The Biosynthesis of Hyoscyamine: the Process by which Littorine Rearranges to Hyoscyamine

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The incorporation of isotope from specifically-labelled 3-phenyllactic acid **4** or littorine **7** into 3α-phenylacetoxytropane **10**, 3α-phenylacetoxy-6β,7β-epoxytropane and 3α-(2′-hydroxyacetoxy)-tropane **9** has been demonstrated. Transformed root cultures of *Datura stramonium* or *Brugmansia* (*Datura*) *candida x B. aurea* incorporated fed (*RS*)-3-phenyl[1,3-$^{13}C_2$]lactic acid **4** into 3α-phenylacetoxytropane **10** and 3α-phenylacetoxy-6β,7β-epoxytropane with the efficient retention of both ^{13}C nuclei. In contrast, no label was incorporated into these two compounds from (*RS*)-3-phenyl[2-^{13}C,2-^{2}H]lactate **4**. From this evidence it can be deduced that 3-phenyllactic acid **4** is not incorporated into 3α-phenylacetoxytropane **10** *via* free phenylacetic acid **6**, a route which would result in the loss of the C-1 of 3-phenyllactic acid **4**. Furthermore, (*RS*)-(3′-phenyl[1′,3′-$^{13}C_2$]lactoyl)[*methyl*-$^{2}H_3$]tropine (littorine **7**) was incorporated into 3α-phenylacetoxytropane **10**, at up to 4% specific incorporation, with the retention of all the ^{13}C and ^{2}H nuclei. Label was also incorporated into 3α-(2′-hydroxyacetoxy)tropane **9** from (*RS*)-3-phenyl[1,3-$^{13}C_2$]lactic acid **4** and (*RS*)-(3′-phenyl[1′,3′-$^{13}C_2$]lactoyl)[*methyl*-$^{2}H_3$]tropine **7**. We propose, on the basis of these observations, a putative process for the rearrangement of littorine **7** to hyoscyamine **8** and suggest that both 3α-phenylacetoxytropane **10** and 3α-(2′-hydroxyacetoxy)tropane **9** arise as by-products of the rearrangement process.

The aromatic moiety of the tropane alkaloids, hyoscyamine **8** and hyoscine, is (*S*)-tropic acid. During the biosynthesis of the tropoyl moiety from (*R*)-phenylalanine, a carbon skeletal rearrangement of the linear propanoid side chain occurs, forming the isopropanoid side chain characteristic of tropic acid.[1] It was clearly demonstrated that this rearrangement is intramolecular, since hyoscyamine **8** isolated from plants of *Datura innoxia* fed (*RS*)-phenyl[1,3-$^{13}C_2$]alanine had contiguous C–C coupling at the C-1′ and C-2′ positions in the NMR spectrum. Furthermore, it was shown[2,3] that a 1,2-vicinal interchange occurs, the carboxy residue migrating to C-3 with retention of configuration and the 3-*pro*-(*S*) proton of (*R*)-phenylalanine migrating in the counter direction.

Aspects of the route by which phenylalanine is incorporated into hyoscyamine **8** have been established by a series of recent studies. It has been demonstrated that 3-phenyllactic acid **4** is an obligatory intermediate;[4,5] alternative putative routes, *via* such intermediates as cinnamic acid,[6] 3-hydroxy-3-phenylpropanoic acid,[6] or 3-amino-2-phenylpropanoic acid[7] have now been discarded as improbable. The intermediacy of 3-phenyllactic acid was effectively shown by feeding (*RS*)-3-phenyl[1,3-$^{13}C_2$]lactic acid **4** both to whole plants of *D. stramonium*[8] and to transformed root cultures of *D. stramonium* or *Brugmansia* (*Datura*) *candida x B. aurea*.[9] In these experiments, contiguity of the C-1′ and C-2′ in the derived hyoscyamine **8** was observed, mimicking the incorporation seen previously from phenyl[1,3-$^{13}C_2$]alanine.[1] That 3-phenyllactic acid **4** is incorporated without the intermediacy of phenylpyruvic acid **5** has also been shown, firstly by the unaltered ^{3}H:^{14}C ratio in the derived hyoscyamine **8** when (*RS*)-3-phenyl[1-^{14}C,2-^{3}H]lactic acid **4** was fed[4] and, unequivocally, by the incorporation from (*RS*)-3-phenyl[2-^{13}C,2-^{2}H]lactic acid **4** of the intact ^{13}C–^{2}H bond into the C-3′ of the derived hyoscyamine **8**.[5]

From these data it may be deduced that the carbon skeletal rearrangement occurs subsequent to the intermediacy of 3-phenyllactic acid **4**. One possibility is that free 3-phenyllactic acid **4** could be rearranged to form free tropic acid. However, we have recently presented evidence[9,10] that this is not the case. Firstly, the effects of tropic acid on the relative extents to which (*RS*)-3-phenyl[1,3-$^{13}C_2$]lactic acid **4** is incorporated into hyoscyamine **8** and littorine **7** by transformed root cultures of *D. stramonium* and *B. candida x B. aurea* are incompatible with free tropic acid being an intermediate in this pathway.[9] Secondly, littorine **7** has been shown to be a direct precursor for hyoscyamine **8**, as (*RS*)-(3′-phenyl[1′,3′-$^{13}C_2$]lactoyl)[*methyl*-$^{2}H_3$]tropine **7** is efficiently converted into hyoscyamine **8** without loss of label in transformed root cultures of *D. stramonium*.[10] Littorine **7** is, therefore, rearranged directly to form hyoscyamine **8**.

We now report the incorporations from (*RS*)-3-phenyl[1,3-$^{13}C_2$]lactic acid **4**, (*RS*)-3-phenyl[2-^{13}C,2-^{2}H]lactic acid **4** and (*RS*)-(3′-phenyl[1′,3′-$^{13}C_2$]lactoyl)[*methyl*-$^{2}H_3$]tropine **7** into 3α-phenylacetoxytropane **10**, 3α-phenylacetoxy-6β,7β-epoxytropane and 3α-(2′-hydroxyacetoxy)tropane **9**, as determined by GC–MS, and discuss the biosynthetic implications of the labelling patterns found. These bases all occur as minor alkaloids in extracts from transformed root cultures of *D. stramonium* or *B. candida x B. aurea*, as well as from other tropane-alkaloid-producing species.[11] 3α-Phenylacetoxytropane **10** could arise from the esterification of tropine **2** with phenylacetyl-coenzyme A **3** (Scheme 1). An enzyme capable of carrying out this esterification has been found[12] at low levels in a number of solanaceous species but has so far proved intransigent to purification. Similarly, 3α-(2′-hydroxyacetoxy)-tropane **9** could arise by the esterification of tropine **2** with 2-hydroxyacetyl-coenzyme A in a manner analogous to that

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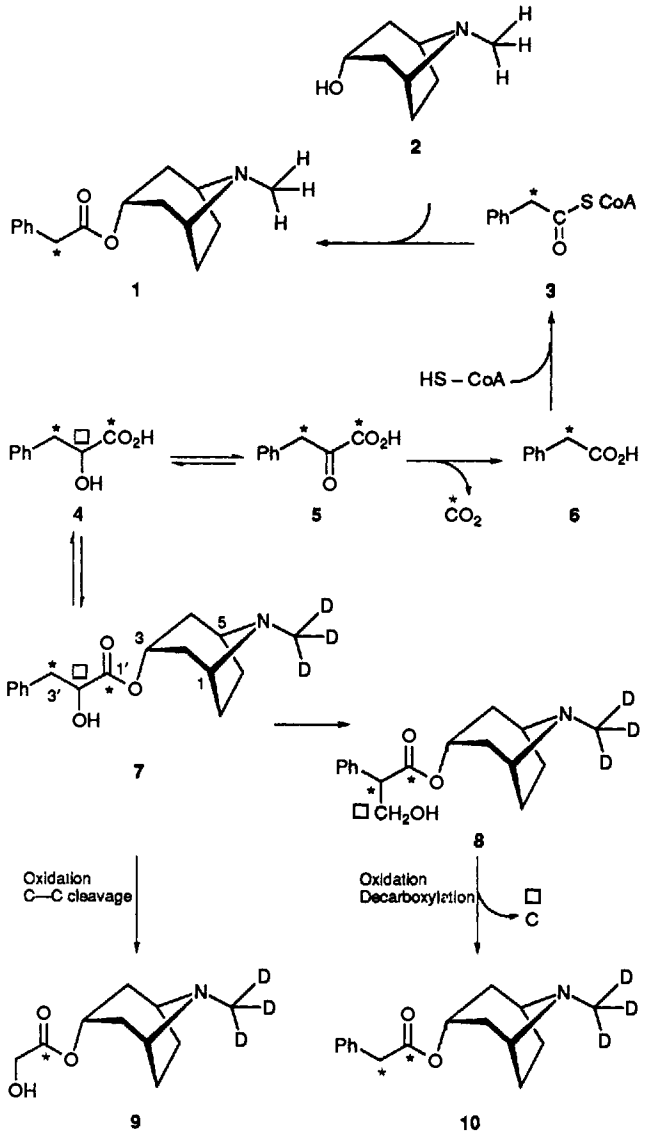
Table 1 The incorporations of (*RS*)-3-phenyl[1,3-$^{13}C_2$]lactic acid into tropane alkaloids in transformed root cultures of *D. stramonium*

Additional acid fed (mmol dm^{-3})[b]	Specific incorporation (%)[a]				
	3α-(2′-Hydroxyacetoxy)-tropane **9**	3α-Phenylacetoxy-tropane **10**		Hyoscyamine **8**	
	M + 1	M + 1	M + 2	M + 1	M + 2
None	43.3	16.4	37.2	6.1	41.0
(*S*)-3-Phenyllactic acid **4** (1.0)	35.7	8.8	33.2	5.0	38.9
(*RS*)-3-Phenyllactic acid **4** (1.0)	45.1	10.2	44.3	7.2	45.4
(*RS*)-Tropic acid (1.0)	48.8	16.0	32.8	7.6	48.5

[a] Specific incorporation (%) = 100 × (Excess of isotope in the isolated product)/(Excess of isotope in the precursor). [b] (*RS*)-3-Phenyl[1,3-$^{13}C_2$]lactic acid **4** was fed to all cultures at 0.25 mmol dm^{-3}.



Scheme 1 Alternative putative pathways for the biosynthesis of 3α-phenylacetoxytropane

shown for the formation of 3α-acetoxytropane, 3β-acetoxytropane and 3β-tigloyloxytropane.[13,14] However, the incorporation patterns observed in 3α-phenylacetoxytropane **10**, 3α-phenylacetoxy-6β,7β-epoxytropane and 3α-(2′-hydroxyacetoxy)tropane **9** are incompatible with the alkaloids arising by such routes. Rather, all compounds contain ^{13}C at levels that indicate that they have arisen as by-products of the rearrangement of littorine to hyoscyamine.

Results and Discussion

(*RS*)-3-Phenyl[1,3-$^{13}C_2$]lactic acid **4** (0.25 mmol dm^{-3}) was fed to 4 day-old transformed root cultures of *D. stramonium* (7 mg per flask; 2 flasks; grown on to 14 days) or *B. candida x B. aurea* (7 mg per flask; 4 flasks; grown on to 7 days). The crude alkaloidal fractions (3.8 mg from 9.6 g fresh mass and 15.4 mg from 19.4 g fresh mass, respectively) were isolated and examined by GC–MS.[9,15] The mass spectra obtained from several replicate feedings to *D. stramonium* all showed a high specific incorporation of the label into 3α-phenylacetoxytropane **10** with the retention of both ^{13}C nuclei (Table 1). The specific incorporation observed was not diminished by diluting the fed phenyllactic acid to only 16% isotopic excess. Nor was the specific incorporation reduced by feeding the culture tropic acid, the acidic moiety of hyoscyamine **8**. In the *Brugmansia* hybrid, both 3α-phenylacetoxytropane **10** and 3α-phenylacetoxy-6β,7β-epoxytropane were labelled (Table 2). Again, there was no substantial diminution of specific incorporation by added tropic acid. This is the first demonstration that 3-phenyllactic acid **4** can act as a precursor for 3α-phenylacetoxytropane **10** and 3α-phenylacetoxy-6β,7β-epoxytropane. The slightly lower incorporation into 3α-phenylacetoxy-6β,7β-epoxytropane is probably indicative of this compound being a metabolite of 3α-phenylacetoxytropane **10**.[16]

The specific incorporations seen in the M + 2 ions of these compounds are comparable with those observed in hyoscyamine **8** and hyoscine (Tables 1 and 2). Therefore, the fed (*RS*)-3-phenyl[1,3-$^{13}C_2$]lactic acid **4** cannot have been metabolised to 3α-phenylacetoxytropane **10** and 3α-phenylacetoxy-6β,7β-epoxytropane *via* free phenylacetic acid **6** (Scheme 1) because decarboxylation at C-1 of (*RS*)-3-phenyl[1,3-$^{13}C_2$]lactic acid **4** would lead to the loss of the enriched nucleus at this position and a consequent lack of a M + 2 ion in any subsequent metabolites; only an M + 1 ion would be observed. In fact, there is a small enhancement over the natural abundance of the M + 1. However, this is much lower than the incorporation at the M + 2 level and some enhancement of the M + 1 is to be expected, as the (*RS*)-3-phenyl[1,3-$^{13}C_2$]lactic acid **4** used contains about 9% (*RS*)-3-phenyl[1-^{13}C]lactic acid and (*RS*)-3-phenyl[3-^{13}C]lactic acid as a result of the synthetic method used in its preparation.[8] Incorporation from this source is strongly suggested by the comparable M + 1 incorporations into hyoscyamine **8** and hyoscine. In *D. stramonium*, the 3α-phenylacetoxytropane **10** does show a higher M + 1 than the hyoscyamine **8**, making it feasible that a route *via* free phenylacetic acid **6** might have made a small contribution in this species. Nevertheless, the data can only imply that it is primarily C-2 of 3-phenyllactic acid **4** that is lost, not

Table 2 The specific incorporations of (*RS*)-3-phenyl[1,3-$^{13}C_2$]lactic acid **4** into tropane alkaloids in transformed root cultures of a *Brugmansia* (*Datura*) *candida* x *B. aurea* hybrid

Additional acid fed (mmol dm^{-3})[b]	Specific incorporation (%)[a]								
	3α-(2′-Hydroxyacetoxy)tropane **9**	3α-Phenylacetoxytropane **10**		Hyoscyamine **8**		3α-Phenylacetoxy-6β,7β-epoxytropane		Hyoscine	
	M + 1	M + 1	M + 2	M + 1	M + 2	M + 1	M + 2	M + 1	M + 2
None	41.7	9.5	40.9	7.5	41.3	8.5	32.3	6.8	33.3
(*RS*)-Tropic acid (0.25)	30.4	7.6	34.1	8.8	35.0	7.5	27.3	6.0	27.9
(*RS*)-Tropic acid (0.75)	30.3	5.4	34.8	9.4	36.9	3.9	14.3	3.6	15.2

[a] See Table 1. [b] (*RS*)-Phenyl[1,3-$^{13}C_2$]lactic acid **4** was fed to cultures at 0.25 mmol dm^{-3}.

Table 3 The percentage isotopic excess in tropane alkaloids extracted from transformed root cultures of *D. stramonium* fed with (*RS*)-(3′-phenyl[1′,3′-$^{13}C_2$]lactoyl)[*methyl*-$^2H_3$]tropine (littorine **7**)

Additional precursor fed (mmol dm^{-3})[b]	Percent isotopic excess[a]									
	3α-(2′-Hydroxyacetoxy)tropane **9**		3α-Phenylacetoxytropane **10**				Hyoscyamine **8**			
	M + 3	M + 4	M + 2	M + 3	M + 4	M + 5	M + 2	M + 3	M + 4	M + 5
None	5.1	13.0[c]	n.d.[d]	n.d.	n.d.	n.d.	3.3	4.8	1.4	4.5
Tropine **2** (0.25)	4.9	18.9	2.2	1.9	0.0	4.0	4.3	3.8	2.0	6.5
(*RS*)-3-Phenyllactic acid **4** (0.25)	4.1	16.0	1.8	1.6	0.0	3.3	4.0	3.5	1.8	6.1

[a] 100 × Enhancement of the isotopic abundance (corrected for natural abundance) relative to the M ion. [b] (*RS*)-(3′-Phenyl[1,3-$^{13}C_2$]lactoyl)-[*methyl*-$^2H_3$]tropine (littorine **7**) was fed to cultures at 0.125 mmol dm^{-3}. [c] Areas under the M + 5 peaks of 3α-(2′-hydroxyacetoxy)tropane **9** are very small. [d] n.d. = insufficient area under the peak for quantitation.

C-1 as would be the case were metabolism to take place *via* **6**.

Confirmatory evidence that the C-2 of 3-phenyllactic acid **4** is not incorporated into 3α-phenylacetoxytropane **10** was obtained in an experiment in which (*RS*)-3-phenyl[2-^{13}C, 2-^2H]lactic acid **4** (0.4 mmol dm^{-3}) was fed to 4 day-old root cultures of *D. stramonium* (1.2 mg, per flask; 9 flasks, pulse-fed; grown on to 19 days).[5] The alkaloid extracted from these cultures (40.5 mg) had no mass enhancement of the extracted 3α-phenylacetoxytropane **10**, even though some oxidation of the fed (*RS*)-3-phenyl[2-^{13}C,2-^2H]lactic acid **4** to phenylpyruvic acid **5** had apparently occurred. In contrast, high incorporations into hyoscyamine **8**, littorine **7** and apoatropine were observed by GC–MS and NMR.[5] If C-1 were lost, as in the route *via* **5** and **6**, then the derived 3α-phenylacetoxytropane **1** would have shown a strong M + 1 enrichment. Thus, it is demonstrated directly that during incorporation C-2 of phenyllactic acid **4** is lost.

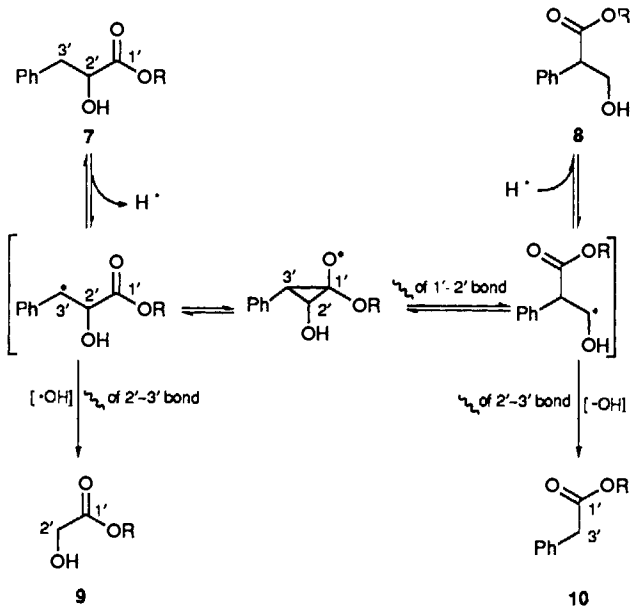
It is therefore indicated that the biosynthesis of 3α-phenylacetoxytropane **10** proceeds *via* a mechanism which does not involve free phenylpyruvic acid **6**. Rather, the close correlations of the levels of incorporation observed into hyoscyamine **8**/3α-phenylacetoxytropane **10** and hyoscine/3α-phenylacetoxy-6β,7β-epoxytropane (Tables 1 and 2) strongly suggest that the mechanism of biosynthesis of these alkaloids is closely linked.

Evidence that hyoscyamine **8** and 3α-phenylacetoxytropane **10** have a common biosynthetic origin is provided by the incorporation patterns seen after feeding (*RS*)-(3′-phenyl[1′,3′-$^{13}C_2$]lactoyl)[*methyl*-$^2H_3$]tropine **7** to cultures of *D. stramonium*.[10] This quintuply-labelled littorine is incorporated effectively into hyoscyamine **8** (Table 3), indicating that littorine **7** has rearranged directly. 3α-Phenylacetoxytropane **10** extracted from these cultures is also found to have a significant simultaneous incorporation of two ^{13}C and three ^2H nuclei to give a M + 5 mass ion (Table 3). The level of incorporation of the quintuply-labelled littorine **7** into the M + 5 ion of 3α-phenylacetoxytropane **10** is lower than into the M + 5 of hyoscyamine **8**, but still much higher than could have occurred *via* hydrolysis and partial re-use of the labelled material. If label from (*RS*)-(3′-phenyl[1′,3′-$^{13}C_2$]lactoyl)[*methyl*-$^2H_3$]tropine **7** only enters tropane alkaloids following hydrolysis and the separate reincorporation of the two parts of the precursor molecule, then products with M + 2 and M + 3 mass spectral peaks should be much more prominent than those with M + 5.[10] Incorporations into the M + 2 and M + 3 of 3α-phenylacetoxytropane **10** are, however, much smaller than into the M + 5 (Table 3) and, as a proportion of the total incorporation observed, are comparable with those determined for the M + 2 and M + 3 of hyoscyamine **8**. Therefore, it can be concluded that littorine **7** acts not only as a direct precursor for hyoscyamine **8**, as shown previously,[10] but also as a precursor for 3α-phenylacetoxytropane **10**.

In addition, we have observed that label from (*RS*)-3-phenyl[1,3-$^{13}C_2$]lactic acid **4** (Tables 1 and 2) or (*RS*)-(3′-phenyl[1′,3′-$^{13}C_2$]lactoyl)[*methyl*-$^2H_3$]tropine **7** (Table 3) is incorporated effectively into a M + 1 or M + 4 ion, respectively, of 3α-(2′-hydroxyacetoxy)tropane **9**. As can be seen, the level to which this base is labelled is comparable with the labelling of both hyoscyamine **8** and 3α-phenylacetoxytropane **10**. This would be extremely improbable were it to be derived by the esterification of tropine **2** with 2-hydroxyacetylcoenzyme A. Furthermore, since no M + 5 ion was seen in 3α-(2′-hydroxyacetoxy)tropane **9** it cannot be derived by the degradation of hyoscyamine **8**, either endogenously or during extraction. Moreover, were 3α-(2′-hydroxyacetoxy)tropane **9** to be derived by the degradation of hyoscyamine, it is likely that 3α-(2′-hydroxyacetoxy)-6β,7β-epoxytropane, the equivalent degradation product of hyoscine, would simultaneously be detected. The absence of 3α-(2′-hydroxyacetoxy)-6β,7β-epoxytropane from the alkaloidal extract of *Brugmansia* supports the argument that 3α-(2′-hydroxyacetoxy)tropane **9** is a natural metabolite.

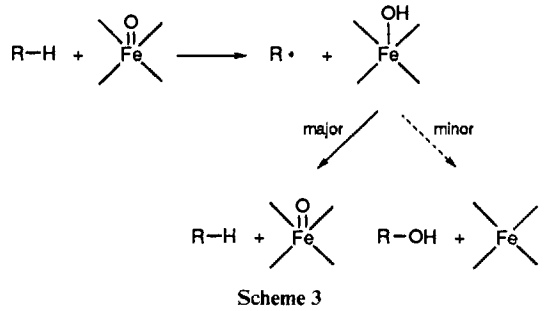
We deduce from these observations that the rearrangement

of littorine **7** to hyoscyamine **8** involves a reaction mechanism which, rarely, results in the loss of the C-2′ of littorine **7**, yielding 3α-phenylacetoxytropane **10** or, again rarely, the loss of the {C-3′ + phenyl}, giving 3α-(2′-hydroxyacetoxy)tropane **9**. A putative process is indicated in Scheme 2. Retention of the C-1′, C-2′ and C-3′ of littorine **7** after direct rearrangement to hyoscyamine **8** is the predominant outcome.



Scheme 2 A putative mechanism for the rearrangement of littorine to hyoscyamine (R = tropine; numbering refers to littorine)

It has been demonstrated[1-3] that the rearrangement involves the migration of the C-1 carboxy to C-3 and the simultaneous counter-migration of the 3-*pro*-(*S*)-H of phenylalanine (and therefore implicitly of 3-phenyllactate **4**) to the C-2′ of hyoscyamine **8**. We suggest, therefore, that the rearrangement is initiated by the abstraction of the 3′-*pro*-(*S*)-H from littorine **7**. This process may be anionic or radical in nature. Anionic carbon skeletal rearrangements are rare in biochemistry, whereas radical processes have precedent.[17] The potential similarity of this vicinal interchange process to methylmalonyl-CoA mutase[18] has been widely discussed.[3,19] Coenzyme B_{12}, however, is not associated with *Datura* plants,[19] which clearly limits development of an hypothesis involving this co-factor. More reasonably, we suggest that the radical process might be initiated by a haem-thiolate enzyme (cytochrome P450) as described for the flavanone–isoflavanone isomerisation.[20] In the light of the evidence discussed by Hakamatsuka *et al.*[20] that such processes may account for several other rearrangements in plants, we favour a free-radical process as outlined in Scheme 2 and summarised in Scheme 3. Homolytic abstraction of the 3′-



Scheme 3

pro-(*S*)-H of littorine **7** would generate a radical at C-3′, which may find stability by transient formation of a cyclopropyl alkoxyl radical. Cleavage of the bond between C-1′ and C-2′ would result in a rearranged free radical at the C-2′ position. Return of the sequestered 3′-*pro*-(*S*)-H proton from the enzyme (or cofactor) to this centre, as demonstrated by Leete,[3] would then generate hyoscyamine **8**. This is the major reaction. Supporting evidence for the involvement of a haem-thiolate protein is provided by the presence of **9** and **10** as minor co-metabolites. In two minor pathways, if the pre- and post-rearranged radicals are quenched by a hydroxyl radical, rather than by a hydrogen radical, then further oxidative processing would account for **9** and **10**, respectively as the end products of these oxidative pathways.

The putative intermediacy of 2-formylphenylacetic acid between hyoscyamine **8** and **10** has also been suggested[21] and is consistent with our hypothesis. The extreme lability of this compound in neutral aqueous solution, however, renders it impossible to use in feeding experiments.

The loss of the hydroxymethyl group of the tropate moiety could in principle occur subsequent to hyoscyamine **8** formation. Small amounts of 3α-phenylacetoxytropane **10** and 3α-phenylacetoxy-6β,7β-epoxytropane can be generated from hyoscyamine and hyoscine, respectively, by thermal degradation in the heated split/splitless injector during analysis by GC–MS.[11] Several lines of evidence, however, indicate that this is not the cause of the isotopic incorporations seen here. Firstly, 3α-phenylacetoxytropane **10** is detected as a natural product in these extracts in GC profiles obtained using a cold on-column injector. Standard hyoscyamine **8** and hyoscine do not degrade under these conditions. Secondly, no 3α-(2′-hydroxyacetoxy)-tropane **9** occurs in standard hyoscyamine analysed by GC–MS. Thirdly, the M + 4 of 3α-(2′-hydroxyacetoxy)tropane **9** isolated following the feeding of quintuply-labelled littorine **7** has a higher level of incorporation than found in the recovered littorine **7** or hyoscyamine **8**: if it were an artifact this could not occur. Thus, it can be concluded that the labelling patterns seen are not due to post-extraction artifacts but arise from the metabolic processes involved in hyoscyamine **8** formation.

In conclusion, we propose that the unique rearrangement by which hyoscyamine **8** is formed from littorine **7** is a free radical process initiated by a haem-thiolate enzyme. Additionally, the pre- and post-rearranged radicals are adventitiously quenched by hydroxyl radicals to generate **9** and **10** after further oxidative processes.

Experimental

General.—(*RS*)-3-Phenyl[1,3-$^{13}C_2$]lactic acid (atom excess: 81 ± 2% at M + 2 ion)[8] (*RS*)-phenyl[2-^{13}C,2-^{2}H]lactic acid (atom excess: 99% at M + 2 ion)[5] and (*RS*)-(3′-phenyl[1′,3′-$^{13}C_2$]lactoyl)[*methyl*-$^{2}H_3$]tropine (atom excess: 0% at M ion, 0% at M + 1 ion, 0.3% at M + 2 ion, 1.8% at M + 3 ion, 18.7% at M + 4 ion, 81 ± 2% at M + 5 ion)[10] were prepared as described. (*RS*)-3-Phenyllactic acid and tropine were from Sigma Chemical Company (Poole, Dorset, UK) and Aldrich Chemical Company (Gillingham, Kent, UK), respectively.

Cultures.—Root cultures of *Datura stramonium* L. D15/5[22] and *Brugmansia* (*Datura*) *candida* x *B. aurea*[23] were grown as described. Feeding experiments were performed as described previously.

Alkaloid Separation and Identification.—Alkaloids were extracted and analysed by GC–MS essentially as described previously.[9,15]

Acknowledgements

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the (*RS*)-(3′-phenyl[1′,3′-$^{13}C_2$]lactoyl)[*methyl*-$^{2}H_3$]tropine-feeding experiment; to John Eagles (IFR) and Louise Tatton (IFR) for assistance with running the GC–MS analyses; to Birgit Dräger for valuable discussions; to Abbi Peerless for maintaining the cultures; to Morteza Ansarin for the synthesis of (*RS*)-3-phenyl[1,3-$^{13}C_2$]lactic acid; to the Nuffield Foundation, London, for financial support to J. G. W. and to the SERC, and the University of Durham for support to N. C. J. E. C.

Note in Proof.—The stereochemistry of this process is now fully established.[24–26]

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