The Biosynthesis of Tropic Acid: The (*R*)-D-Phenyllactyl moiety is processed by the Mutase involved in Hyoscyamine Biosynthesis in *Datura stramonium*

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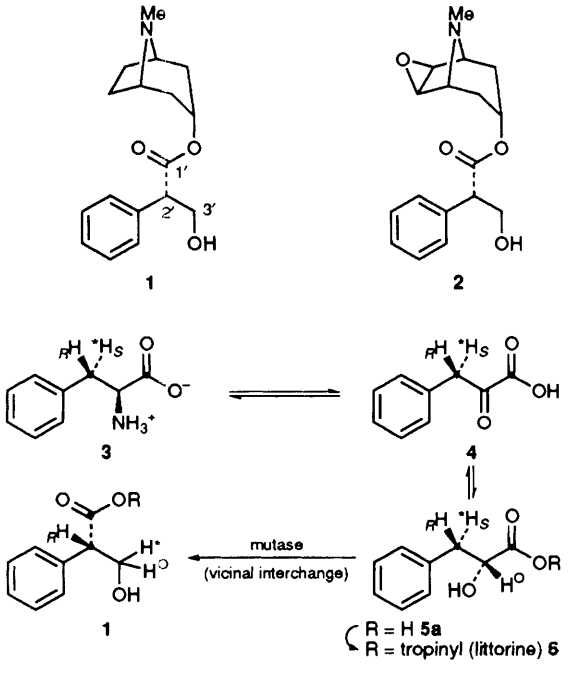
Incubations of (*R*)-D-phenyl[2-^{13}C,^{2}H]lactic acid **5a** and (*S*)-L-phenyl[2-^{13}C,^{2}H]lactic acid **5b** with transformed root cultures of *Datura stramonium* have revealed that the ^{13}C–^{2}H bond is retained, intact at C-3 of the tropate moiety of hyoscyamine, only in the case of the (*R*)-D enantiomer **5a** (the deuterium is lost from the (*S*)-L-enantiomer **5b**); therefore, it is the (*R*)-D-phenyllactate moiety of littorine which is the enantiomer processed by the mutase in hyoscyamine biosynthesis.

The (*S*)-tropate ester moiety is found in the alkaloids hyoscyamine **1** and scopolamine **2** and its biosynthetic origin has been the focus of much interest for many years.[1] In 1975 Leete *et al.* showed[2] that the tropate moiety of hyoscyamine **1** originates from an intramolecular rearrangement of the L-phenylalanine **3** skeleton. Feeding of L-phenyl[1,3-$^{13}C_2$]alanine **3** to *Datura* plants resulted in hyoscyamine **1** with a [1,2-$^{13}C_2$]-labelled tropate moiety. The resultant contiguous arrangement of isotopes established the intramolecular nature of the rearrangement. Leete also demonstrated[3,4] that the carboxylate group of phenylalanine **3** migrates to C-3 with retention of configuration and that the 3-*pro*-*S* hydrogen of L-phenylalanine is abstracted during the rearrangement and is delivered to the hydroxymethyl carbon, C-3′ of **1** (Scheme 1).

Recently, phenyllactate has been identified[5,6] as an obligatory intermediate in tropate biosynthesis in *Datura stramonium*. A radiolabelled study[5] showed that the ^{3}H : ^{14}C ratio from fed (*R*,*S*)-DL-phenyl[1-^{14}C,2-^{3}H]lactate remained essentially the same in the recovered hyoscyamine **1** and scopolamine **2**. In a stable isotope study[6] we have demonstrated that the ^{13}C–^{2}H bond of (*R*,*S*)-DL-phenyl[2-^{13}C,^{2}H]lactate **5** is incorporated intact into C-3′ of **1**. Further, in an exciting development, Robins *et al.* have demonstrated[7] that hyoscyamine **1** is generated by an *intramolecular* rearrangement of littorine **6**, the phenyllactate ester of tropine. The study involved the incorporation of racemic phenyl[1,3-$^{13}C_2$]lactyl-[*N*-methyl-$^{2}H_3$]tropine (littorine) and it was shown that the quintuply-labelled precursor was predominantly incorporated intact, suggesting no requirement for tropate ester hydrolysis. These studies lay to rest speculation[8–11] on the nature of intermediates after L-phenylalanine and establish littorine **6** as the true substrate for the mutase enzyme. The pathway between L-phenylalanine and hyoscyamine is summarised in Scheme 1. In this and the following Communication we report the resolution of the remaining stereochemical questions concerning the rearrangement of the phenyllactate moiety of littorine **6** to the tropate moiety of hyoscyamine **1**.



Scheme 1 Biosynthetic intermediates and stereochemical summary between L-phenylalanine and hyoscyamine in *Datura stramonium*

It became relevant to establish whether (*R*)-D- or (*S*)-L-phenyllactate is the true substrate for the mutase enzyme. To this end we have prepared both sodium (*R*)-D-phenyl[2-^{13}C,^{2}H]lactic acid **5a** and (*S*)-L-phenyl[2-^{13}C,^{2}H]lactic acid **5b**. To achieve this, the dual-labelled racemate was synthesised as previously described[6] and was then resolved into its component enantiomers **5a** and **5b**† by the method of Saigo *et al.*[12] Due to the *in vivo* interconversion of these enantiomers, *via* phenylpyruvate **4**,[13] it was necessary to incorporate deuterium at C-2. Clearly any equilibrium with, or processing *via* phenylpyruvate **4**, will result in loss of the deuterium atom. Also, the double-labelling strategy provides a sensitive probe for deuterium incorporation as demonstrated previously with the dual-labelled racemate of phenyl[2-^{13}C,^{2}H]lactic acid.[6]

The hyoscyamine **1**, which was isolated after separate feeding experiments of **3** and **4** to transformed root cultures of *Datura stramonium*, was analysed by ^{13}C{^{1}H} and ^{13}C{^{1}H,^{2}H} NMR experiments. From the relevant sections of the ^{13}C NMR spectra shown in Fig. 1 it is apparent that the greater part of (*R*)-D-phenyl[2-^{13}C,^{2}H]lactic acid **5a** is incorporated into hyoscyamine with its ^{13}C–^{2}H bond intact. There is a clear α-shift associated with the enriched resonance at δ 64.05 corresponding to C-3′, the hydroxymethyl carbon of the tropate moiety of **1**. Conversely for (*S*)-L-phenyl[2-^{13}C,^{2}H]lactic acid **5b**, there was no α-shift component associated with the enriched C-3′ signal at δ 64, indicating that all of the deuterium had been washed out.

GCMS analysis of the crude alkaloidal extracts, conducted after each experiment, confirmed this isotopic distribution and allowed quantification of the enrichments. The percentage enrichments of the M + 1 and M + 2 ions are shown in Table 1. Such an analysis also allowed isotopic enrichments into the co-produced tropane alkaloids 3α-(2′-hydroxyacetoxy)tropane **7** and 3α-phenylacetoxytropane **8** to be evaluated. These alkaloids are produced at much lower levels and were not observable by ^{13}C NMR. Of some significance was the M + 2 ion (18.4%) evaluated for **7** after the (*R*)-D-phenyl[2-^{13}C,^{2}H]lactic acid **5a** feeding experiment. This demonstrates that one of the C–H bonds of the hydroxymethyl group of **7** derives intact from C-2 of (*R*)-phenyllactate, presumably after C-2/C-3 sission of littorine, and lends further support to a recent proposal[14] from our laboratories defining the metabolic relationship between these alkaloids. Also, in these experiments there was no significant incorporation into 3α-phenylacetoxy-

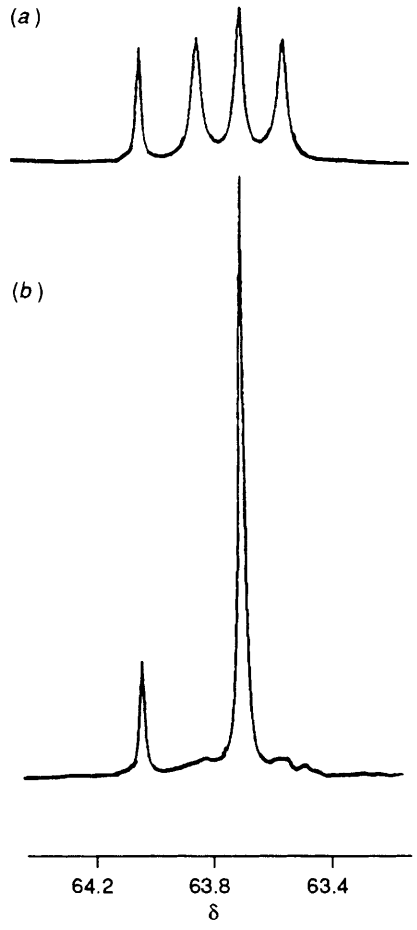


Fig. 1 The C-3′ resonance at δ 64.05 of the tropate moiety of **1** after incorporation of (*R*)-D-phenyl[2-^{13}C,^{2}H]lactic acid **5a**. The intact ^{13}C–^{2}H incorporation is evident by the triplet at δ 63.7 in (*a*) corresponding to the deuterium-induced α-shift, and ^{13}C–^{2}H coupling. This triplet collapses to a singlet in (*b*), the ^{13}C{^{1}H,^{2}H} spectrum.

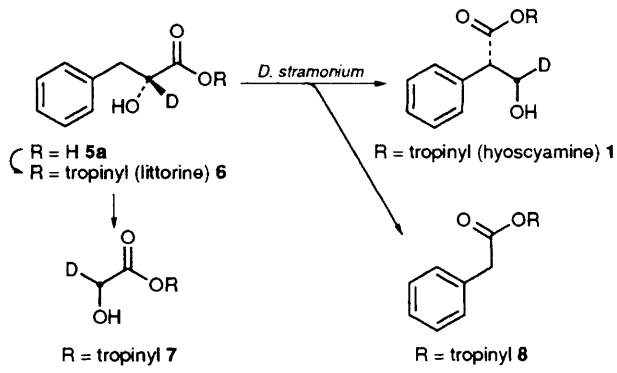
Table 1 Isotope enrichments of alkaloids determined by GCMS analysis after feeding experiments with **5a** and **5b**. Values represent the total isotope excess, corrected for natural abundance.[7]

	Phenyl[2-^{13}C,^{2}H]lactate			
	(*R*)-D **5a**		(*S*)-L **5b**	
	M + 1	M + 2 (%)	M + 1	M + 2 (%)
1 (hyoscyamine)	0.8	21.7	5.1	2.4
6 (littorine)	1.5	28.9	10.3	3.3
7	1.4	18.4	9.8	1.0
8	−1.5[a]	−0.4	−0.6	−0.4

[a] Negative values represent an error in determination of ≈2%.

tropane **8**, from either enantiomer, consistent with our previous evaluation[14] that the phenylacetyl ester moiety originates from hyoscyamine *after* rearrangement of littorine. Adventitious oxidative removal of the C-3′ carbon of the tropate moiety during hyoscyamine formation emerges as the most likely pathway to **8**.

These experiments demonstrate unambigiously that (*R*)-D-phenyllactate **5a** is processed more directly that (*S*)-L-phenyllactate **5b**‡ and are consistent with (*R*)-D-littorine **6** as the true substrate for the mutase as shown in Scheme 2. (*R*)-D-Littorine is shown to be a precursor of hydroxyacetyl tropane and hyoscyamine and by implication[14] of phenylacetoxy tropane. It is already established[16] that littorine **6** has the (*R*)-D-configuration and clearly this stereochemical result is consistent with the direct interconversion of littorine and hyoscyamine in *Datura stramonium*. Implicit in this conclusion is the role of a (*R*)-D-phenyllactate dehydrogenase operating at a pivotal point



Scheme 2 The metabolic relationship between co-produced alkaloids in *Datura stramonium*. The (*R*)-D-phenyllactate moiety of littorine **6** is converted directly to hyoscyamine **1** and also labels the hydroxyacetyl moiety of **7**. No isotope is incorporated into the phenylacetyl moiety of **8**.

between phenylalanine metabolism and alkaloid biosynthesis.

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Footnotes

† (*R*)-D-Phenyl[2-^{13}C,^{2}H]lactate **5a**: mp 119–121 °C (lit.[14] 124–125 °C), $[\alpha]_D^{23}$ = +17.28 (*c* 4.6, EtOH); (*S*)-L-phenyl[2-^{13}C,^{2}H]lactate **5b**: mp 120–121 °C, $[\alpha]_D^{23}$ = −16.0 (*c* 10, EtOH). In both cases unlabelled preparations of **5a** and **5b** gave the higher optical rotation values of +22.5 (*c* 4.4, EtOH) [lit.[14] +19 (*c* 3.1, EtOH)] for **5a** and −21.27 (*c* 23.5, EtOH) for **5b**. We attribute this anomaly to the presence of the deuterium atom at the chiral centre. The optical purity of our samples was found to be *at least* >95% ee in each case after conversion of **5a** and **5b** to their methyl ester acetates, by ^{1}H NMR using the chiral shift reagent tris[3-heptafluoropropylhydroxymethylene)-(+)-camphoratol, europium(III) derivative [Eu(hfc)$_3$].

‡ During the preparation of this manuscript we became aware that Dr J. G. Woolley, De Montfort University, Leicester, has drawn a complementary conclusion after incorporation studies of (*R*)-D- and (*S*)-L-phenyl[1,3-^{13}C$_2$;1-^{14}C]lactate into scopolamine and hyoscyamine from *Datura stramonium* plants.[15]

References

1 E. Leete, *Planta Med.*, 1990, **56**, 97; 1979, **36**, 97.
2 E. Leete, N. Kowanko and R. A. Newmark, *J. Am. Chem. Soc.*, 1975, **97**, 6826.
3 E. Leete, *J. Am. Chem. Soc.*, 1984, **106**, 7271.
4 E. Leete, *Can. J. Chem.*, 1987, **65**, 226.
5 M. Ansarin and J. G. Woolley, *Phytochemistry*, 1993, **32**, 1183.
6 N. C. J. E. Chesters, D. O'Hagan and R. J. Robins, *J. Chem. Soc., Perkin Trans. 1*, 1994, 1159.
7 R. J. Robins, P. Bachmann and J. G. Woolley, *J. Chem. Soc., Perkin Trans. 1*, 1994, 615.
8 W. C. Evans and J. G. Woolley, *Phytochemistry*, 1976, **15**, 287.
9 E. Leete and E. P. Kirven, *Phytochemistry*, 1974, **13**, 1501.
10 E. Leete, *Phytochemistry*, 1983, **22**, 933.
11 R. J. Cox and D. O'Hagan, *J. Chem. Soc., Perkin Trans. 1*, 1991, 2537.
12 K. Saigo, H. Miura, K. Ishizaki and H. Nohira, *Bull. Chem. Soc., Jpn.*, 1982, **55**, 1188.
13 E. Leete and E. P. Kirven, *Phytochemistry*, 1974, **13**, 1501.
14 R. J. Robins, N. C. J. E. Chesters, D. O'Hagan, A. J. Parr, N. J. Walton and J. G. Woolley, *J. Chem. Soc., Perkin Trans. 1*, in the press.
15 M. Ansarin and J. G. Woolley, *J. Chem. Soc., Perkin Trans. 1*, submitted.
16 J. R. Cannon, K. R. Joshi, G. V. Meehan and J. R. Williams, *Aust. J. Chem.*, 1969, **22**, 221.