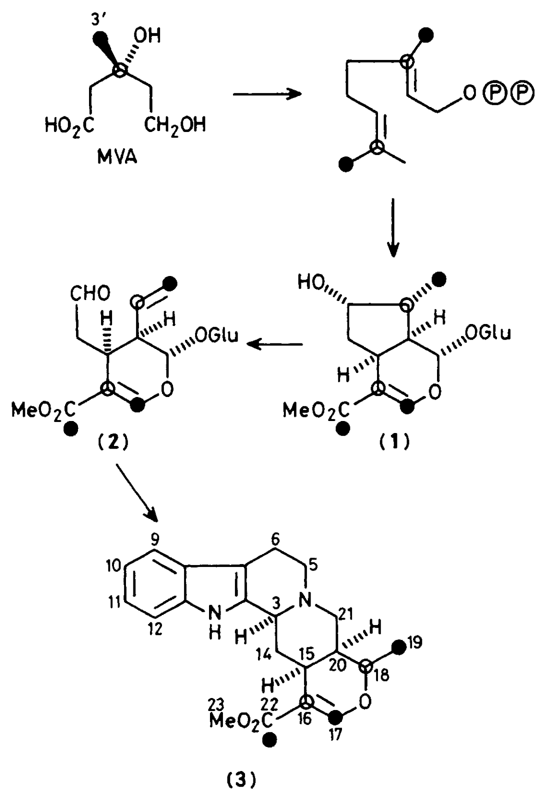
Randomisation and Apparent Regiospecificity in the Incorporation of Acetate into the Monoterpenoid Segment of the *Corynanthe* Alkaloids

Robert L. Baxter* and W. Russell McLauchlan

Department of Chemistry, University of Edinburgh, West Mains Road, Edinburgh, EH9 3JJ, U.K.

Incorporation of ^{13}C-enriched acetates into ajmalicine (**3**) by tissue cultures of *Catharanthus roseus* shows that, although administered [2-^{13}C]- and [1,2-$^{13}C_2$]-acetates are extensively randomised prior to incorporation, the 16-, 17-, 18-, 19-, and 22-positions of the monoterpene skeleton are selectively enriched as a result of equilibration of ^{13}C enrichment in the intermediate 3-hydroxy-3-methylglutaryl (HMG) CoA pool; in contrast, DL-[2-^{13}C]mevalonic acid was incorporated preferentially into the dimethylallylpyrophosphate-derived unit of the monoterpene moiety.

It has been rigorously established by extensive radiolabelling and degradation experiments that the C_{10} unit of the *Corynanthe* alkaloid skeleton is derived from mevalonic acid (MVA) *via* the monoterpenoid iridoid glycosides loganin (**1**) and secologanin (**2**) (Scheme 1).[1] Since acetate appears to be the ubiquitous precursor of mevalonate[2] in plants, it can be reasoned that labelled acetates should be specifically incorporated into the monoterpene unit of these alkaloids. However the results obtained from radiochemical studies of acetate incorporation into ajmalicine (**3**),[3] ajmaline,[4a] cephaeline,[4b] and other indole alkaloids[5] are more consistent with randomisation of ^{14}C from the methyl and carboxylate carbons of acetate than with the regiospecific incorporation predicted on the basis of the acetoacetate–mevalonate pathway. Several factors militate against successful incorporations of common substrates into the terpenoid pathways in intact photosynthetically active plants.[6] A principal difficulty is that the key enzymes of terpene biosynthesis are associated with the chloroplast[7] which is also the site of carbon assimilation *via* the Calvin cycle. This can result in an effective reduction in the utilisation of added precursors. Studies using tissue cultures of plant cells, which lack chloroplasts, have shown however that isotopically enriched acetates,[8] or acetyl CoA precursors,[9] can be regiospecifically incorporated into tri- and sesquiterpenoids. In this communication we describe a re-examination of the role of acetate as a precursor of the monoterpenoid segment of the *Corynanthe* alkaloid, ajmalicine (**3**) in tissue cultures of *Catharanthus roseus* using ^{13}C-enriched acetates.



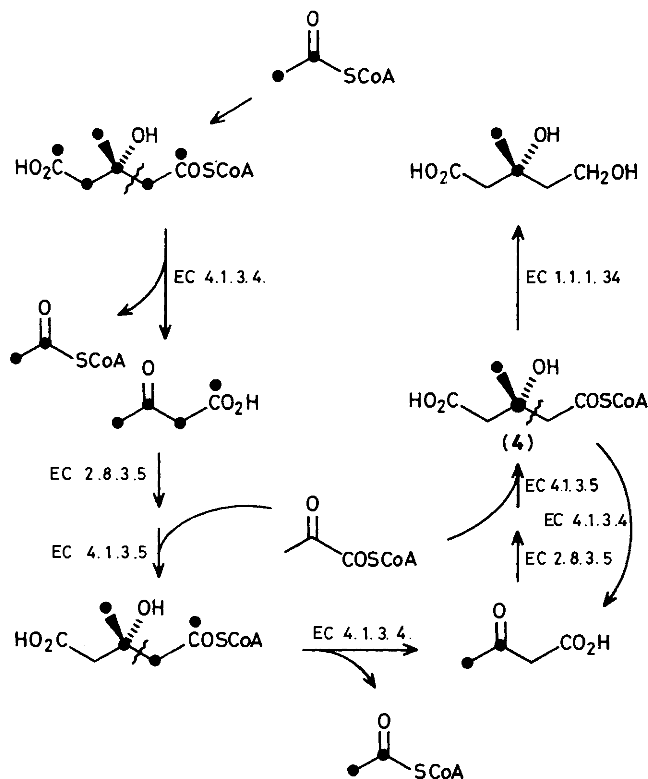
Scheme 1. Origin of the C_{10} unit of ajmalicine (**3**). The fate of the 3- and 3′-carbons of mevalonate (MVA) is shown.

Following a radiochemical assessment of the optimum conditions for [U-^{14}C]acetate incorporation into (**3**) by *C. roseus* cells grown in suspension culture,[10] [1-^{13}C]-, [2-^{13}C]-, and [1,2-$^{13}C_2$]-acetates (1 mg/ml) were pulse fed to cell suspensions (10 × 50 ml) between 10 and 13 days of growth. After 14 days growth the cells were harvested by filtration and extracted with ethanol, and the ajmalicine produced was isolated by successive cation-exchange chromatography and t.l.c. on SiO_2 layers. The $^{13}C\{^1H\}$ spectrum of (**3**) derived from [2-^{13}C]acetate showed enrichment only at the 16-, 17-, 18-, 19-, and 22-positions (1.5, 1.4, 1.8, 2.0, and 1.4 ± 0.2 times natural abundance respectively). In contrast, ajmalicine derived from the [1-^{13}C]acetate experiment showed no significant enhancement of signal intensities over natural abundance. Material derived from the [1,2-$^{13}C_2$]acetate feeding showed a similar pattern of enrichment as for [2-^{13}C]acetate but no multiplet signals corresponding to incorporation of intact acetate units could be detected in the spectrum (Table 1).

Table 1. ^{13}C N.m.r. data for ajmalicine (**3**) produced by tissue cultures of *C. roseus*.[a]

Carbon	Chemical shift and multiplicity[b]	Carbon	Chemical shift and multiplicity
2	134.4 (s)	14	32.9 (t)
3	60.0 (d)	15	30.6 (d)
5	53.1 (t)	16[c,d]	106.7 (s)
6	21.7 (t)	17[c,d,e]	154.5 (d)
7	107.8 (s)	18[c,d]	73.7 (d)
8	127.3 (s)	19[c,d]	14.8 (q)
9	117.9 (d)	20	40.9 (d)
10	121.3 (d)	21	56.8 (t)
11	119.2 (d)	22[c,d,e]	167.3 (s)
12	110.7 (d)	23	50.8 (q)
13	136.0 (s)		

[a] Spectra recorded in $CDCl_3$ at 50 MHz on a Bruker WM-200. [b] Assignment of chemical shifts is based on those reported by R. H. Levin, J. Y. Lallemand, and J. D. Roberts, *J. Org. Chem.*, 1973, **38**, 1983, corroborated by single-frequency ^1H decoupling and DEPT experiments; chemical shifts are expressed in p.p.m. relative to $SiMe_4$, multiplicities [(s), (d), (t), and (q)] correspond to the ^1H coupling observed in the off-resonance decoupled spectrum.[c], [d], and [e] correspond to peaks biosynthetically enriched from [2-^{13}C]- and [1,2-$^{13}C_2$]-acetate and DL-[2-^{13}C]mevalonate respectively.



Scheme 2. Proposed cycle for the equilibration of carbon labelling in HMG CoA derived from doubly enriched acetyl CoA with unenriched acetyl CoA; EC 4.1.3.4 hydroxymethylglutaryl CoA lyase, EC 4.1.3.5 hydroxymethylglutaryl CoA synthetase, EC 2.8.3.5 3-ketoacid CoA transferase, EC 1.1.1.34 hydroxymethylglutaryl CoA reductase.

The results indicate (i) that the MVA precursor to the loganin unit is enriched at C-3 and C-3′ by the methyl carbon of acetate (see Scheme 1) and (ii) that the carboxy carbon of administered acetate is lost prior to MVA formation. The absence of observable coupling between the C-18 and C-19 and the C-16 and C-17 (or C-16 and C-22) carbons indicates that no intact incorporation of *either* administered *or* metabolically derived doubly enriched acetate occurs. Both the loss of acetate ^{13}C-carboxy enrichment and the redistribution of ^{13}C from the methyl group into two adjacent positions, which are *formally* derived from the same acetate molecule, indicate that extensive randomisation of methyl ^{13}C-enriched acetate *via* the Kreb's cycle has occurred to afford a mixture of [1-^{13}C]- and [2-^{13}C]-acetyl CoA prior to MVA formation. Since the tissue culture cells lack an active CO_2 assimilation system uniform reincorporation of label derived from the acetate carboxy, which can occur in intact plants, is not possible.

The regiospecificity of incorporation at the carbon atoms of (**3**) derived from C-3 and C-3′ of MVA, but not at the other positions of the terpenoid segment of the molecule which are derived from C-2, C-4, and C-5 of mevalonate, indicates that further dilution of enrichment at the latter positions must occur during MVA biosynthesis. This can be conjectured to arise through metabolic cycling of the key intermediate 3-hydroxy-3-methylglutaryl CoA (HMG CoA) (**4**) (Scheme 2). Successive degradation and resynthesis of an (initially) randomly ^{13}C-enriched HMG CoA pool in the presence of unenriched acetyl CoA results in the progressive dilution of enrichment at positions 1, 2, 4, and 5 of HMG CoA (**4**) and hence in the production of MVA enriched at only C-3 and C-3′. The existence of such a 'futile' cycle in the acetoacetate–mevalonate pathway permits rationalisation of the variable enrichments obtained in earlier studies of terpenoid biosynthesis in higher plants.[5,11]

The $^{13}C\{^1H\}$ spectrum of ajmalicine derived from administered DL-[2-^{13}C]MVA (Table 1) showed enrichments at C-17 and C-22 (1.3 times natural abundance) indicating that (i) added MVA was incorporated preferentially into the dimethylallylpyrophosphate derived C_5 unit[12] and (ii) the C-17 and C-22 positions are equilibrated at some point in the biosynthesis. This latter effect, which is also observed in the acetate experiments above, is consistent with the original mechanism proposed by Thomas[13] and offers confirmation of radiochemical results obtained with whole plants.[14]

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