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Tropic acid biosynthesis: the incorporation of (*RS*)-phenyl[2-^{18}O,2-^{2}H]lactate into littorine and hyoscyamine in *Datura stramonium*

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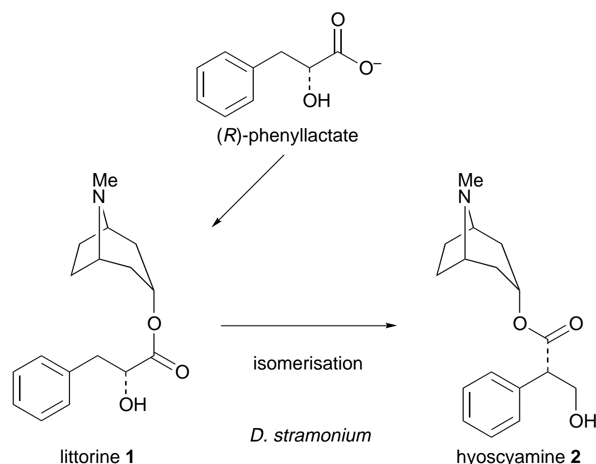
The incorporation of oxygen-18 from (*RS*)-phenyl-[2-^{18}O,2-^{2}H]lactate into the tropane alkaloids littorine 1 and hyoscyamine 2 in *Datura stramonium* reveals that up to 29% of the oxygen-18 is lost during the transformation of 1 to 2.

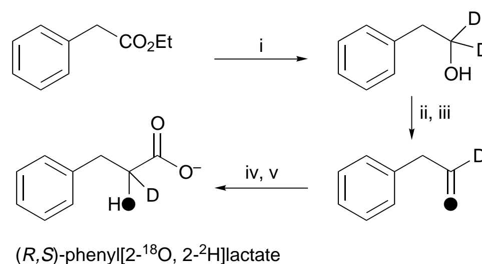
The biosynthetic origin of the tropate ester moiety of the alkaloid hyoscyamine **2** has been the subject of attention for many years.[1,2] It was shown in 1972 that this ester arises as a consequence of a carbon skeleton rearrangement of a phenylpropanoid metabolite derived from L-phenylalanine.[3]

We were able to show in 1995[4] that (*R*)-phenyllactate was the relevant phenylpropanoid metabolite, and in a notable development Robins *et al.* demonstrated[5] that the tropate ester moiety is derived after a *direct isomerisation* of littorine **1**, the (*R*)-phenyllactate ester of tropine, to hyoscyamine **2**, the tropine ester of (*S*)-tropic acid, as represented in Scheme 1. Although the substrate for the isomerisation is now established, the nature of the enzyme and the mechanism of the process remains unknown. There is a superficial similarity of the rearrangement to some coenzyme-B_{12} mediated isomerisations which has led to speculation that this is a coenzyme-B_{12} process,[6,7] however the characteristic vicinal interchange process common to carbon skeleton coenzyme-B_{12} mediated isomerisations[8] *does not occur*[9] in the littorine **1** to hyoscyamine **2** rearrangement.



Scheme 1

In order to explore this process further we have studied the incorporation of sodium (*RS*)-phenyl[2-^{18}O,2-^{2}H]lactate into both of the alkaloids **1** and **2** in transformed root cultures of *Datura stamonium*.[10] The requisite (*RS*)-phenyl[2-^{18}O,2-^{2}H]lactate was prepared as shown in Scheme 2. In the event only *ca.* 50% of the oxygen-18 isotope from $H_2{}^{18}O$ was introduced by exchange into phenyl[1-^{2}H]acetaldehyde, presumably as the hydrate is stable to dehydration under the reaction conditions. This route provided a sample of (*RS*)-phenyl[2-^{18}O,2-^{2}H]lactate which had an M + 1/M + 3 ratio of 1.10 as determined by GC–MS analysis. The material was pulse fed (days 5, 7 and 9) to transformed root cultures[10] of *D. stramonium* at a final concentration of 0.64 mM, and the alkaloids were extracted from the root tissue on days 11, 13, 15 and 17 as previously described. GC–MS analysis of the alkaloid extracts allowed determination of isotope content from the relative abundances of the M + 1 (^{2}H only) and M + 3 (^{2}H + ^{18}O)



Scheme 2 *Reagents and conditions*: i, $LiAlD_4$, Et_2O, 2 h, 73%; ii, PCC, CH_2Cl_2, 2 h, 83%; iii, $H_2{}^{18}O$ (97 atom%), THF, HCl, 120 °C, 5 h; iv, TMSCN, Et_3N, CH_2Cl_2, 2 h, then aq. HCl, 24 h, 69%; v, aq. NaOH, 46%

Table 1 GC–MS derived data for the alkaloids **1** and **2** on four different days after supplementation of *D. stammonium* root cultures with (*RS*)-phenyl[2-^{18}O,2-^{2}H]lactate. For these studies the mass spectrometer was operated in the selected ion monitoring mode (SIM) measuring ion currents at *m/z* 289 (M), 290 (M + 1), 291 (M + 2) and 292 (M + 3). The data are presented after natural abundance levels have been subtracted. The corrections made to each of the ion peak areas for natural isotope abundances were determined experimentally using authentic standard compounds

	Day 11		Day 13		Day 15		Day 17	
	1	**2**	**1**	**2**	**1**	**2**	**1**	**2**
M	83.39	90.58	86.49	90.73	84.39	89.00	87.93	90.01
M + 1	9.19	6.48	7.48	6.53	8.50	7.58	6.74	6.78
M + 3	7.24	3.15	5.88	2.95	6.92	3.61	5.24	3.35
M + 1/M + 3 ratio	**1.27**	**2.06**	**1.27**	**2.21**	**1.23**	**2.10**	**1.29**	**2.02**
^{18}O loss from **1** to **2** (%)	25		29		28		25	

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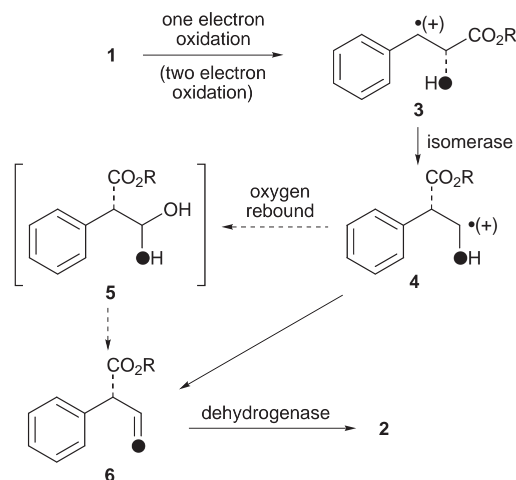
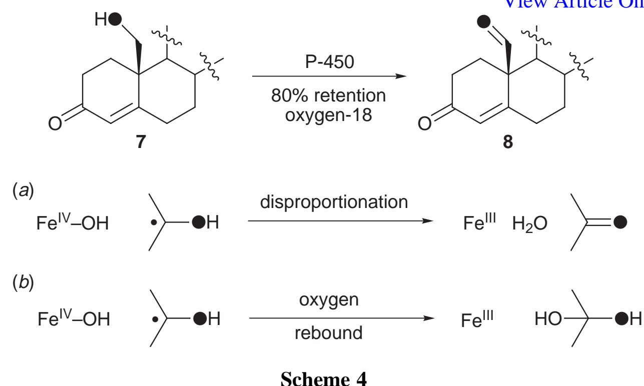


Scheme 3



Scheme 4

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ions. The levels of incorporation were determined by selective ion monitoring (SIM) and the values in Table 1 are corrected for natural isotope abundances. There was no significant incorporation (M + 2 = 0 ± 0.3) into the M + 2 ions and these are not included in Table 1. Of particular significance for this study is the M + 1/M + 3 ratio in the molecular ions of both **1** and **2**, which is independent of total incorporation (12–17%). The extracted littorine **1** on each of the four days had an M + 1/M + 3 = 1.26 ± 0.02, whereas the hyoscyamine had a value of M + 1/M + 3 = 2.1 ± 0.07. This change of ratio represents a loss of *ca.* 25–29% of the M + 3 abundance in hyoscyamine relative to littorine **1** and a corresponding enrichment of the M + 1 abundance of hyoscyamine **2** relative to littorine in going from **1** to **2**. Thus there is an exchange of *ca.* 25–29% of oxygen-18 for oxygen-16 in going from littorine **1** to hyoscyamine **2**.

Our working hypothesis for the rearrangement of **1** and **2** involves two enzymatic activities, an iron–oxo isomerase and a dehydrogenase, as illustrated in Scheme 3. This hypothesis developed from ideas of Sankawa,[11,12] who rationalised a number of isomerisation reactions in terms of iron–oxo enzyme processes. Three mechanistic scenarios emerge in the light of this result. In general, iron–oxo processes are considered to involve radicals and it can be envisaged that a Fe^{IV}–O· species abstracts hydrogen (the 3′-*pro-R* hydrogen)[9] to generate a substrate radical **3**. After rearrangement, the product radical **4** is quenched in a classical manner by delivery of an hydroxyl radical from Fe^{IV}–OH (oxygen rebound) to generate hydrate **5** followed by collapse of the hydrate to give aldehyde **6**. The high retention of oxygen-18 found experimentally is compatible with this process if (i), a partially stereospecific collapse of the hydrate occurs under enzymatic control, or (ii) a fully stereospecific process removes the unlabelled oxygen, but there is some exchange at the aldehyde prior to reduction by a dehydrogenase. A non-stereospecific process would of course result in 50% loss of oxygen-18 and this is not observed.

A similarly high retention of oxygen (80%) was observed previously[13] in the oxidation of **7** to **8** in a P-450 mediated process operating during oestrogen biosynthesis. It was concluded[13,14] in that case that the observation is most consistent with a disproportionation process [Scheme 4(*a*)] involving Fe^{IV}OH and the product radical, rather than the more common oxygen rebound [Scheme 4(*b*)] process. It is poignant that a similar level of oxygen-18 retention is found in this study and such a disproportionation must remain under consideration. The partial loss of isotope can then be attributed to some exchange of the carbonyl oxygen of aldehyde **6** with the aqueous medium prior to reduction by a dehydrogenase. Finally, an additional mechanism would involve a two-electron oxidation of **1** to generate carbocation **3**, as illustrated in the brackets in Scheme 3. Such a process has some precedent with the accepted mechanisms of thromboxane and prostacyclin synthases,[15] where carbocation intermediates are implied, and in some recent mechanistic studies[16] on P-450 systems, where carbocations gave rise to side products during the hydroxylation of cyclopropane substrates. As illustrated in Scheme 3, rearrangement to carbocation **4** followed by a direct collapse to aldehyde **6** prior to reduction would not require any loss of oxygen-18. Some exchange then at the aldehyde level is also consistent with the experimental result. A fuller discussion of the various mechanistic possibilities is given elsewhere.[17]

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Notes and References

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