The Beta-hop:† [2-$^2H_3$,2-$^{13}C_1$]Acetate as a Probe for 1,2-Hydride Shifts in the Biosynthesis of Natural Products

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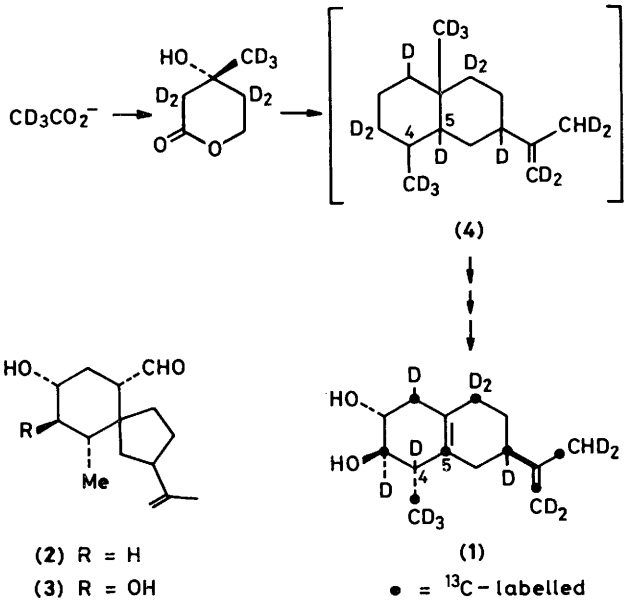
The postulated migration of hydrogen from C-5 to C-4 in the biosynthesis of the potato phytoalexin rishitin (**1**) has been confirmed by incorporation of [2-$^2H_3$,2-$^{13}C_1$]acetate and direct observation of the predicted β-shift of the C-5 resonance due to ^2H at C-4.

The use of stable isotopes in basic precursors, in conjunction with n.m.r. spectroscopy, often leads to solutions to biosynthetic problems which are not readily accessible by classical means. A notable example was the clarification of the nature of a hydride shift in the biosynthesis of the fungal phytotoxin fusicoccin[1] in which the magnetic properties of the nuclei were employed to show that a ^2H atom had migrated to a ^{13}C atom within the same mevalonic acid (MVA) residue.[1] We now report the application of a similar stratagem, but with acetate as an even simpler precursor, and with deuterium migration from one residue to the next.

The biosynthesis of several stress compounds of the *Solanaceae* appears to involve 1,2-hydride shifts from C-5 to C-4.[2] With [4,4-$^2H_2$]MVA as the administered precursor, this has been confirmed for capsidiol in sweet pepper[3] and, in as yet unpublished work, for dihydrolubimin and hence, also lubimin (**2**) in the potato. However, results for rishitin (**1**), another major phytoalexin of the potato, were ambiguous because of inadequate resolution of the observed spectral bands, and a different approach was required. This was particularly important because it has recently been shown by Masamune, Murai, and co-workers[4] that (**1**) is not formed directly from a eudesmane precursor, as postulated earlier by us,[2] but rather *via* lubimin (**2**) and hydroxylubimin (**3**) through an unspecified second rearrangement.

To clarify the situation, we applied 0.03 M sodium [2-$^2H_3$, 2-$^{13}C_1$]acetate (98% ^2H, 90% ^{13}C; Merck, Sharp, and Dohme, Montreal) to potato tubers (20 kg) which had been inoculated with *Monilinia fructicola* a day earlier. Rishitin (20.6 mg) was isolated from the ether-soluble product after another day's incubation as described previously[5] and its ^{13}C n.m.r. spectrum recorded. The eight carbon sites arising from C-2 of the acetate were significantly enriched (*ca.* 4% specific incorporation) as expected from our earlier results using [1,2-$^{13}C_2$]-acetate;[5] these are indicated with dark circles in (**1**). In addition, the absorptions for seven of these sites contained the typical multiplets from directly bonded deuterium. The remaining, fully substituted, enriched centre, C-5, gave rise to

† Beta-hop refers to deuterium migration resulting in an observable β-isotope shift in a ^{13}C n.m.r. spectrum.



the expected closely spaced pair of singlets, clearly indicative of deuterium on a contiguous carbon atom exerting a β-isotope shift[6] (0.045 p.p.m.).

The labelling pattern in the probable eudesmane intermediate in the pathway to (**1**) will be that shown in (**4**). In the subsequent steps to (**1**) *via* (**2**) and (**3**), deuterium migration from C-5 to C-4 will produce an observable β-shift for the ^{13}C atom which was its original partner in an acetate unit. If the 4-^{2}H label had a different origin, *i.e.* a different acetate residue, no shifted component would be observable in the C-5 absorption within the range of normally encountered enrichment levels. The retention of ^{2}H at C-4 also confirms that this centre is not involved in the rearrangement of (**4**) to (**1**).

Thus, the postulated intramolecular migration of hydrogen from C-5 to C-4 in the biosynthesis of the potato phytoalexin rishitin (**1**) is confirmed by the direct observation of the β-shifted component of the C-5 signal. This method, which we term 'beta-hop,' should prove useful for the detection of such 1,2-hydride shifts in other systems.

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