Biosynthesis of Lythraceae Alkaloids: Incorporation of DL-{4,5-C, 6-C}lysine and cis-and trans-4-(3,4-dihydroxyphenyl)-quinolizidin-2-one Into Vertine and Lythrine

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J. Nat. Prod., **1993**, 56 (8), 1259-1267• DOI:
10.1021/np50098a009 • Publication Date (Web): 01 July 2004

Journal of Natural Products
Vol. 56, No. 8, pp. 1259-1267, August 1993

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[1]

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ABSTRACT.—DL-[4,5-$^{13}C_2$, 6-^{14}C]Lysine is incorporated specifically, and via a symmetrical intermediate, into ring A of vertine [**11**] and lythrine [**10**]; the *cis*- and *trans*-quinolizidinones, **25** and **19**, are, respectively and specifically, effective precursors for **11** and **10**, and the corresponding mono-*O*-methyl ethers **15**, **17**, **21**, and **23** are not effectively utilized for alkaloid biosynthesis.

Study of the biosynthesis of the Lythraceae alkaloids of *Decodon verticillatus*, such as decodine [**7**], has shown that they are constituted from one molecule of L-lysine [**1**] (2) and two of phenylalanine [**4**] (3). Lysine, its decarboxylation product cadaverine [**2**] (3) and Δ^1-piperideine [**3**] all serve as precursors for ring A (C-6 to C-10) of decodine [**7**]. Appropriate to cadaverine being an intermediate following lysine, ^{14}C-label at C-2 or C-6 in the amino acid **1** was found to be incorporated into decodine [**7**] in the manner fitting for a symmetrical intermediate: i.e., in each case the label was deduced to be equally spread over C-6 and C-10.

Phenylalanine [**4**] has been shown to be incorporated into decinine [**8**] and decodine [**7**] as two C_6-C_3 units: C-2 and C-11 of **7** and **8** both derive from C-1 of the amino acid (C-4, -3, -13, and -12 also originate in phenylalanine) (3).

Using a different plant, which produces different alkaloids, we have carried out experiments with ^{13}C-labeled lysine in order to garner independent evidence on the manner of lysine utilization in the biosynthesis of Lythraceae alkaloids. We have also obtained results which point to the later stages of biosynthesis being very interesting (1).

RESULTS AND DISCUSSION

The low incorporation generally observed in the study of biosynthesis in plants means the enrichment of a metabolite by a precursor labeled with ^{13}C is difficult to detect by nmr analysis. An ingenious solution to this problem has been pioneered by Eddie Leete (4,5). 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-nmr spectrum of the metabolite; very low levels of enrichment are clearly discernible (4–6). Wick feeding of DL-[4,5-$^{13}C_2$, 6-^{14}C]lysine (99.8% doubly labeled with ^{13}C, 8.5 μCi, 18.7 mg) to *Heimia salicifolia* Link & Otto (Lythraceae) plants gave lythrine [**10**] and vertine [**11**] (analyzed as its *O*-methyl ether **12**) with, respectively, 0.35 and 0.14% enrichment of label as measured by ^{13}C-nmr spectroscopy; closely similar values were obtained through radioactivity measurements. In the ^{13}C-nmr spectrum of each alkaloid (Figure 1) the three highest field signals, and these only, showed satellites arising from ^{13}C-^{13}C coupling, i.e., labeling by the precursor. These signals were clearly assignable to C-7, C-8, and C-9 (Table 1), first from published values for model compounds (7), particularly of relevance to **10**, and by selective off-resonance proton decoupling on **12**. Second, since for each alkaloid one of

[1]This paper is dedicated to the affectionate memory of Professor Edward Leete.

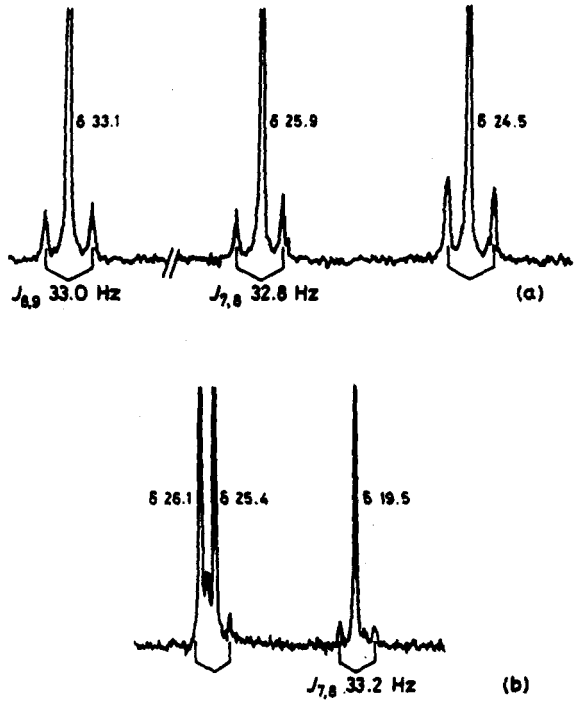
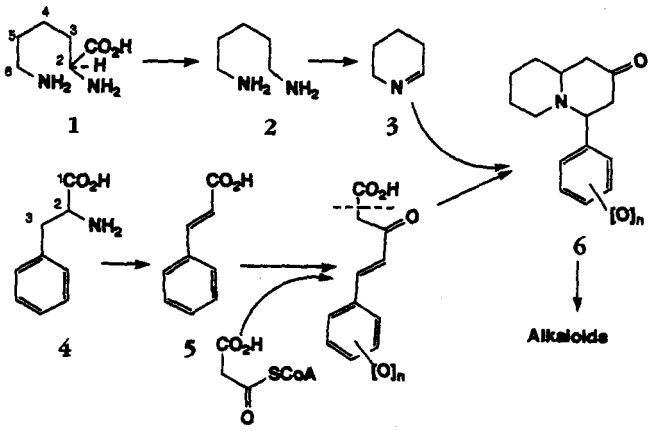


FIGURE 1. ^{13}C-nmr signals enriched by [4,5-$^{13}C_2$]lysine on incorporation (a) into lythrine [**10**] (100 MHz ^{13}C-nmr spectrum), and (b) into *O*-methylvertine [**12**] (22.5 MHz ^{13}C-nmr spectrum).

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 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 **10** and **12**.) The γ-gauche interactions expected for C-7/C-4 and C-9/C-4 in the *cis*-quinolizidine ring system of **12**, absent in the *trans*-quinolizidine ring system of **10**, are reflected in the appropriate chemical shift differences for these carbon atoms in **10** and **12**. The X-ray crystal structure of vertaline



SCHEME 1

TABLE 1. Assignment of ^{13}C resonances in *O*-methylvertine [**12**] and lythrine [**10**].

Carbon	Compound	
	12	**10**
C-7	19.5	25.9
C-8	25.4	24.5
C-9	26.1	33.1
C-1	34.5	34.5
C-2	71.6	71.0
C-3	40.5	39.8
C-4	48.0	60.2
C-6	50.3	54.9
C-10	57.4	61.4

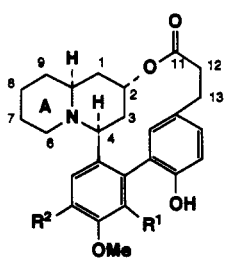
(8), an alkaloid which like **12** has a *cis*-quinolizidine ring system, shows that this alkaloid is an undistorted *cis*-quinolizidine, and so, we conclude, is **12**.

It is apparent from Figure 1 that C-8 of lythrine [**10**] 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 [**12**] 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. Leete (4)]. The satellites for **10** and **12** showed the appropriate upfield shifts relative to natural abundance singlets.

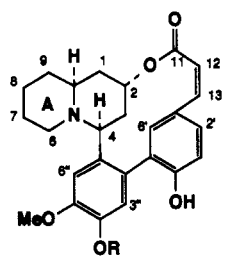
These results clearly demonstrate that lysine provides C-6 to C-10 of vertine [**11**] and of lythrine [**10**], and that this amino acid is utilized in biosynthesis via a symmetrical intermediate (cadaverine [**2**]), in accord with the earlier results discussed above.

It is notable that for some piperidine alkaloids such as the Lythraceae bases, L-lysine that is administered to the plants is incorporated via the symmetrical compound cadaverine [**2**], now proved independently by our results, while for some piperidine bases such as sedamine [**13**], lysine is incorporated without passing through any symmetrical intermediates (9,10).

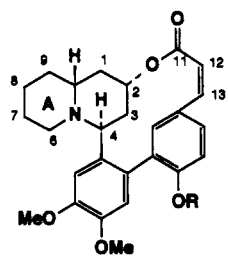
From our results and those of others it follows as likely that the Lythraceae skeleton in, e.g., **10** is biosynthesized by the route shown in Scheme 1. A putative key intermediate in this route is the quinolizidinone **6**, examples of which are naturally occurring (11). The role of **6** in biosynthesis was examined in a set of precursor feeding experiments. The racemic quinolizidinones **15** (X=^{3}H), **21** (X=^{3}H), **17** (X=^{3}H), and **23** (X=^{3}H), labeled as shown, were synthesized in a similar, straightforward way following well-delineated precedent (12). Δ^1-Piperideine [**3**] was prepared from cadav-



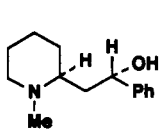
7 R^1=OH, R^2=H
8 R^1=H, R^2=OH



9 R=H
10 R=Me



11 R=H
12 R=Me



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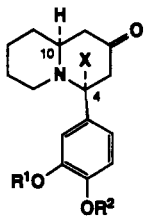
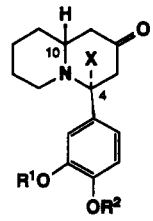
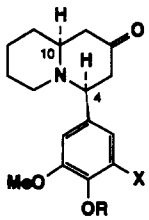
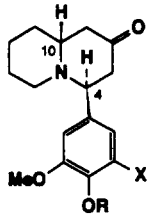




14 R^1=$PhCH_2$, R^2=Me
15 R^1=H, R^2=Me
18 R^1=R^2=$PhCH_2$
19 R^1=R^2=H

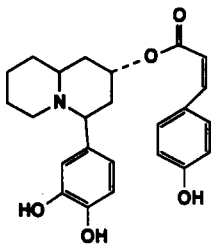
20 R^1=$PhCH_2$, R^2=Me
21 R^1=H, R^2=Me
24 R^1=R^2=$PhCH_2$
25 R^1=R^2=H





16 R=$PhCH_2$
17 R=H

22 R=$PhCH_2$
23 R=H



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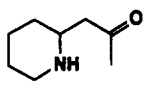

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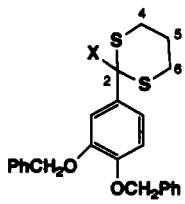

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erine by incubation with pea seedling diamine oxidase. Condensation in situ with acetoacetic acid yielded pelletierine [**27**]. [For other applications of this diamine oxidase in organic synthesis see Herbert and co-workers (13,14).] The pelletierine was condensed with the appropriate tritiated aldehyde to give the required quinolizidinones after removal of the protecting groups. Preparation of the tritiated aldehydes followed either of two excellent published methods: proton exchange (15) of the starting aldehyde sufficed for the preparation of **17** (X=^{3}H) and **23** (X=^{3}H) but because of the site of coupling in the putative formation of the alkaloids from **15** (X=^{3}H) and **21** (X=^{3}H) a different labeling site and method (16) were required.

To our surprise, none of these quinolizidinones were incorporated into **9** and **11** in *H. salicifolia* on one occasion and on another at a very low level into **9–11** when in a parallel experiment lysine was satisfactorily incorporated. However, the dihydroxyquinolizidinones **19** (X=^{3}H) and **25** (X=^{3}H) were found to act as satisfactory precursors. The cis-isomer **25** (X=^{3}H) almost exclusively labeled the cis-alkaloid, vertine [**11**] (0.5% incorporation; lysine in a parallel experiment 0.3% incorporation into both alkaloids) and the trans isomer **19** (X=^{3}H) predominantly labeled the trans-alkaloid, lythrine [**10**] (0.07% incorporation), though this result is much less satisfac-

tory. Even though only singly labeled 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 center in each racemic pair would have allowed conversion of cis-precursor into trans-precursor, and vice versa.

It follows from the combined results with **25** (X=^{3}H), **19** (X=^{3}H), **17** (X=^{3}H), **23** (X=^{3}H), **15** (X=^{3}H), and **21** (X=^{3}H) that it is likely that the two phenolic hydroxy groups in **25** and **19** remain unmethylated until after phenol oxidative coupling, which indicates that **26** is the intermediate on which coupling occurs. Additional experiments are needed to establish or refute this hypothesis. So far our further results have been inconclusive (17,18). If phenol oxidative coupling occurs on **26**, this will be only the second example where the joining of aromatic rings in this way requires two free phenolic hydroxy groups on one of the aromatic rings. The other example is found in the biosynthesis of hasubanonine and protostephanine (19). The mechanism of coupling may well differ from the coupling of two phenolic radicals deduced for the biosynthesis of a host of other secondary metabolites [recently so elegantly proved for the biosynthesis of morphine (20)]. In the case of hasubanonine and protostephanine and maybe the Lythraceae alkaloids a different mechanism involving the substitution of a phenol onto an *ortho*-quinone is an interesting possibility.

EXPERIMENTAL

GENERAL EXPERIMENTAL PROCEDURES.—General procedures were as previously published (13,14,21). Diamine oxidase was isolated following a published procedure and was purified to the stage prior to hydroxyapatite chromatography (22,23). This enzyme was used for the preparation of pelletierine [**27**] from cadaverine plus acetoacetic acid essentially as previously described (24). The pelletierine was isolated as its hydrochloride (27%), mp 143–145° [lit. (24) 141–143°].

trans-*and* cis-*4-(3',4'-Dimethoxyphenyl)-quinolizidin-2-one.*—To a solution of pelletierine hydrochloride (30 mg, 0.17 mmol) was added aqueous NaOH (5%, 2 ml) followed by veratraldehyde (28 mg, 0.17 mmol) and MeOH (1 ml). The mixture was heated under N_2 at 100° (bath temperature) for 5 h. It was cooled and acidified to Congo Red with dilute H_2SO_4. The solution was concentrated in vacuo, H_2O (10 ml) was added, and this solution was washed with Et_2O. The aqueous solution was basified (concentrated aqueous NH_3) and was extracted with $CHCl_3$. The organic extract was dried and evaporated to give a brown oil. Chromatography ($CHCl_3$) gave *trans*-4-(3',4'-dimethoxyphenyl)-quinolizidin-2-one as a colorless oil (30 mg, 61%): ν max ($CHCl_3$) 2795, 2750, 1720 cm^{-1}; δ ($CDCl_3$) 1.2–1.8 (6H, m), 2.2–2.9 (7H, m), 3.2 (1H, dd, J=12 and 5 Hz, H-4), 3.85 (3H, s), 3.88 (3H, s) 6.8–7.0 (3H, m); *m/z* $[M]^+$ 289.16715 ($C_{17}H_{23}NO_3$ requires 289.16778). The cis isomer was also isolated (8%) after the chromatography using 2% MeOH in $CHCl_3$.

cis-4-(3',4'-Dimethoxyphenyl)-quinolizidin-2-one was obtained from a mixture of pelletierine hydrochloride (30 mg, 0.17 mmol), veratraldehyde (28 mg, 0.17 mmol), aqueous NaOH (5%, 2 ml), and MeOH (2 ml), stirred in ice for 5 h. Isolation and chromatography as above gave the *cis*-quinolizidinone as a colorless oil (25 mg, 51%): ν max ($CHCl_3$) 1710 cm^{-1}; δ ($CDCl_3$) 1.2–1.8 (6H, m), 2.1–3.0 (7H, m), 3.85 (6H, s), 4.2 (1H, dd, J=6 and 4 Hz, H-4), 6.6–6.9 (3H, m); *m/z* $[M]^+$ 289.16743 ($C_{17}H_{23}NO_3$ requires 289.16778). The trans isomer was also isolated (10%).

trans-*and* cis-*4-(3'-Hydroxy-4'-methoxyphenyl)-quinolizidin-2-one.*—The trans-isomer **15** (X=^{1}H) was obtained (10 mg, 52%) from isovanillin (17 mg, 0.11 mmol) and pelletierine hydrochloride (20 mg, 0.11 mmol) as described for the trans-dimethoxy compound above. ν max ($CHCl_3$) 3540, 2800, 2760, 1720 cm^{-1}; δ ($CDCl_3$) 1.2–1.9 (6H, m), 2.1–3.0 (7H, m), 3.21 (1H, dd, J=12 and 5 Hz, H-4), 6.7–7.0 (3H, m); *m/z* $[M]^+$ 275.15153 ($C_{16}H_{21}NO_3$ requires 275.15213).

The cis isomer **21** (X=^{1}H) was obtained (12 mg, 39%) from isovanillin (17 mg) and pelletierine hydrochloride (20 mg) as described for the cis dimethoxy compound above. ν max ($CHCl_3$) 3540 and 1715 cm^{-1}; δ ($CDCl_3$) 1.2–1.8 (6H, m), 2.1–3.0 (7H, m) 3.85 (3H, s), 4.19 (1H, dd, J=6 and 4 Hz, H-4), 4.8 (1H, brs, OH), 6.6–6.9 (3H, m); *m/z* $[M]^+$ 275.15153 ($C_{16}H_{21}NO_3$ requires 275.15213).

trans-*and* cis-*4-(4'-Benzyloxy-3'-methoxyphenyl)-quinolizidin-2-one.*—The trans isomer **16** (X=^{1}H) (24 mg, 58%) was prepared from *O*-benzylvanillin (28 mg, 0.11 mmol) and pelletierine hydrochloride (20 mg, 0.11 mmol) as described for the dimethoxy compound above. ν max ($CHCl_3$) 2800, 2760, 1720 cm^{-1};

δ ($CDCl_3$) 1.2–1.9 (6H, m), 2.2–2.9 (7H, m), 3.21 (1H, dd, J=12 and 6 Hz, H-4), 3.9 (3H, s), 5.1 (2H, s), 6.7–7.0 (3H, m), 7.2–7.5 (5H, m); *m/z* $[M]^+$ 365.19871 ($C_{23}H_{27}NO_3$ requires 365.19908).

The cis isomer **22** (X=^{1}H) (18 mg, 43%) was prepared as follows. An ethereal solution (0.3 ml) of *O*-benzylvanillin (28 mg, 0.11 mmol) was added to a solution of pelletierine hydrochloride (20 mg, 0.11 mmol) in EtOH (2 ml). Aqueous NaOH (5%, 0.6 ml) was added to the mixture with care to avoid precipitation of the aldehyde. The solution was stirred in ice for 5 h. Isolation and purification were as above. ν max ($CHCl_3$) 1710 cm^{-1}; δ ($CDCl_3$) 1.2–1.8 (6H, m), 2.2–3.0 (7H, m), 3.85 (3H, s), 4.2 (1H, dd, J=6 and 4 Hz, H-4), 5.1 (2H, s), 6.6–6.9 (3H, m), 7.2–7.5 (5H, m); *m/z* $[M]^+$ 365.19836 ($C_{23}H_{27}NO_3$ requires 365.19908).

trans- *and* cis-*4-(4'-Hydroxy-3'-methoxyphenyl)-quinolizidin-2-one.*—Debenzylation of each isomer (15 mg) of the benzyloxy derivatives prepared above was achieved by hydrogenation in EtOH (4 ml) at amospheric pressure and room temperature with Pd-C (10%, 3 mg). The trans isomer **17** (X=^{1}H) (80% after chromatography): ν max ($CHCl_3$), 3540, 2800, 2760, 1720 cm^{-1}; δ 1.2–1.9 (6H, m), 2.2–2.9 (7H, m), 3.21 (1H, dd, J=12 and 6 Hz, H-4), 3.8 (3H, s), 6.7–6.9 (3H, m); *m/z* $[M]^+$ 275.15099 ($C_{16}H_{23}NO_3$ requires 275.15213).

The cis isomer **23** (X=^{1}H) (74% after chromatography): ν max ($CHCl_3$) 3540 and 1710 cm^{-1}; δ ($CDCl_3$) 1.1–1.9 (6H, m), 2.2–3.0 (7H, m), 3.85 (6H, s), 4.2 (1H, dd, J=6 and 4 Hz, H-4), 5.05 (1H, br s, OH), 6.6–6.9 (3H, m); ms *m/z* $[M]^+$ 275.15108.

trans-*4(-3',4'-Dibenzyloxyphenyl)-quinolizidin-2-one.*—To a solution of pelletierine hydrochloride (0.4 g, 2.25 mmol) in aqueous NaOH (5%, 30 ml) and MeOH (20 ml) was added a solution of 3,4-dibenzyloxybenzaldehyde (25,26) (0.8 g, 2.5 mmol) in dioxan (10 ml); more dioxan (40 ml) was added to dissolve the resulting precipitate. The mixture was heated under N_2 on a steam bath for 5 h. The volume of the mixture was reduced by half by evaporation in vacuo. H_2O (100 ml) was added. The mixture was acidified (dilute H_2SO_4), washed with Et_2O, basified (concentrated aqueous NH_3), and extracted with $CHCl_3$. Drying of the $CHCl_3$ extract followed by evaporation in vacuo gave a pale yellow oil which was purified by chromatography (20% Et_2O in C_6H_6). The required quinolizidinone **18** (X=^{1}H) (0.8 g, 80%) was isolated as a pale yellow oil which crystallized on standing: mp 122–124° (from EtOAc/hexane or EtOH/H_2O); ν max ($CHCl_3$) 3000, 2930, 2860, 2800, 2760, 1715 cm^{-1}; δ_H ($CDCl_3$) 1.1–1.8 (6H, m), 2.1–2.9 (7H, m), 3.15 (1H, dd, J=12 and 4 Hz, H-4), 5.1 (2H, s), 5.13 (2H, s), 6.2–7.1 (3H, m), 7.1–7.6 (10H, m); δ_C ($CDCl_3$) 24.2 (C-8), 25.8 (C-7), 34.3 (C-9), 48.7, 50.8, 52.7 (C-1, -3, -6), 62.3 (C-10), 69.7 (C-4), 71.3, 71.4 (benzyl CH_2), 114.1, 114.9 (C-2', -5'), 120.4 (C-6'), 135.9 (C-1'), 148.6 (C-3'), 149.1 (C-4'), 207.6 (C-2'), 127.7, 128.4, 127.3, 127.5, 137.2, 137.3 (aromatic carbons of benzyl groups) (assignment assisted by pseudo INEPT and ^{2}H at C-4); *m/z* $[M]^+$ 441.23174 ($C_{29}H_{31}NO_3$ requires 441.23038). Found C 78.9, H 6.85, N 3.4%; $C_{29}H_{31}NO_3$ requires C 78.7, H 7.0, N 3.2%. A small amount (6.5%) of the cis-isomer (see below) was also isolated.

cis-*4-(3',4'-Dibenzyloxyphenyl)-quinolizidin-2-one.*—To a solution of pelletierine hydrochloride (0.48 g, 2.2 mmol) in aqueous NaOH (5%, 20 ml) and EtOH (30 ml) was added a solution of 3,4-dibenzyloxybenzaldehyde (0.89 g, 2.8 mmol) in dioxan (40 ml). The mixture was stirred at room temperature in the dark for 48 h. The volume of the mixture was reduced to a half by evaporation in vacuo, and H_2O (100 ml) was added. Isolation and chromatographic purification were as above for the trans isomer. The *cis*-quinolizidinone was obtained (0.57 g, 48%) as a pale yellow oil which could be crystallized: mp 104–107° (from EtOAc/hexane or EtOH/H_2O); ν max ($CHCl_3$) 3000, 2930, 2860, 1710 cm^{-1}; δ_H ($CDCl_3$) 1.2–1.8 (6H, m), 2.2–3.1 (7H, m), 4.12 (1H, dd, J=7 and 4 Hz, H-4), 5.13 (4H, s), 6.5–7.0 (3H, m), 7.4 (10H, s); δ_C ($CDCl_3$) 23.2 (C-7), 24.5 (C-8), 32.5 (C-9), 46.4, 47.6 (C-1, -3), 51.4 (C-6), 53.5 (C-10), 64.1 (C-4), 71.0, 71.1 (benzyl CH_2), 114.0, 115.8 (C-2', -5'), 121.8 (C-6'), 131.8 (C-1'), 147.9 (C-3'), 148.3 (C-4'), 209.4 (C-2), 127.2, 127.6, 127.7, 128.3, 128.4, 137.2 (aromatic carbons of benzyl groups) (assignment assisted by pseudo INEPT and ^{2}H at C -4); *m/z* $[M]^+$ 441.23115 ($C_{29}H_{31}NO_3$ requires 441.23038). Found C 78.75, H 7.2, N 3.3%; $C_{29}H_{31}NO_3$ requires C 78.9, H 7.0, N 3.2%. A small amount (12%) of the trans isomer (see above) was also isolated.

trans- *and* cis-*4-(3',4'-Dihydroxyphenyl)-quinolizidin-2-one.*—Treatment of solutions (EtOH) of the benzyl compounds **18** (X=^{1}H) and **24** (X=^{1}H) with H_2 at room temperature and atmospheric pressure in the presence of Pd-C (10%) gave the dihydroxy compounds **19** (X=^{1}H) and **25** (X=^{1}H), respectively. They were obtained after filtration and evaporation of the solvent and were unstable to further handling. The trans compound **19** (X=^{1}H) was obtained as an oil which crystallized on addition of EtOAc: ν max (Nujol) 3410, 2800, 1720 cm^{-1}; *m/z* $[M]^+$ 261.13685 ($C_{15}H_{19}NO_3$ requires 261.13648).

The cis compound **25** (X=^{1}H) was obtained as a gum which solidified: ν max (Nujol) 3550, 1710 cm^{-1}; *m/z* $[M]^+$ 261.13535 ($C_{15}H_{19}NO_3$ requires 261.13648).

LABELED COMPOUNDS.—The tritiated quinolizidinones used in the feeding experiments were obtained from the corresponding tritiated aldehydes. In each case the deuteriated aldehyde was first prepared to check

the site and extent of labeling. Thus [5-^{2}H]vanillin was prepared following a published procedure (15) (complete deuteriation by ms and δ 7.05 ppm signal for H-5 missing in ^{1}H nmr). Tritiation: vanillin (0.5 g), tritiated H_2O (1 ml, 0.5 Ci), and triethylamine heated at 100° for 40 h in a sealed tube. It was benzylated ($PhCH_2Cl$, aqueous KOH, EtOH, 4 h reflux) to give [5-^{3}H]-*O*-benzylvanillin (0.45 g, 17 mCi) which was used to give *cis*- [**17**] (X=^{3}H) and *trans*-[5′-^{3}H]-4-(4′-hydroxy-3′-methoxyphenyl)-quinolizidin-2-one and [**23**] (X=^{3}H), following the procedure for unlabeled material (above).

[Formyl-^{2}H]-*O*-benzylisovanillin was prepared from *O*-benzylisovanillin in a way closely similar to that described for [formyl-^{2}H]-3,4-dibenzyloxybenzaldehyde below. The ^{1}H-nmr showed no aldehyde resonance (δ 9.85), and deuteriation by ms was complete. [Formyl-^{3}H]-*O*-benzylisovanillin was prepared similarly and was used to synthesize *cis*- [**15**] (X=^{3}H) and *trans*-[4-^{3}H]-4-(3′-hydroxy-4′-methoxyphenyl)-quinolizin-2-ones [**21**] (X=^{3}H) by simple adaptation of the method used above to give unlabeled compounds.

cis- *and* trans-*[4-^{3}H]-4-(3′,4′-Dihydroxyphenyl)-quinolizidin-2-one.*—Formyl labeled 3,4-dibenzyloxybenzaldehyde was prepared following a method published for labeled benzaldehyde (16). In the following the synthesis of deuteriated material is described; only slight modification was needed for the preparation of tritiated material.

2-(3′,4′-Dibenzyloxyphenyl)-1,3-dithiane [**28**].—To a stirred solution of 3,4-dibenzyloxybenzaldehyde (2 g, 6.3 mmol) and toluene-*p*-sulfonic acid (0.8 g) in glacial HOAc (30 ml) was added 1,3-propanedithiol (0.9 g, 8.3 mmol). The resulting white precipitate of the dithiane **28** (X=^{1}H) was collected and washed with H_2O and dilute aqueous NaOH. It was recrystallized (EtOH): mp 107.5–108.5° [lit. (27) 109–110°]; δ_C ($CDCl_3$) (partial) 23.03 (C-5), 32.02 (C-4, -6), 50.98 (C-2); *m/z* $[M]^+$ 408.12335 ($C_{24}H_{24}O_2S_2$ requires 408.12176).

To a stirred solution of *n*-butyl lithium (1.6 M, 1 ml) at −78° under dry N_2 was added a solution of the above dithiane (0.5 g, 1.22 mmol) in dry THF (30 ml). After stirring for 2.5 h, D_2O (1.5 ml) was added and the temperature of the mixture was allowed to rise to room temperature. The solvent was removed in vacuo, and the residue was taken up in $CHCl_3$ and washed with dilute aqueous HCl and saturated brine. The $CHCl_3$ solution was taken to dryness after drying to yield [2-^{2}H]-2-(3′,4′-dibenzyloxyphenyl)-1,3-dithiane [**27**] (X=^{2}H) (82 % after recrystallization). Analysis by ms showed approx. 70% deuteriation, and the signal at δ 51.03 (C-2) in the ^{13}C nmr was of markedly reduced intensity. Tritiated material was prepared similarly, from tritiated H_2O.

[Formyl-^{2}H]-3,4-dibenzyloxybenzaldehyde.—To a warm solution of the deuteriated dithiane (above) (150 mg, 0.37 mmol) in MeCN (10 ml) and H_2O (0.5 ml) was added mercuric oxide (80 mg, 0.37 mmol). A solution of aqueous $HgCl_2$ (200 mg, 0.74 mmol in 2 ml H_2O) was added, and the mixture was heated under N_2 under reflux for 4 h. The mixture was filtered, and the filtrate was taken to dryness. The residue was taken into $CHCl_3$ which was washed with 5% aqueous NaOAc and with saturated brine. After drying, the $CHCl_3$ solution was evaporated to give [formyl-^{2}H]-3,4-dibenzyloxybenzaldehyde (90%) needing no further purification; unlabeled material was identical with an authentic sample. Analysis by ms showed approximately 65% deuteriation; in the ^{13}C nmr a triplet signal at δ 190.37 (J=26.6 Hz) was apparent for the carbonyl group (isotope shift 6.1 Hz).

[4-^{2}H]-trans-4-(3′,4′-dibenzyloxyphenyl)-quinolizidin-2-one [**18**] (X=^{2}H).—This compound showed a large reduction in the signal at δ 69.7 (C-4) in its ^{13}C nmr, and the ^{13}C nmr of [4-^{2}H]-*cis*-4-(3′,4′-dibenzyloxyphenyl)-quinolizidin-2-one [**24**] (X=^{2}H) similarly showed a large reduction in the signal for C-4 at δ 64.1.

ALKALOIDS: ISOLATION, PURIFICATION, AND CHARACTERIZATION.—Crude alkaloid (typically 0.25 g from 150 g of plant material) was obtained in the usual way (28) from an EtOH extract of macerated whole plants of *H. salicifolia*. Cc on neutral alumina (50 g) with 2% MeOH in C_6H_6 gave a mixture of vertine [**11**] and lythrine [**10**]. Elution with 10% MeOH in C_6H_6 gave lyfoline [**9**] plus many minor components. The lyfoline could be purified by preparative tlc (10% MeOH/$CHCl_3$ + drops of concentrated aqueous NH_3). For convenience of repeated crystallization it was handled as its diacetyl derivative (Ac_2O/pyridine). The mixture of lythrine and vertine was separated by cc on Kieselgel G60 (20 g). Lythrine (50 mg) was eluted with 2% MeOH/$CHCl_3$ and vertine (90 mg) with 4% MeOH/$CHCl_3$.

Diacetyllyfoline.—Mp 210–211° from MeOH; ν max (Nujol) 1770, 1710 cm^{-1}; ^{1}H nmr δ ($CHCl_3$) 1.2–2.7 (13H, *m*), 2.05 (3H, s), 2.31 (3H, s), 3.58 (1H, d, J=12 Hz, H-4), 3.8 (3H, s), 5.32 (1H, brs), 5.95 (1H, d, J=13 Hz), 6.76 (1H, d, J=13 Hz), 6.95 (1H, s), 7.15 (1H, s), 7.2–7.35 (3H, m); *m/z* $[M]^+$ 505.20981 ($C_{29}H_{31}NO_7$ requires 505.21004). Found C 69.0, H 6.3, N 2.8%; Calcd for $C_{29}H_{31}NO_7$, C 68.9, H 6.15, N 2.75%.

Vertine.—Mp 240–242° from MeOH [lit. (29) mp 250–252° (245–247°)]; ν max ($CHCl_3$) 3540, 1710 cm^{-1}; ^{1}H nmr δ ($CDCl_3$) 3.3 (13H, m), 3.85 (6H, s), 4.7 (1H, d, J=12 Hz, H-4), 5.3 (1H, brs), 5.75 (1H,

d, J=13 Hz), 6.75 (1H, d, J=13 Hz), 6.95–7.2 (4H, m), 7.63 (1H, s); *m/z* $[M]^+$ 435.20384 ($C_{26}H_{29}NO_5$ requires 435.20456). Found C 71.4, H 6.65, N 3.34; calcd for $C_{26}H_{29}NO_5$, C 71.7, H 6.6, N 3.2%.

O-Methylvertine.—Prepared from vertine with CH_2N_2, purified on hplc, silica with 3.5% MeOH+1% NH_3 saturated MeOH in $CHCl_3$: mp 222–226° from EtOAc [lit. (26) 225–228°]; ^{1}H nmr δ (400 MHz, $CDCl_3$) 0.46 (1H, tq, J=13 and 7.5 Hz, H-7ax), 0.75 (1H, d, J=13 Hz, H-7eq), 1.01 (1H, d, J=13 Hz, H-9ax), 1.14–1.34 (1H, m, H-8ax), 1.34–1.42 (1H, m, H-8eq), 1.65 (1H, dq, J=13 and 4.5 Hz, H-9eq), 1.68 (1H, d, J=15 Hz, H-1ax), 1.91 (1H, t, J=12.5 Hz, H-3ax), 2.04–2.13 (1H, m, H-1eq), 2.31 (1H, td, J=14 and 2.3 Hz, H-3eq), 2.45 (1H, dt, J=13.5 and 2.5 Hz, H-6ax), 2.92 (1H, m, H-6eq), 3.1–3.18 (1H, m, H-10), 3.78, 3.88, and 3.91 (each 3H, s), 4.6 (1H, d, J=11 Hz, H-4), 5.36 (1H, m, H-2), 5.87 (1H, d, J=13 Hz, H-12), 6.81 (1H, d, J=13 Hz, H-13), 6.95–6.99 (1H, m, H-3″), 6.98 (1H, d, J=9 Hz, H-3′), 7.02 (1H, s, H-6″), 7.19 (1H, d, J=2.5 Hz, H-6′), 7.26 (1H, dd, J=9 and 2.5 Hz, H-4′); ^{13}C nmr δ (100 MHz, $CDCl_3$), 19.5 (C-7), 25.4 (C-8), 26.1 (C-9), 34.5 (C-1), 40.5 (C-3), 48.0 (C-4), 50.3 (C-6), 55.5, 55.9, and 56.1 (3×OMe), 57.4 (C-10), 71.6 (C-2), 109.4, 111.0, and 114.1 (C-3′, -3″, -6″), 119.4 (C-12), 125.95, 127.42, and 128.54 (C-1′, -1″, -5′), 129.95 and 131.8 (C-4′, -6′), 132.2 (C-2″), 135.4 (C-13), 146.9 (C-5″), 149.5 (C-4″), 157.5 (C-2′), 167.8 (C-11); ms *m/z* $[M]^+$ 449.22000 ($C_{27}H_{31}NO_5$ requires 449.22021).

Lythrine.—Mp 242–244° from CH_2Cl_2, [lit. (29) 243–245°]; ν max 3400, 2930, 2350 (Bohlmann), 1695 cm^{-1}; ^{1}H nmr δ ($CDCl_3$) 1.0–2.5 (12H, protons on C-1, -3, -6, -7, -8, -9), 2.55–2.8 (1H, m, H-10), 3.66 (1H, dd, J=10 and 2.5 Hz, H-4), 3.88 and 3.94 (each 3H, s), 5.35 (2H, m, H-13), 5.87 (1H, d, J=12.5 Hz, H-12), 6.80 (1H, d, J=12.5 Hz, H-13), 6.94 (1H, s, H-3″), 7.0–7.2 (4H, m, H-3′, -4′, -6′, -6″); ^{13}C nmr (100 MHz, $CDCl_3$) 24.5 (C-8), 25.9 (C-7), 33.1 (C-9), 34.5 (C-1), 39.8 (C-3), 54.9 (C-6), 56.2 and 56.4 (2×OMe), 60.2 (C-4), 61.4 (C-10), 71.0 (C-2), 110.9 and 114.5 (C-3″, -6″), 115.9 (C-3′), 119.45 (C-12), 135.0 (C-2″), 135.4 (C-13), 124.9, 126.1, and 126.3 (C-1′, -5′, -1″), 130.5 and 131.0 (C-4′, -6′), 148.1 (C-5″), 150.1 (C-4″), 153.6 (C-2′), 168.2 (C-11); *m/z* $[M]^+$ 477.21462 ($C_{28}H_{31}NO_6$ requires 477.21512).

FEEDING EXPERIMENTS.—The *H. salicifolia* were grown in a greenhouse in Leeds from seed supplied by Kew Gardens. Experiments were conducted in the summer. Aqueous solutions of precursor were assimilated into the plants through cotton wicks inserted at the base of the plant stems. The solution was taken up in 12–24 h, residues being washed with fresh H_2O. Isolation took place 10–14 days later. Radioactivity was measured by scintillation counting. Except for the experiment immediately below all alkaloids were crystallized to constant radioactivity.

Experiment with [4,5-$^{13}C_2$,6-^{14}C]-DL-lysine.—The aqueous solution (1–2 ml) of the lysine hydrochloride (18.7 mg, 1.87×10^7 dpm, 99.8% doubly labeled with ^{13}C) was fed to one small plant. The alkaloids were isolated and separated by cc as described above, but they were then purified using hplc to conserve material. Lythrine was purified on Whatman 10 μm silica, 250×10 mm, eluting with 1.75% MeOH/0.5% MeOH saturated with NH_3 in $CHCl_3$; radioactivity coincided with the lythrine fraction. Vertine was converted into *O*-methylvertine which was also purified by hplc (see above). Lythrine 18 mg, 0.3% total ^{14}C incorporation, 0.35% by nmr; *O*-methylvertine 45 mg, 0.18% total ^{14}C incorporation, 0.14% by nmr.

Experiments with the tritiated quinolizidinones.—First summer: **17** (X=^{3}H) (3 mg, 92 μCi) vertine (25 mg), diacetyllyfoline (15 mg); **23** (X=^{3}H) (2 mg, 65 μCi) vertine (29 mg), diacetyllyfoline (17 mg); **15** (X=^{3}H) (2 mg, 387 μCi) vertine (20 mg), diacetyllyfoline (12 mg); **21** (X=^{3}H) (2 mg, 388 μCi) vertine (23 mg), diacetyllyfoline (14 mg). All of the samples were inactive. Second summer: **17** (X=^{3}H) (1.4 mg, 43.2 μCi) vertine (0% incorporation), acetyllythrine (0.007%), diacetyllyfoline (0.013), total 0.02%; **15** (X=^{3}H) (0.42 mg, 83 μCi) vertine (0), acetyllythrine (0.005), diacetyllyfoline (0.005), total 0.1%; **23** (X=^{3}H) (2.9 mg, 93.9 μCi) vertine (0), acetyllythrine (0.001), diacetyllyfoline (0.013), total 0.014%; **21** (X=^{3}H) (0.74 mg, 143 μCi) vertine (0), acetyllythrine (0.01), diacetyllyfoline (<0.01), total <0.02%. [6-^{14}C]-DL-Lysine: vertine, 0.07% incorporation. Third summer: **25** (X=^{3}H) (323 μCi) vertine (0.49), acetyllythrine (0.016), total 0.51%; **19** (X=^{3}H) (372 μCi) vertine (0.012), acetyllythrine (0.071), total 0.08%. [6-^{14}C]-DL-lysine: vertine (0.18), acetyllythrine (0.12), total 0.3%.

ACKNOWLEDGMENTS

We thank Dr. B.E. Mann, University of Sheffield, for nmr spectra and invaluable assistance, Professor E. Leete for a very generous gift of DL-[4,5$^{13}C_2$,6-^{14}C]lysine, and the S.E.R.C. for financial support.

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Received 5 October 1992