

## **The Synthesis and Separation of [3-<sup>2</sup>H]Tropine and [3-<sup>2</sup>H]Pseudotropine ( $\psi$ -Tropine)**

Barbara A. Bartholomew, Michael J. Smith, Paul J. Darcy, Peter W. Trudgill and David J. Hopper

Department of Biochemistry, University of Wales, Aberystwyth, Dyfed SY23 3DD,  
United Kingdom

### **SUMMARY**

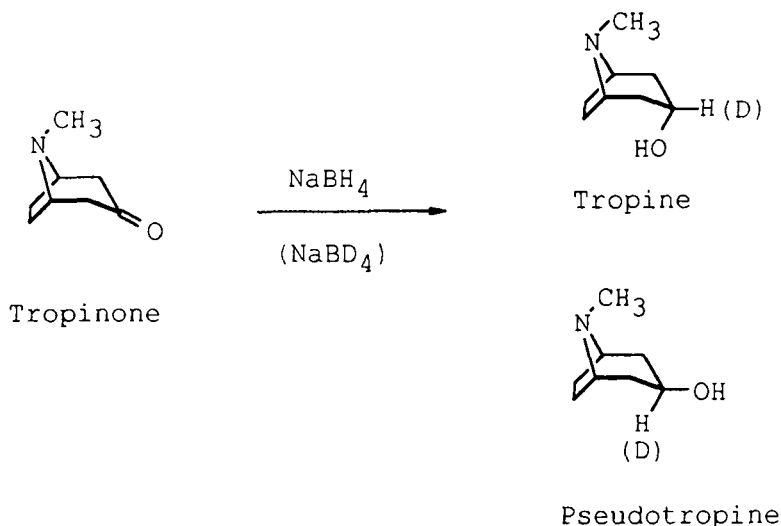
Tropinone was reduced using sodium borodeuteride to give a mixture of the epimers [3-<sup>2</sup>H]tropine and [3-<sup>2</sup>H]pseudotropine with the latter compound predominating and constituting about 70% of the mixture. Crystallisation of the product from diethyl ether gave crystals of pure [3-<sup>2</sup>H]pseudotropine and a supernatant solution containing a mixture of the epimers. Acetylation of this mixture using acetyl chloride preferentially acetylated the pseudotropine and the acetylated products were separated from the tropine by flash chromatography to leave a sample of pure [3-<sup>2</sup>H]tropine.

**Key Words:** Tropinone, Tropine, Pseudotropine.

### **INTRODUCTION**

Tropine and pseudotropine are epimers differing in the orientation of the hydroxyl group at C3 (Scheme 1) (1). They both occur naturally and a major source of tropine is the alkaloid, atropine, in which it is esterified with tropic acid. Several studies have shown that the first step in the bacterial metabolism of atropine is its hydrolysis by an esterase to yield these two separate components (2,3,4) but the pathway for the further breakdown of the tropine component is still obscure. In our studies on atropine metabolism we have demonstrated the presence, in atropine-grown bacteria, of a tropine dehydrogenase that oxidises tropine to tropinone and, in an investigation into the significance of this enzyme, samples of both tropine and pseudotropine labelled with deuterium in the C3 position were required.

Both epimers can be synthesised by the reduction of tropinone (Scheme 1) and the particular epimer produced depends on the reducing agent used (1). Reduction using sodium borohydride yields a mixture of the two alcohols with pseudotropine predominating. Because [ $^2\text{H}$ ]-sodium borohydride is readily available commercially we decided to use this to reduce tropinone and then to obtain pure deuterium-labelled tropine and pseudotropine by separation of the products.



**Scheme 1 Reduction of tropinone by sodium borohydride**

## EXPERIMENTAL

### Materials

Tropinone and tropine were from Sigma Chemical Co. Ltd. (Poole, Dorset, U.K.). Sodium [ $^2\text{H}$ ]borohydride was from Aldrich Chemical Co. Ltd. (Gillingham, Dorset, U.K.).

### Methods

The GC-MS analyses were performed on a Hewlett-Packard 5890 instrument with a 5971 mass selective detector using an HP-5 (cross-linked 5% phenyl methyl silicone) column (25 m x 0.2 mm x 0.33  $\mu\text{m}$  film). Ionization was by electron impact. The carrier gas was helium and a temperature programme of 2 min at 70  $^\circ\text{C}$  rising at 10  $^\circ\text{C}/\text{min}$  to 250  $^\circ\text{C}$  for 1 min was used.

NMR spectra were recorded on a Jeol FX 60Q instrument operating at 60 MHz for proton and 15.1 MHz for carbon spectra. Samples were run in solution in deuteriochloroform with TMS as internal standard.

Flash chromatography was performed on a silica gel 60 (230-400 mesh; Merck 9385) column (16.5 cm x 2 cm) with methanol/aq. ammonia (sp.g. 0.88)(100:4 v/v) as solvent.

Fractions of 7.5 ml were collected. Products were detected by spotting samples on to filter paper and spraying with Dragendorff's reagent (5) with which tropine and pseudotropine give a red colour.

TLC was performed on precoated silica gel GHLF plates (Analtech, Newark, NJ, U.S.A.) with methanol/aq. ammonia (sp.g. 0.88)(100:4 v/v) as solvent. Compounds were detected by the red colour obtained when plates were sprayed with Dragendorff's reagent (5).

#### *Reduction of tropinone.*

Tropinone (1 g) was dissolved in a mixture of 10 ml of water and 5 ml of ethanol. Sodium borohydride or sodium [<sup>2</sup>H]borohydride (0.5 g) dissolved in 5 ml of water was added dropwise to the stirred solution over a period of 30 min and the solution left to stir for a further 60 min. The reaction mixture was then acidified by the dropwise addition of 5M HCl to pH 5.5. It was then made alkaline to pH >12 by addition of 5M NaOH and extracted five times with equal volumes of chloroform. The pooled extracts were dried over anhydrous Na<sub>2</sub>SO<sub>4</sub> and the chloroform then removed on a rotary evaporator to leave a clear syrup.

#### *Separation of products.*

The products of reduction were dissolved in 40 ml of diethyl ether and left at -20 °C for 24 h. This yielded a crop of white crystals. The supernatant solution was decanted and the crystals washed with cold diethyl ether which was added to the supernatant. The crystals were dried giving 519 mg of material (pseudotropine).

The supernatant solution containing a mixture of tropine and residual pseudotropine was concentrated to about 5 ml and partially acetylated by addition of 0.15 ml of acetyl chloride. This resulted in copious precipitation. The reaction was warmed and after 5 min the ether was evaporated under a stream of nitrogen. The residue was dissolved in 5 ml of chloroform which was then extracted with an equal volume of 1M NaOH. The NaOH was back-extracted with chloroform which was added to the original chloroform solution. The chloroform was dried over anhydrous Na<sub>2</sub>SO<sub>4</sub> and then evaporated under a stream of nitrogen. The extent of acetylation was monitored by GC-MS and a second treatment with acetyl chloride was required to achieve the desired degree of conversion.

The residue was dissolved in methanol/ aq. ammonia (sp.g. 0.88) (100:4 v/v) and separated by flash chromatography. The fractions were examined by TLC and those containing tropine, fractions 12-25, were pooled and the methanol removed on a rotary evaporator. The residue was taken up in chloroform which was dried over anhydrous Na<sub>2</sub>SO<sub>4</sub> and then evaporated, yielding 91 mg of a syrup which slowly crystallised.

## RESULTS AND DISCUSSION

The products of reduction of tropinone by sodium borohydride or by sodium [ $^2\text{H}$ ]borohydride, when examined by GC-MS, gave two peaks with retention times of 9.83 min and 10.19 min. The first peak corresponded to that given by authentic tropine and, from the reduction using sodium borohydride, gave the same mass spectrum with a molecular ion at  $m/z$  141. The second peak gave a very similar spectrum (Fig. 2) as would be expected for the other reported reduction product, pseudotropine. Products from the reduction with sodium [ $^2\text{H}$ ]borohydride had molecular ions at  $m/z$  142 as predicted for the deuterium labelled compounds. Integration of the peaks showed that the product contained 70% pseudotropine and 30% tropine.

Crystallisation of this mixture from diethyl ether yielded 519 mg of the labelled material which, when examined by GC-MS, gave a single peak corresponding to the retention time for pseudotropine.

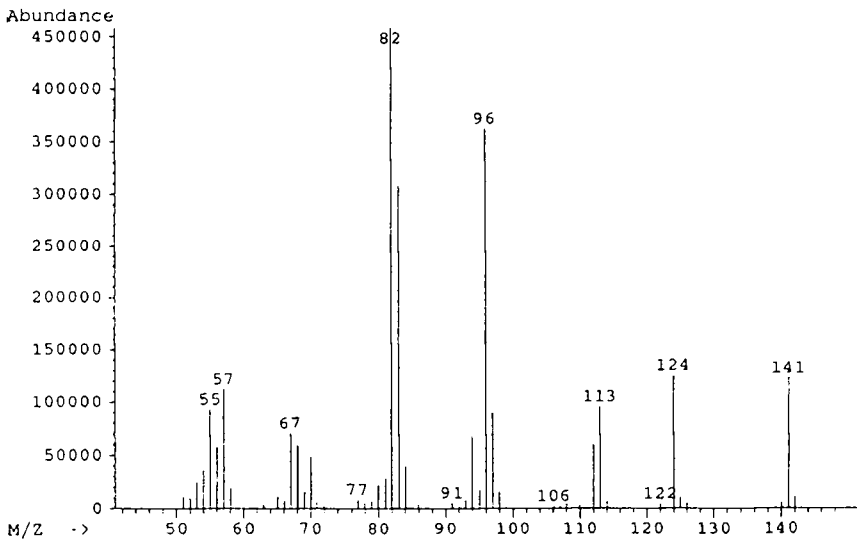
The supernatant solution from the crystallisation of the [ $^2\text{H}$ ]pseudotropine was examined by GC-MS which showed it now to be enriched for [ $^2\text{H}$ ]tropine (68%) over [ $^2\text{H}$ ]pseudotropine (32%). Further attempts at crystallisation proved unsuccessful and chromatography of the epimers by TLC or on paper gave separations too small to be useful preparatively. However, it was established that treatment of a tropine/pseudotropine mixture with acetyl chloride resulted in a preferential acetylation of the pseudotropine and this was better separated from tropine by TLC. When methanol/aq. ammonia (sp.g. 0.88)(100:4 v/v) was used as TLC solvent the  $R_F$  values were tropine, 0.23, pseudotropine, 0.41, acetyltropine, 0.46 and acetylpsudotropine, 0.63. These differences were sufficient to facilitate separation of the acetylpsudotropine from tropine by flash chromatography. Therefore, the residue from the crystallisation of [ $^2\text{H}$ ]pseudotropine was reacted with a small amount of acetyl chloride and the degree of acetylation monitored by GC-MS. After a second treatment most of the [ $^2\text{H}$ ]pseudotropine was in the form of acetyl[ $^2\text{H}$ ]pseudotropine (retention time, 12.11 min and molecular ion at  $m/z$  184) and, although there was some acetyl[ $^2\text{H}$ ]tropine (retention time, 11.96 min) there was still a considerable amount of free [ $^2\text{H}$ ]tropine. The relative amounts were tropine 35%, pseudotropine 4%, acetyltropine 24% and acetylpsudotropine 37%. These products were separated by flash chromatography, which not only separated the acetylpsudotropine from the tropine but also from the acetyltropine and the trace of unacetylated pseudotropine, yielding, from the labelled products, 91 mg of [ $^2\text{H}$ ]tropine. Examination by GC-MS gave a major peak,

constituting >99% of the material, with a retention time corresponding to tropine and a molecular ion at  $m/z$  142.

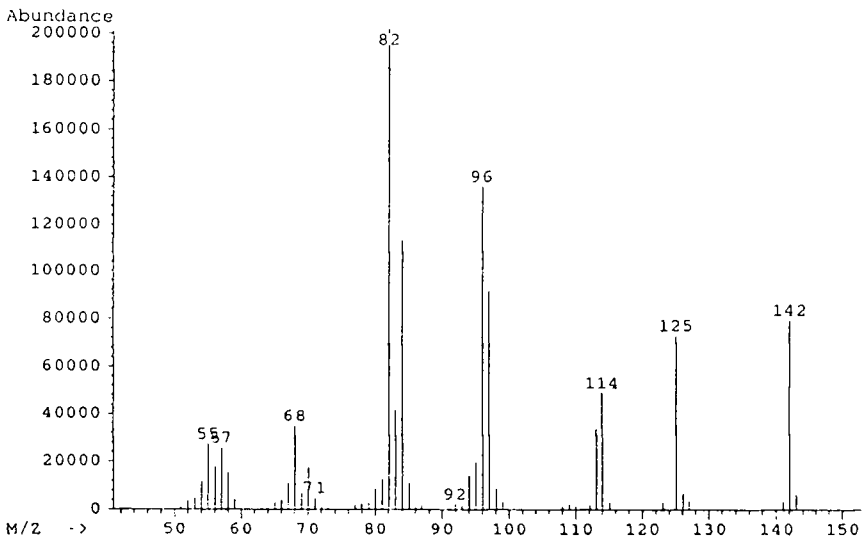
The mass spectra for the labelled and unlabelled tropine are shown in Fig. 1 and for labelled and unlabelled pseudotropine in Fig. 2. Confirmation that the labelling was at the C3 position,

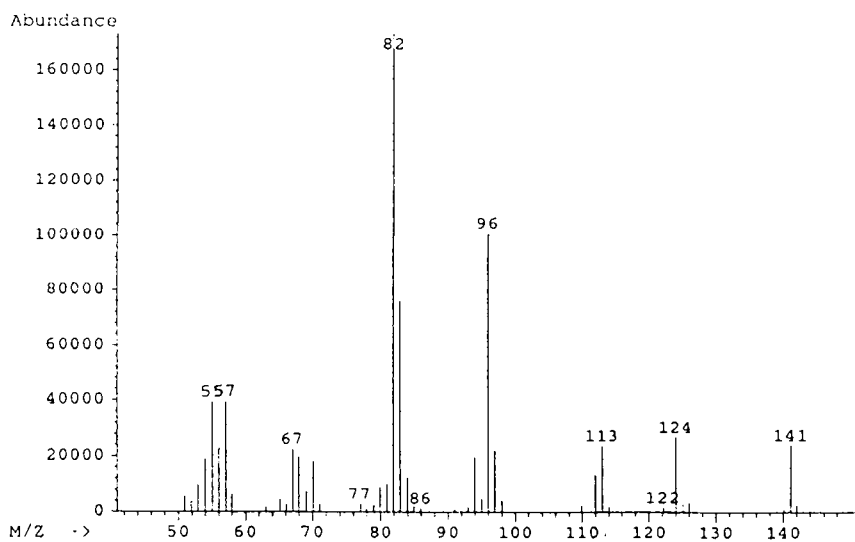
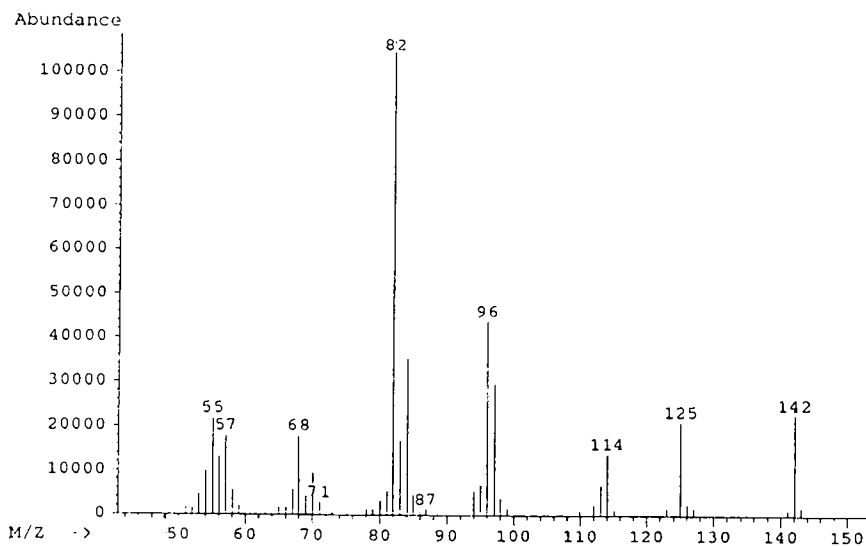
**Fig.1** Mass spectra of (1) tropine and (2) the synthesised [3-<sup>2</sup>H]tropine

1. Tropine



2. [3-<sup>2</sup>H]Tropine



**Fig.2 Mass spectra of (1) pseudotropine and (2) [3-<sup>2</sup>H]pseudotropine****1. Pseudotropine****2. [3-<sup>2</sup>H]Pseudotropine**

as expected for reduction of the ketone, came from NMR spectra of the products. The <sup>1</sup>H NMR spectra of both products were identical to those of the corresponding unlabelled compounds except for the absence for the deuterated compounds of a signal due to the hydrogen on C3 at 4.0 ppm (tropine) or 3.5 ppm (pseudotropine). The <sup>13</sup>C NMR spectrum of unlabelled tropine showed a signal due to C3 at 64.6 ppm; this was absent from the spectrum of the deuterated derivative but the expected triplet due to the deuterium on C3 could not be observed due to the low concentration of the sample. More of the [<sup>2</sup>H]pseudotropine was available and its <sup>13</sup>C NMR spectrum did show a triplet centred at 63.5 ppm due to the deuterium on C3. The signal for C3 in unlabelled pseudotropine falls at 64.1 ppm, the slight difference being due to the deuterium isotope effect.

#### ACKNOWLEDGEMENTS

This work was supported by a grant from the Science and Engineering Research Council (SERC) Biotechnology Directorate.

#### REFERENCES

1. Finar, I.L. - Organic Chemistry vol. 2 (5th ed.) pp726-728 Longman, London, (1975)
2. Niemer, H. and Bucherer, H. - Z. Physiol. Chem. 326: 9-11 (1961)
3. Stevens, W.F. and Rorsch, A. - Biochim. Biophys. Acta 320: 204-211 (1971)
4. Long, M.T., Hopper, D.J. and Trudgill, P.W. - FEMS Microbiol. Lett. 106: 111-116 (1993)
5. Harborne, J.B. - Phytochemical Methods - A Guide to Modern Techniques in Plant Biochemistry. Chapman and Hall, London, (1984)