Tropane alkaloid biosynthesis. A century old problem unresolved

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1 Introduction

The modern natural products era, certainly in terms of structure elucidation, emerged around 150 years ago during the mid-19th century. Identifying the structure of the “vegetable alkalis” or the plant alkaloids as they subsequently became known, was a major focus of organic chemistry research. Methods in isolation could deliver analytically pure samples of compounds from plants, and the leading German and British organic chemistry laboratories in the 19th century set about solving the constitution of natural products by synthesis. Structure elucidation, emerged around 150 years ago during the mid-19th century. Identifying the structure of the “vegetable alkalis” from plants, and the leading German and British organic chemistry laboratories in the 19th century set about solving the constitution of natural products by synthesis. Structure elucidation was as much then as it has been all through the 20th century, a driving force in the development of organic chemistry laboratories.

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1 Introduction

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The tropane alkaloids, such as hyoscyamine and scopolamine, were prominent and attracted attention as they had accumulated a significant folklore where extracts of plants, e.g. henbane (Hyoscyamus niger) and mandrake (Mandragora officinarum), were used in the Middle Ages as hallucinogens and the toxic effects of thorn-apple (Datura stramonium), also known as jimson-weed were well known. They also have a characteristic and potent mydriatic action. Atropine (racemic hyoscyamine) causes the dilation of eye pupils at 1 in 130 000 parts in water and is used widely in ophthalmics as a pupil dilatory agent. During the Renaissance fashionable ladies would drop belladonna extract (from Atropa belladonna) into their eyes to make themselves appear more attractive.

Scopolamine, which is a co-product of hyoscyamine in Datura plants, remains a significant premedication administered before surgery under general anaesthetic, to arrest salival and mucous secretions.

Cocaine was first isolated from the leaves of the Peruvian Erythroxylon coca plant by Niemann in 1860. The plant was well known for its local anaesthetic properties but it also stimulates the central nervous system and improves physical endurance. These are attractive properties and led to the drug being widely administered in Europe in various medications before the addictive properties of cocaine were fully realised.

2 Tropane structure elucidation and synthesis

The synthesis of u-conine by Ladenburg in 1886 is of particular historical significance in the history of organic chemistry as this was the first laboratory synthesis of an aliphatic alkaloid, although Baeyer had prepared indigo as early as 1870. The elucidation of the structure of the tropane ring system, found in hyoscyamine and cocaine, was very much

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on the agenda during this early period, but it was a more challenging problem and it was not until 1901 that the structure was secured with the completion of Willstätter’s extensive synthetic work on this alkaloid group. The tropanes were the first of the key alkaloids, represented by nicotine (in 1904 by Pictet and Rotschy), quinine (in 1944 by Woodward and Doering) and morphine (in 1956 by Gates and Tscudi), to succumb to total synthesis.

Plate 1 Richard Martin Willstätter (1872–1942). Established the structures of the tropane alkaloids and cocaine. His tropine synthesis of 1903 was an outstanding milestone in the history of organic chemistry. He won the Nobel prize for chemistry of 1915 largely for solving the structure of chlorophyll and other plant pigments.

In 1863 Kraut recovered atropic acid 5 and tropine 6 after boiling a sample of atropine (racemic hyoscyamine) with BaSO₄ solution (baryta). The following year Lossen showed that the initial product of the hydrolysis reaction was tropic acid 7, and that this had undergone a dehydration to atropic acid 8. The esterification process can be reversed if tropic acid and tropine are treated with dry HCl, as was demonstrated by Ladenburg 1879. It was not until many years later, in 1961, that the absolute stereochemistry of (S)-tropic acid was deduced. A similar hydrolysis of cocaine was shown to result in benzoic acid and ecgonine 8. At that time, of course, the structures of these fragments were unknown in terms of their connectivity, thus successful synthesis presented a formidable challenge. Ladenburg and Rugheimer are credited with the first synthesis of tropic acid 7 in 1880. Willstätter carried out degradation studies on tropine and simultaneously on ecgonine 8, and revealed that they both resulted in tropinic acid 9 from ecgonine 8 was optically active (dextrorotatory) but that from tropine 6 (a meso compound) was inactive, observations which could only be rationalised at a much later date. More exhaustive oxidation gave N-methylsuccinimide 10 in each case.

Willstätter’s preparation of the tropane ring system is a masterpiece in organic synthesis, particularly if we project ourselves back to the late 19th century. This synthesis is lengthy and perhaps it has been somewhat overshadowed by the elegance of Robinson’s short and much more direct tropine synthesis over a decade later in 1917 (vide infra). Robinson’s route has been widely applauded and has become, possibly, the first synthesis classic, but the Willstätter route to tropinone and tropine which is shown in Scheme 1 certainly merits a similar accolade, if not for its elegance then certainly for persistence in winning through. The route starts with cycloheptatriene 12, although the synthesis (not shown) of this compound from cycloheptanone 11, which also involved several steps, was an important and necessary part of the process. Addition of bromine and dimethylamine to cycloheptadiene 12 generated the dimethylamino-cycloheptadiene 13, and then reduction of one double bond (Na in alcohol) gave 14. In a truly historic reaction, double bond dibromination primed a cyclisation to generate the tropane salt 15, and thereby the first preparation of the tropine nucleus. Alkali treatment of 15 generated 16 and then 17, and subsequent HBr addition allowed isolation of, presumably the β-bromide 18. Treatment of bromide 18 with sulfuric acid in a sealed vessel at 200 °C (!) gave β-tropine 19, but it was the wrong stereoisomer. The relevant α-isomer (tropine 6) was obtained by Willstätter after oxidation of 19 to tropinone 20 and then reduction of the ketone with alcoholic sodium.
pyrrolidine precursors. Willstätter’s complete synthesis and optical resolution of cocaine, starting from tropinone, is illustrated in Scheme 2.

Treatment of tropinone 20 in alcoholic sodium, followed by a carbon dioxide quench gave egeninone 21. The methyl ester of egeninone 22 was reduced with sodium amalgam to give two diastereomeric products, the relevant one for cocaine synthesis being 23. Benzoyl formation by reaction with the anhydride of benzoic acid, generated racemic cocaine, which was resolved by recrystallisation of the d-ditartrate salt. Release of the free base of one enantiomer gave a compound with identical physical properties to cocaine.

These outstanding achievements of Willstätter merit a profile in any Millennium discussion on progress in organic synthesis. He won the Nobel Prize for Chemistry in 1915 at the age of 43, not only for his contributions to alkaloid structure elucidation but also for his significant contributions to chlorophyll chemistry, which became a major focus of his research in the early 1900s.

3 Biosynthesis of the tropane ring

3.1 The Robinson hypothesis

In 1917 Robert Robinson published the synthesis of tropinone 20 in one pot by the addition of succinaldehyde 24 to an aqueous solution of methylamine, and acetone. The yield improved significantly (42%) at physiological pH, when acetone was replaced by the calcium salt of acetonedicarboxylic acid 25 (Scheme 3). This observation stimulated a proposal by Robinson in 1955 suggesting that the biosynthesis of tropinone might occur via an analogous route involving an amino acid to provide the pyrrolidine ring moiety and an “acetone equivalent” to furnish C-2, C-3 and C-4 of the tropane ring. The exact details of how N-methylpyrrolidine condenses with an acetate-derived subunit, delivering C-2, C-3 and C-4 of the ring system, are unknown (vide infra). However, it is particularly noteworthy that after many years of investigation since Robinson’s original proposal, the evidence which has emerged over the last decade or so points to the involvement of acetonedicarboxylate as the true biosynthetic intermediate to C-2, C-3 and C-4 of tropinone 20 (vide infra). The original proposal is holding remarkably firm today.

3.2 The pyrrolidine moiety in tropane ring biosynthesis

To Edward Leete must be attributed many of the early revelations in tropane alkaloid and cocaine biosynthesis. He first confirmed the involvement of ornithine 26 as a precursor to the amino acid portion of the tropane ring. Arginine can clearly contribute to this pathway too as ornithine and arginine have a close metabolic relationship. After a feeding experiment with radiolabelled [2-14C]ornithine where the label was incorporated into hyoscyamine 1 in D. innoxia plants, Leete proposed a pathway from ornithine to putrescine 27. The N-methylation of putrescine to generate 28 followed by oxidative transamination would yield the aminoaldehyde 29, a compound which is primed for cyclisation to a N-methylpyrrolinium intermediate 30. Condensation with the as yet unknown acetate-derived moiety would then deliver tropinone 20 as illustrated in Scheme 4.
Hemscheidt and Spenser reported unresolved and intriguing stereochemical questions. In 1992 pyrrolinium along the tropane pathway, then there remain some intermediate description of the biosynthesis of the such as putrescine the pathway proceeded Equal incorporation of CD\(^2\)\(^-\)acetate into root cultures of \(D.\) \(stramonium\) produced hyoscyamine that showed the symmetrical incorporation of \(1\)\(^3\)CD\(_2\) methylene groups into C-6 and C-7 of the tropane ring. \[2\]\(^{-}\]13C into the C-1, C-5, C-6, C-7 portion of alkaloids of \(D.\) \(stramonium\) fed with \([1,2\]\(^{13}\)C\]acetate and more recently the incorporation of \([2\]\(^{-}\]15\)C, \(\text{H}_2\]acetate into root cultures of \(D.\) \(stramonium\) produced hyoscyamine that showed the symmetrical incorporation of \(1\)\(^3\)CD\(_2\) methylene groups into C-6 and C-7 of the tropane ring. \[2\]\(^{-}\]13C \(\text{H}_2\]Acetyl-CoA 31 is metabolised via the citric-acid cycle and can be fed into ornithine biosynthesis as shown in Scheme 5, producing a glutamate that is selectively labelled with two deuterium atoms at C-4. Equal incorporation of CD\(_2\) units at C-6 and C-7 would arise if the pathway proceeded via a relevant symmetrical intermediate such as putrescine 27. This appears to be the most satisfactory description of the biosynthesis of the N-methylpyrrolinium intermediate 30 at present.

If we consider the biochemical events from N-methylpyrrolinium along the tropane pathway, then there remain some unresolved and intriguing stereochemical questions. In 1992 Hemscheidt and Spenser reported\(^{27}\) that deuterium from experiments in this system and in other plants, e.g. \(Hyoscyamus albus\)\(^{22}\) and \(Erythroxylum coca\)\(^{12,24}\) have shown equal labelling into C-1 and C-5. The weight of the accumulated evidence suggests a symmetrical rather than a regiochemical incorporation. Walton \textit{et al.} have identified\(^{18}\) high levels of the enzyme putrescine N-methyltransferase in root cultures of \(D.\) \(stramonium\), suggesting that the most likely route to N-methylputrescine 28 in this species is, in fact, via putrescine 27, a symmetrical intermediate.

Hemscheidt has also reported\(^{25}\) a symmetrical distribution of \(^{13}\)C into the C-1, C-5, C-6, C-7 portion of alkaloids of \(D.\) \(stramonium\), suggesting that the most likely route to \(N\)-methylputrescine 28 in this species is, in fact, via putrescine 27, a symmetrical intermediate.

3.3 The acetate-derived fragment of the tropane ring

The biosynthesis of the tropane ring system clearly requires the condensation of an appropriate acetate-derived intermediate with \(N\)-methylpyrrolidinium. Leete and co-workers observed\(^{26,29}\) that \([2\]\(^{-}\]15\)C,\(^{13}\)N\]-N-methylpyrrolinium chloride 30b becomes incorporated into methyl ecegonine 23a, the heterocyclic portion of leaves of \(Erythroxylum coca\) (Scheme 6). The resultant cocaine 3 was labelled at C-5 and not C-2, (the anticipated position). At the outset it had appeared most reasonable that the first condensation of an acetate-derived moiety would be to the side of the molecule carrying the carboxylate group in cocaine, but this proved not to be the case.

Candidates for the acetate-derived moiety suggested themselves from Robinson’s original hypothesis and thus acetone, acetate and acetoxacetic acid have all been considered as potential intermediates. For many years hygrine 32, a widely distributed alkaloid which is formally the product of an acetone condensation with \(N\)-methylpyrrolidinium, was discussed as a relevant biosynthetic intermediate. This was based on early radiochemical incorporation studies.\(^{30,31}\) However, more recent studies\(^{44}\) using stable isotopes do not support a role for hygrine 32 and the general consensus is that it is not involved in tropane ring biosynthesis.

The most advanced intermediate that has become successfully incorporated into the tropane ring moieties of both scopolamine and hyoscymine is racemic ethyl \([2,3\]\(^{13}\)C\]\(-\)4-(\(N\)-methyl-2-pyrrolidinyl)-3-oxobutanoate 33\(^{32,33}\). In an intriguing observation it was concluded that both enantiomers of ethyl \([2,3\]\(^{13}\)C\] 33 can become incorporated into the tropane alkaloids hyoscymine 1 (and scopolamine 2) as illustrated in Scheme 8. Scopolamine 2 isolated from hydroponic cultures of \(Datura innoxia\) showed a substantial incorporation of 33 into C-2, C-3 and C-4, with incorporation at C-4 being significantly (40\%) higher than at C-2. This result was taken as an indication that both (R)- and (S)-enantiomers of 33 were
converted directly into tropane alkaloids by the organism, with a slight preference for the (R) enantiomer over the (S). Robins has since reported\textsuperscript{33} that there is no preference for the formation of tropinone from (R)-33 over (S)-33 in D. stramonium. This was deduced by equal incorporations into C-2/C-3 and C-3/C-4, an observation which cannot be explained by the racemisation of one enantiomer of 33 into the other. The current explanation is somewhat contra-intuitive in that it requires that each enantiomer is processed equally by the ring oxidation/cyclisation enzyme system. Thus there are two stereochemical conundrums which remain unanswered in the later stages of tropane ring assembly. Firstly there is the observation that both enantiomers of 33 are accepted as intermediates in tropane alkaloid biosynthesis (Scheme 8) and secondly there is the observed\textsuperscript{27} scrambling of label from [2-\textsuperscript{2}H\textsubscript{1}]-N-methylpyrrolidine into C-1 and C-5 of the tropane ring (Scheme 6). Perhaps a unifying explanation will emerge to rationalise these observations, but that answer is not clear at present. For cocaine,\textsuperscript{28} the situation is different because the presence of the extra chiral centre at C-2 places a restriction on the possible cyclisation paths. Only the (S)-enantiomer of 4-(N-methyl-2-pyrrolidinyl)-3-oxobutanoate (S)-33 can cyclise to generate the tropane ring system of cocaine 3a as shown in Scheme 9.

The ester 33 appears to be a bona fide intermediate in tropane ring assembly presumably after in vivo hydrolysis to the free carboxylate 36 as shown in Scheme 10.\textsuperscript{28,29,30} It is tempting to speculate that 36 is derived from the sequential condensation of two acetate units, perhaps from malonyl-CoA, in a polyketide type assembly process.\textsuperscript{28} However, it is pertinent too that, although the oxobutanoate 33 results in excellent incorporation into target alkaloids, the shorter monoacetate derivative 34 does not.\textsuperscript{31} So a step wise process of one malonate/acetate condensation following another is not borne out experimentally. Perhaps of course there is a polyketide enzyme that accepts N-methylpyrrolinium 30 and is fully committed to two acetate/malonate condensations before the oxobutanoate product 36 is released from the enzyme. This may explain why the monoaacetate adduct 34 does not access the assembly process.

Alternatively acetoacetate 35 may become involved in a direct condensation with N-methylpyrrolinium. This too is conceptually attractive but again experimental support is lacking. [1,2,3,4-\textsuperscript{13}C\textsubscript{4}]Acetoacetate 35 is not incorporated intact into these systems. In all cases where this has been investigated, acetoacetate is metabolised back to acetate and the isotope re-introduced\textsuperscript{de novo via [1,2,3,4-\textsuperscript{13}C\textsubscript{4}]-acetate.\textsuperscript{27,34}}

Hemscheidt has discussed all of these issues in an excellent and recent review\textsuperscript{27} and proposes that Robinson’s acetonedi-carboxylate 25 emerges as a potential intermediate in tropane alkaloid biosynthesis. The experimental support for this comes from a biosynthetic study\textsuperscript{33,34} on another alkaloid family. A feeding experiment\textsuperscript{36} with [2,3,4-\textsuperscript{13}C\textsubscript{2}]acetonedicarboxylate 25a to whole plants of Lycopodium tristachyum resulted in a predominant and contiguous incorporation of the three isotope into two separate locations in the alkaloid lycopodine 40, as shown in Scheme 11. It was concluded that there is a condensation between the piperidinium ring system 37 and acetonedicarboxylate 25 to generate intermediate 39 and a decarboxylated product 38. Condensation of 38 and 39 then provides lycopodine 40. Clearly a similar condensation between N-methylpyrrolinium 30 and acetonedicarboxylate 25 would generate the oxobutyrate 36 as shown in Scheme 12, for further conversion into tropine. Thus the Robinson synthesis of 1917, which has been accepted as a good general hypothesis for tropane biosynthesis, may become more aligned in detail as further information emerges on tropane ring assembly.
the substrate result in the different stereoselectivity of the two TRs. Site-directed mutagenesis at just five key positions was found to be sufficient to reverse the stereoselectivity of the purified enzymes.42

It is notable that, although both TRI and TRII show appreciable activity in vitro, very little accumulation of alkaloid products resulting from the action of TRII is observed in root cultures of Datura.43 This has been explained44 by the differing kinetic and pH/activity characteristics of the enzymes and by the substantially higher (ca. 25-fold) activity of the TRI over the TRII isolated from Datura root cultures.

4 Tropate ester biosynthesis

The (S)-tropic acid 7 ester moiety is a structural feature of the alkaloids hyoscyamine 1 and scopolamine 2. The biosynthetic origin of tropic acid 7 has been the subject of continued discussion from the early part of the last century and remains an unresolved issue, certainly in the detail. Again we must revisit Robinson who highlighted the general problem of tropic acid biosynthesis in 192744 and then a little more comprehensively in his monograph on The Structural Relations of Natural Products in 1955.45 It was recognised that tropic acid 7 had a branched carbon skeleton and that it must derive either from an isomerisation of a phenylpropionoid moiety or by a special synthesis. In a definitive experiment46 Leete was able to show that it derived by isomerisation after feeding [1,3-13C]phenylalanine to Datura innoxia. The resultant hyoscyamine 1e now had the isotopes adjacent to each other in positions C-1 and C-2 of the alkaloid as shown in Scheme 14. This observation established a direct relationship between phenylalanine metabolism and an intramolecular isomerisation involving carboxylate migration to generate the tropate skeleton. However, phenylalanine is readily metabolised to phenylpyruvate 42, and phenylpyruvate can be reduced to phenyllactate 43 as shown in Scheme 15. Indeed (R,S)-[1,3-13C]phenyllactate was also shown46,47 to efficiently label the tropate ester of hyoscyamine in a contiguous manner similar to that in the [1,3-13C]phenylalanine experiment. These labelling results did not delineate which of these intermediates was closest to tropic acid. This was achieved by a set of labelling experiments48 which involved the incorporation of (S)- and (R)-[2-13C, 2-2H]phenyllactates, as 43, into C-3’ of the tropate ester of 1. It was demonstrated that the deuterium atom at C-2 of (R)-[2-13C, 2-2H]phenyllactate was retained in the resultant tropic acid. The deuterium atom from the (S)-[2,13C, 2-2H]phenyllactate enantiomer was lost. This clearly indicated that (i) (S)-43 is incorporated via (R)-43, and (ii) (R)-phenyllactate 43 is a closer precursor than phenylalanine 41 and phenylpyruvate 42 as, necessarily, deuterium from (R)-[2-13C, 2-2H]phenyllactate would have been lost in transamination to (S)-phenylalanine 41 via phenylpyruvate 42 as shown in Scheme 15.

Now that (R)-phenyllactate 43 had been identified as the most immediate precursor thus far to the tropic acid ester moiety of hyoscyamine 1, attention turned to identifying which form of phenyllactate is the substrate for the isomerisation. An obvious candidate was a direct isomerisation of (R)-phenyllactate to tropic acid. However, several reports emerged with no convincing evidence for a role for free tropic acid in hyoscyamine biosynthesis.50,51 When isotopically labelled tropic acid was administered to plant and root systems, incorporations into the resultant alkaloids was low. Another potential substrate for the rearrangement was (R)-phenyllactate-S-CoA 44. The role of this putative intermediate was stimulated by the similarity of the rearrangement with co-enzyme B12-mediated isomerisations, e.g. methylmalonyl-CoA mutase.49 The notion of the involvement of co-enzyme B12 in tropate ester biosynthesis has persisted to the present day from Leete’s original discussion of the issue in 1987;52 however, there is no evidence that B12 is involved in this transformation (vide infra).

A potential role for (R)-phenyllactoyl-S-CoA 44 was superceded by the important observation of Robins, Bachmann and Woolley53 that the tropane alkaloid littorine 45 (an ester of tropine and (R)-phenyllactate) was directly converted into hyoscyamine 1 in biosynthetic experiments. In an elegant and conclusive experiment they showed that a penta-labelled [H1-N-methyl, 1'-3-13C]littorine 45 was converted into [H2-N-methyl, 1'-2-13C]hyoscyamine 1e in D. stramonium tissue cultures as shown in Scheme 16. GC-MS analysis indicated that the intensity of the M+5 ion in hyoscyamine 1 was sufficiently high that it could not be accounted for by hydrolysis to (R)-phenyllactate 44 and then rearrangement and recombination with tropine. This was a groundbreaking experiment as it revealed that Robinson’s elusive “phenylpropionoid” substrate, required for tropic acid ester biosynthesis, was now the well-known alkaloid littorine 45.

4.1 Stereochemical course of the rearrangement of littorine to hyoscyamine

During the rearrangement of littorine to hyoscyamine two bonds are broken and two are formed. It has emerged that both of these bonds are broken and formed with an overall inversion
of configuration at each migration terminus. These conclusions were gleaned from two sets of experiments. Firstly, feeding experiments with (2R,3R)- and (2S,3S)-[3-^3H]phenyllactates were carried out in *D. stramonium* transformed root cultures. In the event the deuterium at the 3-*pro-R* site was removed during the transformation, and the deuterium at the 3-*pro-S* site was retained. The resultant tropate ester has the (S) configuration so it was deduced that there is an overall inversion of stereochemistry at C-3 of (R)-phenyllactate when it is converted into C-2 of hyoscyamine as illustrated in Scheme 17. This result is consistent too with an earlier one where (2S,3R)- and (2S,3S)-[3-^3H]phenyllalaines were fed to *D. innoxia* plants. The tritium was retained only from the 3-*pro-R* labelled amino acid.

In a second study the stereochemical course at the other migration terminus, i.e. where C-2’ of littorine is converted into C-3’ of hyoscyamine, was solved using chiral methyl group methodology. A feeding experiment to root cultures of *D. stramonium* using radiolabelled [3-^3H]phenyllactate generated a sample of littorine with tritium located at the C-2’ position as shown in Scheme 18. After in vivo isomerisation to hyoscyamine, the stereochemical configuration of the tritium in littorine was determined by chemical conversion, via alcohol, into chiral [^1H,H,^1H]acetic acid (Scheme 18). Analysis of the resultant chiral acetic acid revealed that it possessed the (R) configuration with an enantiomeric excess of 96% ee. This outcome requires that the tritium at C-3’ in hyoscyamine occupies the 3’-*pro-S* site. Clearly, therefore, there is an overall inversion of configuration in going from littorine to hyoscyamine at this migration centre.

### 4.2 B$_{12}$ or not B$_{12}$?

The isomerisation of a phenylactoyl ester (littorine 45) to generate a tropyl ester (hyoscyamine 1) has a clear similarity to some co-enzyme-B$_{12}$ mediated isomerisations. It is a feature of the B$_{12}$ systems that they mediate a vicinal interchange process i.e. the group that migrates also implicates back migration of a hydrogen atom. Thus for methylmalonyl-CoA mutase, which interconverts (R)-methylmalonyl-CoA and succinyl-CoA, the carboxylthioester migrates to the methyl group of (R)-methylmalonyl-CoA, and a hydrogen from the methyl group is retained to the original carboxylate position, as summarised in Scheme 19.

Leete was very aware of a potential role for co-enzyme B$_{12}$ in tropic acid biosynthesis, although he noted that there was no evidence that vitamin-B$_{12}$ is present in plants. An analysis of *D. innoxia* plant tissue for cobalt did not reveal significant levels of the metal in the plant material. However, in an experiment where (2S,3R)- and (2S,3S)-[3-^3H]phenyllalaines were fed to *D. innoxia* plants, it was concluded that tritium from the 3-position of phenyllalane had become located at the 3’-position of the resultant hyoscyamine. This observation was consistent with the migration of a hydrogen (tritium) atom in a vicinal interchange process concomitant with the carboxylate migrating in the opposite direction, a process characteristic of a co-enzyme B$_{12}$ mediated process. However, it should be noted that the determination of the incorporation relied on chemical degradation of the resultant alkaloid and that the level of incorporation was very low (0.2%). A subsequent $^{13}$C NMR study, examining the incorporation of 3(R)- and 3(S)-[2-^13C,3-^3H](R)-phenyllactates, did not reveal any evidence for deuterium migration during the isomerisation process, despite high incorporation levels (15–20%). So this and the earlier study must remain contradictory, but in view of the higher incorporation levels and improved analytical methods it appears unlikely that a vicinal interchange process is operating during the isomerisation of littorine to hyoscyamine.

The absence of vitamin-B$_{12}$ in plants prompted an exploration examining a role for S-adenosylmethionine (SAM) in the rearrangement of littorine to hyoscyamine. SAM has been described as “the poor man’s B$_{12}$” as it too can be activated to an adenosyl radical, the relevant activated intermediate in co-enzyme-B$_{12}$ mediated processes, to act as a co-enzyme-B$_{12}$ surrogate. The mechanisms are therefore similar and the SAM mediated isomerisation should also display a vicinal interchange process, although this is not observed. A cell free extract of *D. stramonium* was used to evaluate the co-factor requirement for the isomerisation reaction. It was concluded that the addition of SAM stimulated a significant increase in hyoscyamine levels in the cell extract. In the study SAM, labelled with tritium at the adenosyl methyl group, was incubated in a cell free extract with littorine, but tritium from this methyl group was not relocated in the resultant tropic acid ester moiety of hyoscyamine. The absence of this isotope delivery from SAM to hyoscyamine is unexpected as the mechanistic hypothesis predicts hydrogen transfer from an intermediate adenosyl methyl group to quench the product radical. It was suggested that perhaps the radiolabel was washed out by a reversible dehydrogenase activity in the extract, but this was not explored further. So a role for SAM in the transformation of littorine 45 to hyoscyamine 1 remains to be confirmed.

### 4.3 A P$_{450}$ type process?

There is some circumstantial evidence that a P$_{450}$ type enzyme process may be operating in the rearrangement of littorine into hyoscyamine. Chlortrimazole, a P$_{450}$ inhibitor, has been shown to inhibit the conversion of littorine to hyoscyamine in...
whole cell incubations. Oxygen-18 labelling to generate [2-18O, 2H]littorine 45c resulted in up to a 30% loss of oxygen isotope relative to deuterium in going from littorine 45c to hyoscyamine 1h in transformed roots of D. stramonium, as shown in Scheme 20.\(^\text{62}\) This could be accounted for by the intermediary of aldehyde 50 as shown in Scheme 21, if some loss of isotope occurred by the exchange of oxygen with water. Aldehyde 50 could arise by an oxygen rebound process after a one-electron oxidation and radical rearrangement (pathway a, Scheme 21) typical of \(P_{450}\) oxidations. Alternatively a carboxylation type migration, arising from a \(P_{450}\) mediated two-electron oxidation \(^4\) (pathway b, Scheme 21) could occur which would result in direct formation of aldehyde 50. The intermediacy of 50 requires that the aldehyde be reduced (by NADPH)\(^3\) to deliver the tropane ester.

Littorine has emerged as the substrate for tropic acid ester biosynthesis.\(^5\) In that it is the immediate precursor to hyoscyamine, but the mechanistic details of this intriguing rearrangement constitute yet another area of tropane alkaloid biosynthesis where there remains much to discover.

### 5 The biosynthesis of scopolamine

The epoxide scopolamine 2 is generated by direct oxidation of hyoscyamine without the intermediary of a double bond. The enzyme responsible for the transformation has been purified\(^6\) from *Hyoscyamus niger* L. (\(M_r = 38\ 999\)) and shown to require Fe\(^{3+}\) and 2-oxoglutarate. Surprisingly it mediates a two step reaction to generate the epoxide.\(^4\)\(^5\)\(^6\) The first step involves hydroxylation of hyoscyamine to introduce a 6β-hydroxy group on the tropane ring to give 51, and then the same enzyme mediates epoxide ring closure to generate scopolamine 2 (Scheme 22) \(^6\)

The cloned enzyme\(^6\)\(^5\) was also able to convert hyoscyamine into scopolamine in *E. coli*, again indicating that the single activity mediates both steps.

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**Scheme 22**

\[\text{Mo} \quad N \quad O \quad \text{Mo} \quad N \quad O \]

30% loss of oxygen-18

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6 Conclusion

The tropane alkaloids have had a significant folklore and influence in medicine for hundreds of years and their presence has contributed in no small part to the development of organic chemistry since the middle of the 19th century. In view of the attention that these alkaloids have received it is perhaps surprising that the biosynthesis of the tropanes still holds several undisclosed and intriguing secrets. What is the source of C-2, C-3 and C-4 of the tropane ring system? How can we rationalise the symmetrical stereochemical distribution of isotopes in feeding experiments involving labelled \(N\)-methylpyrrolinium 30a and racemic butanoate 33? What is the mechanism of the rearrangement of littorine to hyoscyamine?

These questions will perhaps be answered in due course, but, as has always been the case, the tropane alkaloids continue to present us a challenge. There can be no doubt that the answers are worth securing in that they will reveal new insights into mechanistic enzymology and biosynthesis.

### 7 References
