The Biosynthesis of Tropic Acid: The Stereochemical Course of the Mutase involved in Hyoscyamine Biosynthesis in *Datura stramonium*

Nicola C. J. E. Chesters,[a] David O'Hagan,*[a] Richard J. Robins,[b] Allen Kastelle[c] and Heinz G. Floss[c]

[a] *The Department of Chemistry, University of Durham, Science Laboratories, South Road, Durham, UK DH1 3LE*
[b] *Plant Biotechnology Group, Department of Genetics and Microbiology, Institute of Food Research, Norwich Research Park, Colney, Norwich, UK NR4 7UA*
[c] *Department of Chemistry, University of Washington, Seattle, Washington, 98195, USA*

Incubation of (*R,S*)-DL-phenyl[2-^{3}H]lactic acid with *Datura stramonium* generates hyoscyamine **2** with the tritium isotope located at C-3′ of the tropic acid ester moiety; the C-3′ hydroxymethyl group of **2** is converted into a chiral methyl group and is oxidised to generate chiral sodium acetate, with the (*R*) configuration (96% ee); the tritium is therefore located at the 3′-*pro*-S site of **2**; it follows that the 3′-*pro*-*R* hydrogen is introduced with *inversion* of configuration by the mutase operating during hyoscyamine biosynthesis.

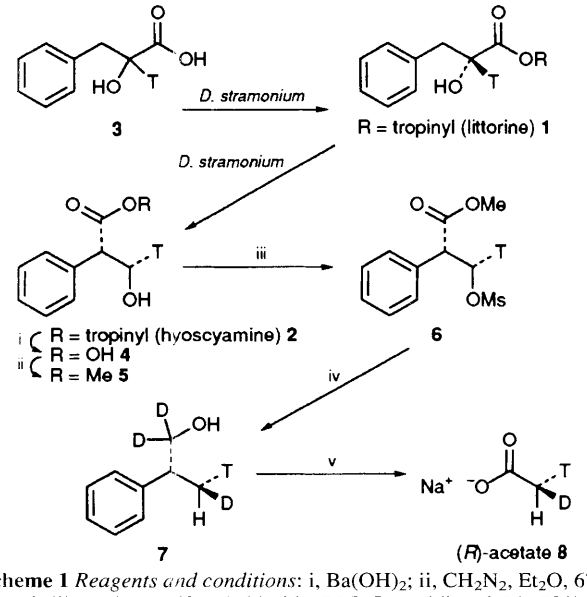
In the preceding communication[1] we report that (*R*)-D-phenyllactate is the stereoisomer processed by the mutase involved in the interconversion of littorine **1** and hyoscyamine **2**. During the rearrangement, the 3′-*pro*-*S* hydrogen of the phenyllactate moiety of littorine migrates in a vicinal interchange process, with the carbonyl group, to generate tropate.[2] The carbon hydrogen bond at C-3′ of the phenyllactate is broken and the new carbon–carbon bond is formed with retention of configuration.[3] The stereochemical course of the delivery and the location of the 3′-*pro*-*S* hydrogen after its delivery, to C-3′ of the tropate moiety have not previously been evaluated and are now reported.

To solve this problem we deployed chiral methyl group methodology and our approach is summarised in Scheme 1. (*R,S*)-DL-Phenyl[2-^{3}H]lactic acid **3** [specific activity 204 μCi mmol^{-1}) was incubated with transformed root cultures of *Datura stramonium*. The isolated hyoscyamine (50 mg) was diluted tenfold with cold hyoscyamine (500 mg) and then subjected to barium hydroxide hydrolysis to release the tropate moiety. Treatment of an acidic ether extract with diazomethane allowed recovery of the tropic acid **4** as its methyl ester **5**. Conversion of the alcohol moiety of **5** to a mesylate generated **6**, which was then reduced with $LiAlD_4$ (98 atom%). The resultant 2-phenyl[1-$^2H_2$, 3-^{3}H, ^{2}H]propan-1-ol **7** now posessed a chiral methyl group at C-3 of **7**, which was chiral by virtue of the presence of three isotopes of hydrogen. Oxidation of **7** with KIO_4–$KMnO_4$, and then steam distillation, allowed chiral acetic acid to be isolated.[4] After neutralisation with dilute NaOH followed by lyophilisation, sodium acetate **8** and sodium formate (ratio, acetate : formate 2 : 1, as determined by ^{1}H NMR) were recovered as a mixture. ^{1}H NMR analysis of this mixture [specific activity 6.0 μCi mmol^{-1}] distinguished two populations of acetate, singly deuteriated and unlabelled, in a 3 : 4 ratio. We have deduced that the unlabelled acetate arose from another source during the oxidation reaction. Reanalysis of the ^{1}H NMR spectrum recorded of **7** prior to its oxidation, revealed a trace of diethyl ether, the solvent used in the $LiAlD_4$ reduction. We therefore attribute the resultant unlabelled acetate to oxidation of this material. In the event, it was determined that the acetate molecules containing three different isotopes of hydrogen had predominantly a single configuration. The chiral purity of the acetate sample was assayed in the usual manner[5] by the coupled malate synthase/fumarase assay, and indicated the (*R*)-configuration for the acetic acid, with an enantiomeric excess of 96% (*F* value = 77.9).

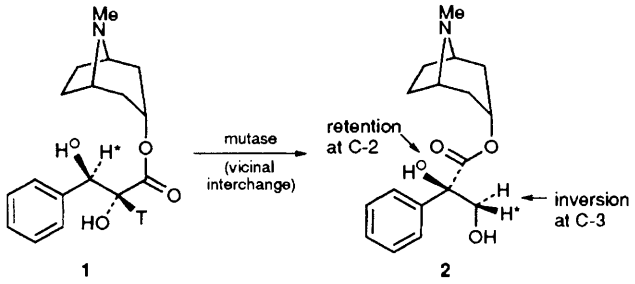
Taking into account the stereochemical inversion at carbon during the $LiAlD_4$ reduction, it is deduced that the tritium isotope occupied the 3′-*pro*-*S* site in the tropate moiety of hyoscyamine **2**. Consequently the migrating hydrogen from the 3′-*pro*-*S* site of phenyllactate must rest in the 3′-*pro*-*R* site of tropate after the vicinal interchange process. It can be further deduced, from a knowledge that (*R*)-D-phenyllactate is processed,[1] that the new C–H bond at C-3′ of the tropate moiety, replaces the old C^1–C^2 bond of phenyllactate with *inversion* of configuration.

All of the stereochemical features of the rearrangement of littorine to hyoscyamine are now evaluated and are summarised in Scheme 2. The vicinal interchange process has obvious similarities to the coenzyme-B_{12}-mediated rearrangements of methylmalonyl–CoA mutase[6] and isobutyryl–CoA mutase.[7,8] In both of these cases, however, the migrating hydrogen atom replaces the COSCoA group with retention of configuration. Therefore the steric course in this respect is opposite.

We thank Dr Peter Bachmann for assistance with the feeding experiments and are grateful to the EPSRC for a studentship



Scheme 1 *Reagents and conditions*: i, $Ba(OH)_2$; ii, CH_2N_2, Et_2O, 67% from **2**; iii, methanesulfonyl chloride, DMAP, pyridine, 0.5 h, 78%; iv, $LiAlD_4$, Et_2O, 2 h, 82%; v, KIO_4–$KMnO_4$, 2 h, steam distillation then lyophilisation, 16%



Scheme 2 Summary of the stereochemical course of the rearrangement of littorine **1** and hyoscyamine **2** in *Datura stramonium*

(N. C. J. E. C.) and the University of Durham and the US Public Health Service (NIH Grant GM 32333) for financial support.

Received, 25th October 1994; Com. 4/06542E

References

1 N. C. J. E. Chesters, D. O'Hagan and R. J. Robins, preceding paper.
2 E. Leete, *J. Am. Chem. Soc.*, 1984, **106**, 7271.
3 E. Leete, *Can. J. Chem.*, 1987, **65**, 226.
4 J. T. Kealey, S. Lee, H. G. Floss and D. V. Santi, *Nucleic Acids Res.*, 1991, **19**, 6465.
5 H. G. Floss and M. D. Tsai, *Adv. Enzymol.*, 1979, **50**, 243.
6 M. Sprecher, M. Y. Clark and D. B. Sprinson, *Biochem. Biophys. Res. Commun.*, 1964, **15**, 581; *Biol. Chem.*, 1966, **241**, 872.
7 K. A. Reynolds, D. O'Hagan, D. Gani and J. A. Robinson, *J. Chem. Soc., Perkin Trans. 1*, 1988, 3195.
8 G. Brendelberger, J. Retey, D. M. Ashworth, K. A. Reynolds, F. Willenbrock and J. A. Robinson, *Angew Chem., Int. Ed. Engl.*, 1988, **27**, 1089.