Biosynthesis of Lythraceae Alkaloids: Incorporation of DL-[4,5-$^{13}C_2$,6-^{14}C]Lysine and *cis*- and *trans*-4-(3,4-Dihydroxyphenyl)-quinolizidin-2-one into Vertine and Lythrine

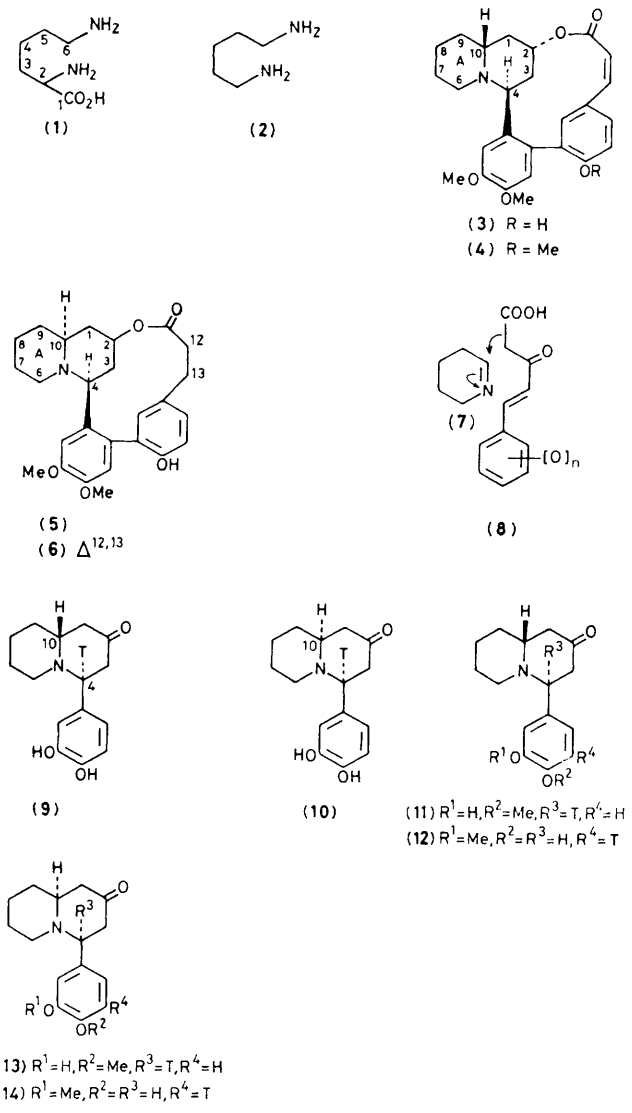
Stuart H. Hedges, Richard B. Herbert,* and Peter C. Wormald

Department of Organic Chemistry, The University, Leeds LS2 9JT, U.K.

DL-[4,5-$^{13}C_2$,6-^{14}C]Lysine is incorporated specifically, and *via* a symmetrical intermediate, into ring A of vertine (**3**) and lythrine (**6**); the *cis*- and *trans*-quinolizidinones, (**9**) and (**10**), are, respectively and specifically, effective precursors for (**3**) and (**6**), and the corresponding mono-*O*-methyl ethers (**11**)—(**14**) are not utilized for alkaloid biosynthesis.

On the basis of results obtained with radioactive precursors in *Decodon verticillatus*, it has been concluded that C-6 to C-10 of the Lythraceae alkaloids, decodine and decinine (**5**), are derived specifically from L-lysine (**1**) and cadaverine (**2**), and that utilization of lysine is necessarily through a symmetrical intermediate (cadaverine).[1] Using a different plant, which produces different alkaloids, we have carried out experiments with ^{13}C-labelled lysine in order to provide independent evidence on the manner of lysine utilization in the biosynthesis of Lythraceae alkaloids. This work is an essential preliminary to detailed probing of the biosynthesis of alkaloids with the decinine (**5**)/vertine (**3**) skeleton, and indeed of other, related alkaloids.

The low incorporation generally observed in the study of biosynthesis in plants means that enrichment of a metabolite by a precursor labelled with ^{13}C is difficult to detect by n.m.r. analysis. An ingenious solution to this problem has been pioneered by Leete.[2,3] It involves the use of two contiguous labels in the precursor. Enrichment of the metabolite is then detected by the presence of satellites flanking the natural abundance singlets in the ^{13}C n.m.r. spectrum of the metabolite; very low levels of enrichment are clearly discernible.[2,3] Wick feeding of DL-[4,5-$^{13}C_2$,6-^{14}C]lysine (99% doubly labelled with ^{13}C; 8.5 μCi; 18 mg) to *Heimia salicifolia* plants gave lythrine (**6**) and vertine (**3**) [analysed as its *O*-methyl ether (**4**)] with, respectively, 0.35 and 0.14% enrichment of label as measured by ^{13}C n.m.r. spectroscopy; closely similar values were obtained through radioactivity measurements. In the ^{13}C n.m.r. spectra of each alkaloid (Figure 1) the three highest field signals, and these only, showed satellites arising from ^{13}C–^{13}C coupling, *i.e.* labelling by the precursor. These signals were clearly assignable to C-7, C-8, and C-9 (see Table 1) firstly from published values for model compounds,[4] particularly of relevance to (**6**) and by selective off-resonance proton decoupling on (**4**). Second, since for each alkaloid one of the three highest field signals shows coupling to the other two, the three carbon atoms giving rise to these signals must be contiguous and C-7, C-8, and C-9 are the only three adjacent



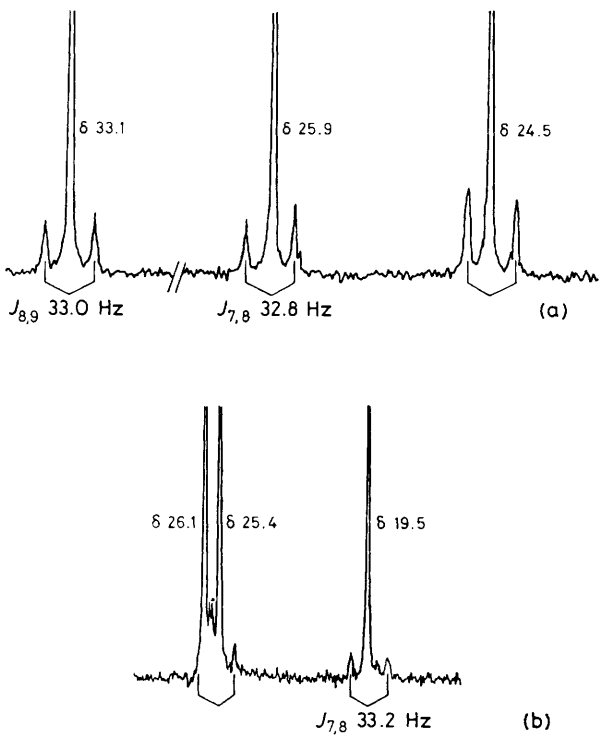


Figure 1. ^{13}C N.m.r. signals enriched by [4,5-$^{13}C_2$]lysine on incorporation (a) into lythrine (**6**) (100 MHz ^{13}C n.m.r. spectrum), and (b) into vertine [22.5 MHz ^{13}C n.m.r. spectrum obtained on (**4**)].

Table 1. Assignment of ^{13}C resonances in *O*-methylvertine (**4**) and lythrine (**6**).

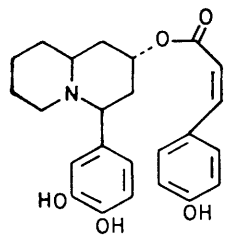
Position	δ/p.p.m. [in (**4**)]	δ/p.p.m. [in (**6**)]
7	19.5	25.9
8	25.4	24.5
9	26.1	33.1
1	34.5	34.5
2	71.6	71.0
3	40.5	39.8
4	48.0	60.2
6	50.3	54.9
10	57.4	61.4

carbon atoms of C-1 to C-10 which together could have such high field resonances. [Table 1 also includes the assignment made of the other resonances associated with the quinolizidine rings in (**4**) and (**6**).] The *γ-gauche* interactions expected for C-7/C-4 and C-9/C-4 in the *cis*-quinolizidine ring system of (**4**), absent in the *trans*-quinolizidine ring system of (**6**), are reflected in the appropriate chemical shift differences for these carbon atoms in (**4**) and (**6**). The *X*-ray crystal structure of vertaline,[5] an alkaloid which like (**3**) has a *cis*-quinolizidine ring system, shows that this alkaloid is an undistorted *cis*-quinolizidine and so, we conclude, is (**3**).

It is apparent from Figure 1 that C-8 of lythrine (**6**) is coupled separately to C-7 and C-9 with a very similar coupling constant; the integration of the satellites for C-8 is twice that for each of the other two. The spectrum of *O*-methylvertine (**4**) is slightly more complex. A clear doublet is apparent for C-7 but only one half of the corresponding doublet is visible on C-8; the proximity of the resonances for C-8 and C-9 means that only the inner lines of the satellite signals can be seen (*cf.* ref. 2). The satellites for (**4**) and (**6**) showed the appropriate upfield shifts relative to natural abundance singlets.

These results clearly demonstrate that lysine provides C-6 to C-10 of vertine (**3**) and of lythrine (**6**), and that this amino-acid is utilized in biosynthesis *via* a symmetrical intermediate [cadaverine (**2**)], in accord with earlier results.[1] Experiments designed to answer further questions about the early stages of the biosynthesis of (**3**) and (**6**) can now follow. In particular why are some piperidine alkaloids, *e.g.* (**3**) and (**6**), formed from lysine through a symmetrical intermediate whereas others, *e.g.* sedamine, are formed without the intervention of any symmetrical intermediates?[6] At the moment there are problems with an attractive model which seeks to explain these differences.[7]

It has been deduced that the biosynthesis of Lythraceae alkaloids proceeds from (**2**) to (**7**)[1] which condenses with (**8**) followed by ring-closure to give quinolizidinone intermediates [as (**9**)],[8] examples of which are naturally occurring.[9] The racemic quinolizidinones (**11**), (**12**), (**13**), and (**14**), labelled as shown, were tested as precursors for (**3**) and (**6**), in *H. salicifolia*, but were not incorporated when lysine was, in a parallel experiment. However, the dihydroxyquinolizidinones (**9**) and (**10**), labels as shown, were found to act as satisfactory pre-



(15)

cursors (the precursors were racemic). The *cis*-isomer (**9**) almost exclusively labelled the *cis*-alkaloid, vertine (**3**) (0.5% incorporation; lysine in a parallel experiment: 0.3% incorporation into both alkaloids) and the *trans*-isomer (**10**) predominantly labelled the *trans*-alkaloid, lythrine (**6**) (0.07% incorporation). Even though only singly labelled precursors were used, the selectivity in the incorporation of each isomer indicates that they were utilized intact. No significant epimerization at either C-10 or C-4 could have occurred because the precursors were racemic and a change at either centre in each racemic pair would have allowed conversion of *cis*-precursor into *trans*-precursor, and *vice versa*.

It follows from the combined results with (**9**) to (**14**) that it is likely that the two phenolic hydroxy-groups in (**9**) and (**10**) remain unmethylated until after phenol oxidative coupling which indicates that (**15**) is the intermediate on which coupling occurs. Experiments to test this are in hand. Only two examples are so far known where three hydroxy-groups are required for phenol oxidative coupling.[10]

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