^{13}C - ^{1}H)[3]. Translocation of ^{2}H to an enriched ^{13}C centre, during biosynthesis, can only be observed if the mevalonoid precursor is optimally labelled with ^{2}H and ^{13}C, such that ^{2}H translocates to an incorporated ^{13}C centre within a discrete isoprenoid residue. Since the labelled precursor would be diluted with endogenous unlabelled precursor, the probability that the biosynthesised polyprenyl intermediate would contain more than one $^{2}H/^{13}C$-labelled isoprenoid residue is negligible. Using this approach it should therefore be possible to distinguish between 1, 2- and 1,3-hydride (deuteride) shifts, since, in terpenoid biosynthesis, the former normally occur within discrete isoprenoid residues whilst the latter often take place across separate isoprenoid units.

We have used the technique to examine further the mechanism of carbocyclic ring formation in the biosynthesis of (1). Ascochlorin (133mg), enriched with $^{2}H/^{13}C$, was isolated from 7 day old shaken cultures (8 x 100ml.) of Nectria coccinea CMT 120337C[1] which had been supplemented with [3-^{13}C, 4-$^{2}H_2$]-

mevalono-lactone (380mg; 90 atom % ^{13}C, 98 atom % ^{2}H)[2], 3 days post inoculation. The proton-noise decoupled F.T. ^{13}C n.m.r. spectrum of $^{13}C/^{2}H$ enriched and unlabelled (1) were recorded under identical instrument conditions and a comparison of peak integrals in the two spectra was made. Signal integrals were normalised with respect to C-8, since this carbon atom is not derived from C-3 of mevalonate and provides a strong signal in the ^{13}C n.m.r. spectrum. The results are collected in the Table.

In the spectrum of $^{13}C/^{2}H$ enriched (1) the C-9 signal appeared as two lines, separated by 2 Hz, and showed a 1.2 fold enhancement relative to natural abundance, consistent with its derivation from a C-3 mevalonoid carbon atom. The line to higher field was due to $^{2}H/^{13}C$ enriched molecules of (1) containing ^{13}C at C-9 and ^{2}H at C-10. The line to lower field was due to natural abundance ^{13}C at C-9 in unenriched molecules of (1). This is in accord with the previously observed secondary isotopic substitution effect (i.e. $^{13}C-C-^{2}H$ vs. $^{13}C-C-^{1}H$)[4]. The signals due to C-1 and C-5, which would also derive from C-3 mevalonoid carbon atoms, showed negligible enhancement relative to the corresponding signals in the natural abundance ^{13}C spectrum of (1). The collapse of the ^{13}C signals due to C-1 and C-5, relative to C-9, in $^{2}H/^{13}C$ enriched (1) must therefore be a consequence of the migration of (4R)-mevalonoid ^{2}H atoms to the former positions during the biosynthesis of (1). Substitution of ^{1}H by ^{2}H at a nucleus, in addition to causing the collapse of

Table

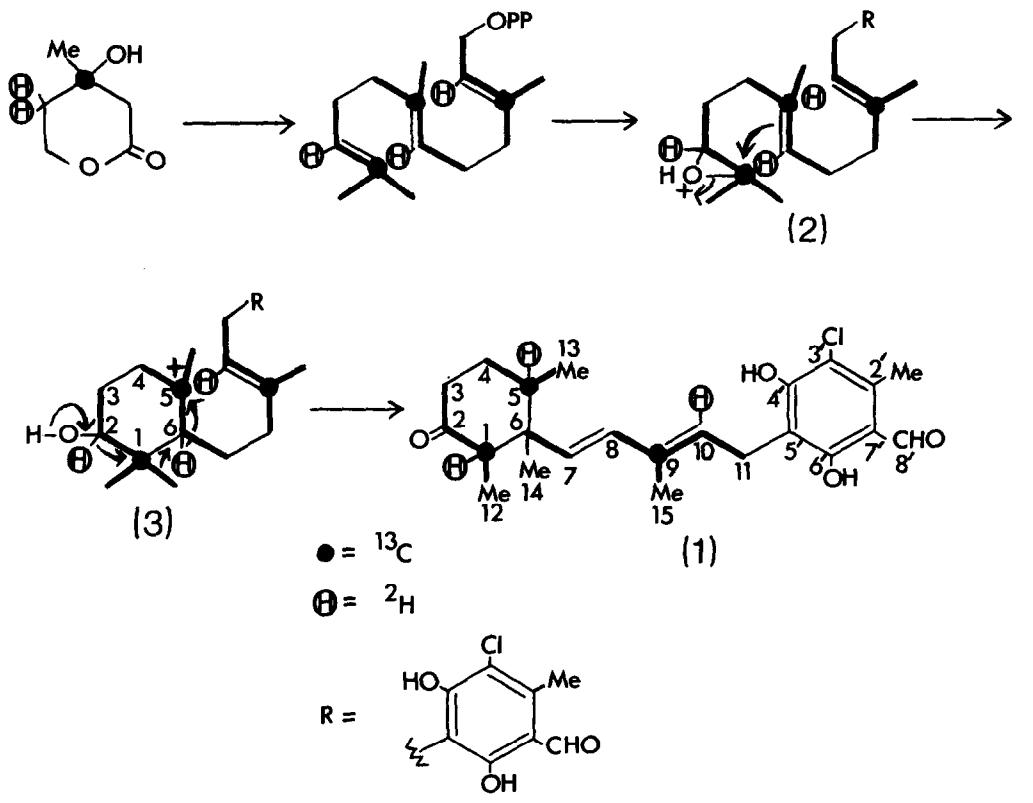
Assignment[a]		Chemical Shift[b]	Enrichment[c]
C-1		53.79	1.22
C-5		41.01	1.19
C-9	$-C(Me)(^{2}H)-$	134.43	
			2.21
	$-C(Me)(^{1}H)-$	134.54	
Rest		-	1.04 ± 0.08

a) made from Ref. 1

b) ^{13}C n.m.r. spectra were recorded in CCl_4 on a Varian XL-100-12 n.m.r. spectrometer at 25.2 MHz; signals in p.p.m. to low field of Me_4Si.

c) Peak integral = $\frac{\text{enriched sample normalised w.r.t. C-8}}{\text{natural abundance sample normalised w.r.t. C-8}}$

Scheme



the ^{13}C signal to a triplet, also results in an upfield shift of the ^{13}C signal by ca. 0.3ppm per ^{2}H atom[5]. Two lines of the ^{13}C-^{2}H C-5 triplet, centered at 40.65ppm and 0.36ppm upfield from the natural abundance ^{13}C signal, were clearly visible (J23.9Hz) but of weak intensity. The remaining line of the triplet was obscured by the natural abundance C-5 signal. The triplet due to C-1 in the spectrum of $^{2}H/^{13}C$ enriched (1) could not be discerned in the baseline noise of the spectrum, presumably due to the longer relaxation time of this carbon atom.

The above results eliminate the interisoprenoid translocation of (4R)-mevalonoid hydrogen atoms in the cyclisation of (3) and provide strong evidence for a concerted series of 1,2-shifts in the rearrangement of (3) to (1), in which (4R)-mevalonoid hydrogen atoms migrate from C-2 to C-1 and C-6 to C-5 respectively and one of the C-1 geminal methyl groups translocates to C-6.

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