The Biosynthesis of Tropic Acid: A Reevaluation of the Stereochemical Course of the Conversion of Phenyllactate to Tropate in *Datura stramonium*

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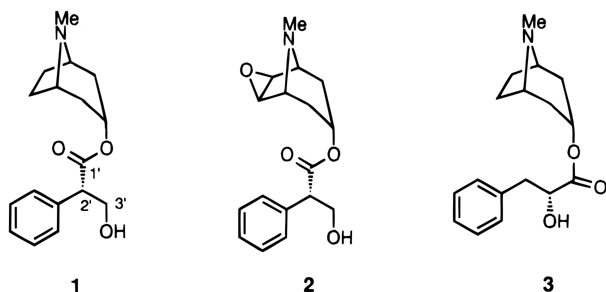
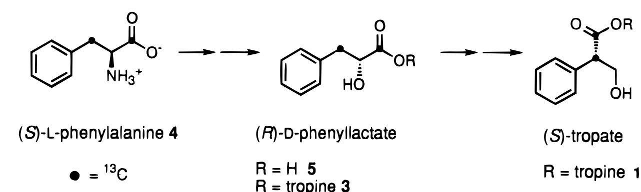
The biosynthetic origin of the tropate ester moiety of hyoscyamine (**1**) and scopolamine (**2**) has attracted a lot of interest over many years,[1] yet many details of the process remain obscure. In 1975 Leete *et al.* showed[2] that the tropate moiety of **1** originates by an intramolecular rearrangement of the (*S*)-phenylalanine (**4**) skeleton. Incorporation studies with (*S*)-[1,3-$^{13}C_2$]phenylalanine (**4**) in *Datura* plants resulted in **1** with a 1′,2′-$^{13}C_2$-labeled tropate moiety as shown in Scheme 1. The resultant contiguous arrangement of isotopes established the intramolecular nature of the rearrangement.



We have recently demonstrated[3] that (*R*)-phenyllactate **5** is a closer precursor than (*S*)-phenylalanine (**4**) or (*S*)-phenyllactate to the tropate ester moiety, and in an illuminating study Robins *et al.*[4] have presented evidence which implicates the alkaloid littorine (**3**), the (*R*)-phenyllactate ester of tropine, as the substrate for the rearrangement. This counters the long-held contention that the rearrangement occurs at the coenzyme A ester level, which extended the analogy from methylmalonyl-CoA mutase which catalyzes a coenzyme B_{12} mediated rearrangement.[5]

Using chiral methyl group methodology we have shown[6] that the carboxylate group at C-2 of (*R*)-phenyllactate, which migrates in the process, is replaced by a hydrogen atom at C-3 of the (*S*)-tropate ester moiety of **1**, with *inversion of configuration*. The stereochemistry at the other migration terminus has been probed in two previous but contradictory studies[7,8] utilizing appropriately labeled (2*S*,3*R*(or 3*S*))-[2-^{14}C,3-$^3H_1$]-phenylalanines. Haslam *et al.*[7] concluded that the 3-*pro-R* hydrogen was lost during the rearrangement and thus, the new C−C bond was formed with inversion of configuration. However, in a reevaluation of this issue, Leete[8] concluded that the 3-*pro-S* hydrogen was replaced with retention of configuration. Leete argued that tritium at C-2′ in the resultant tropate ester was susceptible to washout, during the base hydrolysis required to release free tropic acid, prior to scintillation counting. The hyoscyamine was stirred in either NaOH or $Ba(OH)_2$ solution for 30 min prior to workup. Thus, if this is taken into account in the Haslam study,[7] then the absence of tritium at this site was not a consequence of the stereochemical course of the reaction, but of chemical manipulation. A further conclusion of Leete's study[8] was that the 3-*pro-S* hydrogen underwent an intra- or intermolecular vicinal interchange with the migrating carboxylate group and was returned to C-3′ of the tropate ester moiety. This further strengthened the analogy between this rearrangement and methylmalonyl-CoA mutase, where such a vicinal interchange is well established.[5]

Scheme 1



Since our recent stereochemical study has established an inversion of configuration at the other migration terminus, the enzyme appeared to mediate a retention/inversion process, which is difficult to reconcile with a vicinal interchange process. Such a process would require the putative mutase to remove and deliver the migrating hydrogen to opposite faces of the substrate. We therefore decided to reinvestigate this issue. The knowledge that (*R*)-phenyllactate is a more proximate precursor of **1** than is **4**, and the availability of transformed root cultures which give much higher levels of precursor incorporation (20−45% for **5** into **1**),[3] allowed a more definitive examination of the reaction stereochemistry than was possible at the time of the earlier studies. Using dual labeled (^{2}H and ^{13}C) samples of **5**, the regiospecific location of the deuterium atoms in the resultant samples of **1** could be determined directly by ^{13}C-NMR spectroscopy by employing a ^{13}C label at C-2 of **5** as a reporter atom, without recourse to hydrolysis and isolation of tropic acid. Deuterium directly attached to or one bond removed from a carbon-13 atom induces a detectable α- or β-upfield-shifted signal, respectively, in the $^{13}C\{^1H,^2H\}$-NMR spectrum.[9]

(2*R*,3*S*)-[2-^{13}C,3-$^2H_1$,*phenyl*-$^2H_5$]-Phenyllactate (**5a**) carrying deuterium at the 3-*pro-S* site and (2*R*,3*R*)-[2-^{13}C,2,3-$^2H_2$]-phenyllactate (**5b**) carrying deuterium at the 3-*pro-R* site were prepared by appropriate modifications to the route described by Fryzuk and Bosnich.[10] In each case [2-^{13}C]glycine was used as a starting material. For **5a** [$^2H_6$]benzaldehyde[11] replaced benzaldehyde, and for **5b** deuterium gas was used in place of hydrogen gas. The stereochemical integrity of this synthetic protocol is already demonstrated, and as expected, the ee's of compounds **5a** and **5b** were judged to be 96% by GC analysis of the Mosher's ester derivatives of the methyl esters of the phenyllactates. Compounds **5a** and **5b** were then introduced into submerged cultures of *D. stramonium*, and the resultant crude hyoscyamine samples were purified by chromatography[3b] and analyzed by $^{13}C\{^1H,^2H\}$-NMR. The results are summarized in Scheme 2. In the case of **5a** a β-shift (Δppm = 0.064 ppm) associated with the carbon-13 signal (δ_C = 64 ppm) for C-3′

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(9) Garson, M. J.; Staunton, J. *Chem. Soc. Rev.* **1979**, 539.
(10) Fryzuk, M. D.; Bosnich, B. *J. Am. Chem. Soc.* **1977**, *99*, 6262.
(11) The first sample synthesized, the (2*R*,3*S*) isomer **5a**, also carried five atoms of deuterium in the aromatic ring to facilitate analysis of the product by mass spectroscopy, should this become necessary.

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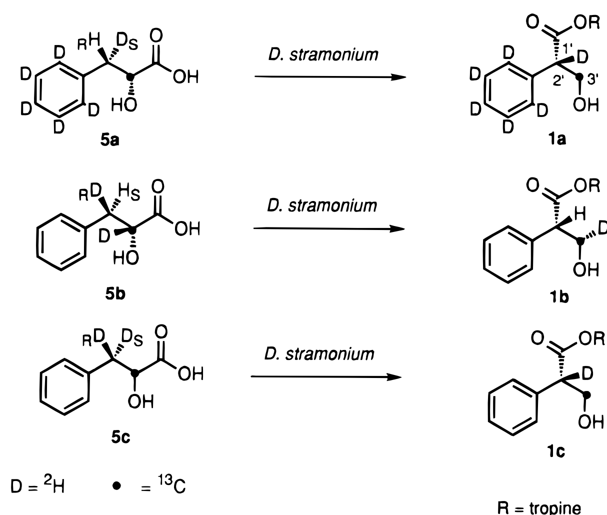
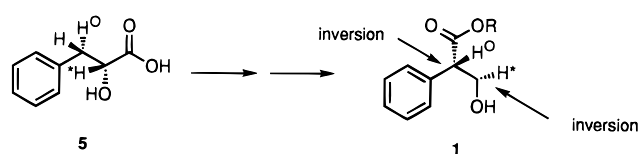
Scheme 2



of hyoscyamine is indicative of **1a** as a component (42%) of the alkaloids where deuterium occurs only on the adjacent C-2′ carbon. Thus, from this experiment it is deduced that the 3-*pro-S* hydrogen to carbon bond remains intact during the rearrangement. In the case of **5b** the isolated alkaloids contained **1b** (20.8%). The enriched ^{13}C-NMR signal has a single α-shift (Δppm = 0.35 ppm) associated with it, consistent with the retention of the $^{13}C-^{2}H$ bond from **5b**. Significantly there was no evidence of an additional β-shift, and thus there was no indication of deuterium retention at C-2′ of **1b**. Thus, the 3-*pro-R* hydrogen is lost during the rearrangement process; it does not migrate to the adjacent carbon.

The results of the feeding experiments with **5a** and **5b** are unambiguous and demonstrate that the 3-*pro-R* hydrogen is removed and the 3-*pro-S* hydrogen retained during the rearrangement of (*R*)-phenyllactate to (*S*)-tropate. The rearrangement proceeds with stereochemical *inversion* at this migration terminus. Additionally there is no evidence of a vicinal interchange process. The 3-*pro-R* deuterium of **5b** was not returned at a detectable level to C-3′ of the tropate skeleton. In order to further reinforce this conclusion we challenged the system with (*R*,*S*)-[2-^{13}C,3-$^{2}H_2$]phenyllactate (**5c**), in which both of the C-3 hydrogen atoms are replaced by deuterium. This compound was prepared by a modification of our previous method[3], and the result of the feeding experiment is summarized in Scheme 2. The ^{13}C-NMR spectrum of the resultant hyoscyamine showed a β-shift (Δppm = 0.063 ppm) associated with the enriched C-3′ carbon atom (64 ppm) indicative of the presence of **1c** (45%). In the light of the above results this observation is consistent with the loss of the 3-*pro-R*, and

Scheme 3



retention of the 3-*pro-S*, deuterium atom. There was again no evidence of an intact $^{13}C-^{2}H$ bond (α-shift) at C-3′ of the tropate ester; thus we can exclude the operation of a vicinal interchange process.

We can only speculate why Leete reached a different conclusion in his work.[8] Both previous studies[7,8] were carried out with appropriately labeled (*S*)-phenylalanines rather than the more immediate precursor, (*R*)-phenyllactate. Some stereospecific loss of tritium during the transamination of (2*S*,3*R*(or 3*S*))-[2-^{14}C,3-$^{3}H_1$]phenylalanines cannot be excluded and could complicate the interpretation of the previous results. Furthermore, the enantiomeric purity of the labeled (2*S*,3*R*(or 3*S*))-[2-^{14}C,3-$^{3}H_1$]phenylalanines at the C-3 stereogenic center in both of the previous studies was only about 90% ee and the incorporation of the precursors into the alkaloids was very low (<1%). Perhaps more importantly, the previous analyses[7,8] relied on ^{3}H/^{14}C ratios and the definitive conclusion was dependent on the complete postbiosynthetic washout of tritium at C-2 of the tropate ester moiety of hyoscyamine. If a kinetic isotope effect resulted in only partial washout during the relatively short (30 min) hydrolysis treatments, then this would validate Haslam's original conclusions.[7]

In summary, all of the stereochemical aspects of the D-phenyllactate to tropate rearrangement have been evaluated and are summarized in Scheme 3. During carboxylate migration an inversion of configuration occurs at both migration termini, and we can exclude the back migration of a hydrogen atom in a vicinal interchange process, as previously suggested.[8a]

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Supporting Information Available: ^{13}C- and ^{1}H-NMR spectra for the synthesized compounds described in the text and the relevant sections of the ^{13}C-NMR spectra of the isotopically enriched hyoscyamines showing α- and β-shifts (9 pages). This material is contained in many libraries on microfiche, immediately follows this article in the microfilm version of the journal, can be ordered from the ACS, and can be downloaded from the Internet; see any current masthead page for ordering information and Internet access instructions.

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