JOURNAL OF LABELLED COMPOUNDS AND RADIOPHARMACEUTICALS
J Label Compd Radiopharm 2002; **45**: 191–198.
DOI: 10.1002/jlcr.543

Research Article

Isotopically labelled tropane alkaloids. The synthesis of (*RS*)-[3′, 3′-$^{2}H_2$]- and (*RS*)-[1′-^{13}C, 3′, 3′-$^{2}H_2$]- hyoscyamines for metabolism studies in plants

Stephen Patterson and David O'Hagan*
School of Chemistry, Centre for Biomolecular Sciences, University of St. Andrews, North Haugh, St. Andrews, Fife, KY16 9ST, UK

Summary

A synthetic route to isotopically labelled forms of the tropane alkaloid hyoscyamine, including (*RS*)-[3′, 3′,-$^{2}H_2$]- (**2a**) and (*RS*)-[1′-^{13}C, 3′, 3′,-$^{2}H_2$]- (**2b**) hyoscyamines, involving the reaction between phenylacetyl tropine and formaldehyde is described. The isotopically labelled products enable the metabolism of hyoscyamine to be studied in plants such as *Datura stramonium*. Copyright © 2002 John Wiley & Sons, Ltd.

Key Words: tropane alkaloids; tropic acid; hyoscyamine; *Datura stramonium*; biosynthesis

Introduction

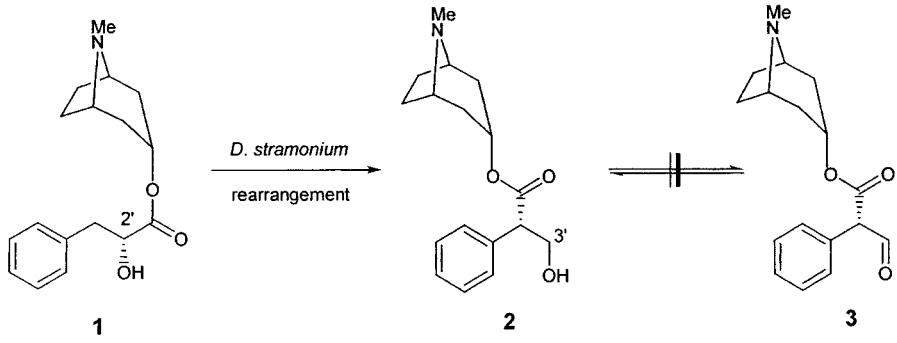
The tropane alkaloids littorine (**1**) and hyoscyamine (**2**) are produced by several plant species including henbane (*Hyoscyamus niger*), mandrake (*Mandragora officinarum*) and thornapple (*Datura stramonium*). Hyoscyamine has a rich folklore as a hallucinogenic alkaloid and has been a

*Correspondence to: D. O'Hagan. School of Chemistry, Center for Biomolecular Sciences, University of St. Andrews, North Haugh, St. Andrews, Fife KY16 9ST UK.
E-mail: dol@st-andrews.ac.uk

Received 22 October 2001
Accepted 24 October 2001

topic of medical research primarily due to its potency as a muscarinic antagonist and its myriadic properties in optometery.[1]

We and others have been investigating the biosynthesis of hyoscyamine for a number of years,[2–5] and in particular the origin of the tropic acid ester moiety. It has been shown that littorine (**1**) is the immediate precursor to hyoscyamine (**2**).[6] The conversion of littorine (**1**) to hyoscyamine (**2**) involves an intriguing intra-molecular isomerization shown in Scheme 1. Despite a number of proposals[7–9] the mechanism of the rearrangement is unknown.

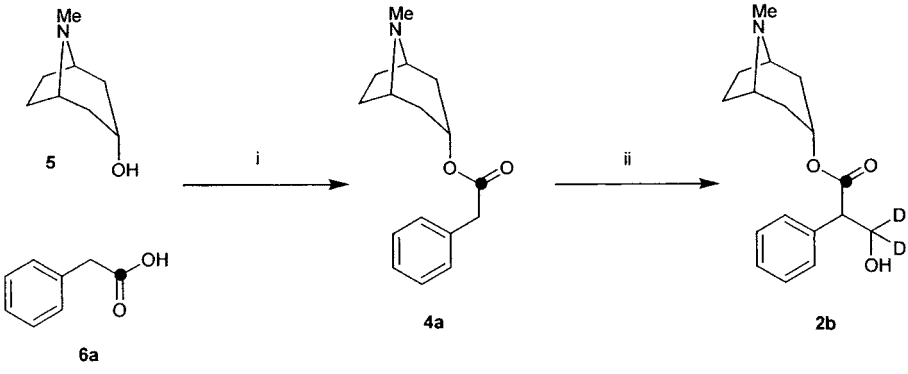


Scheme 1. Hyoscyamine (2) is formed by an intramolecular rearrangement of littorine (1). There is no evidence for the reversible oxidation of hyoscyamine (2) to aldehyde (3)

Previous results from our laboratory involving isotopic labelling experiments with [2′-^{18}O, ^{2}H]-littorine[10] in *D. stramonium*, demonstrated a 71% retention of ^{18}O at the C-3′oxygen of hyoscyamine during the rearrangement of littorine (**1**) to hyoscyamine (**2**). Liberation of the ^{18}O (29%) could arise either during the rearrangement[8] or has been suggested[9] as a consequence of the subsequent reversible metabolism of hyoscyamine (**2**) by a redox enzyme to aldehyde (**3**). Clearly if aldehyde (**3**) is generated then the ^{18}O could have exchanged to some extent with the aqueous medium in the plant cells. In an attempt to delineate these two processes a synthetic route has been developed which allowed the preparation of hyoscyamine **2** carrying deuterium at the 3′ positions. The level of reversible *in vivo* oxidation in *D. stramonium* plants, at the C3′ hydroxyl group could then be assessed by monitoring the loss of deuterium at C3′ by mass spectrometry. The present paper reports the preparation of (*RS*)-[3′, 3′-$^{2}H_2$]- and [1′-^{13}C, 3′, 3′-$^{2}H_2$]-hyoscyamines using a novel two step strategy for hyoscyamine synthesis.

Results and discussion

A synthetic method was developed for the preparation of (*RS*)-[3′, 3′-$^2H_2$] and [1′-^{13}C, 3′, 3′-$^2H_2$]-hyoscyamine as illustrated for (*RS*)-[1′-^{13}C, 3′, 3′-$^2H_2$]-hyoscyamine in Scheme 2.



Scheme 2. Synthesis of (RS)- [1′-^{13}C, 3′, 3′-$^2H_2$]-hyoscyamine. i HCl(g) 145°C. 3 h. 79% (ii) (a) LDA/THF –78°C, 30 min. (b) [$^2H_2$]-formaldehyde(g) and (c) H_2O, 56%

[1′-^{13}C]-Phenylacetyl tropine (**4a**) was prepared following the classical preparation of tropine esters by acid catalysed esterification of tropine (**5**) and [1-^{13}C]-phenylacetic acid (**6a**) using dry HCl gas.[6] The product ester was dissolved in dilute sulfuric acid, treated with base and extracted into an organic solvent. The ester was recovered by this method in good yield. The resultant [1′-^{13}C]-phenylacetyl tropine (**4a**) was treated with LDA in dry THF at −78°C. This generates the enolate anion, which was then quenched with [$^2H_2$]-formaldehyde gas.[11] The gaseous formaldehyde was generated by the depolymerization of paraformaldehyde at 150°C and bubbled into the reaction using a stream of dry nitrogen. Aqueous workup and extraction into an organic solvent gave a mixture of hyoscyamine and unreacted phenylacetyl tropine (44 : 56). The resultant hyoscyamine was purified by preparative thin layer chromatography (t.l.c.). This synthetic methodology was also used to prepare a sample of [3′, 3′-$^2H_2$]-hyoscyamine from unlabelled phenylacetyl tropine. The method is particularly attractive due to the commercial availability of [1-^{13}C]-phenylacetic acid and [$^2H_2$]-paraformaldehyde, and accordingly hyoscyamine can now be prepared in a variety of labelled combinations.

A previous synthetic route to hyoscyamine has involved the acid catalysed esterification of tropic acid and tropine.[12] However, direct esterification is complicated by a competing inter-molecular esterification of tropic acid, due to its reactive primary alcohol. Indeed, to circumvent this problem hyoscyamine has recently been prepared from tropic acid using a four step protection, activation, coupling and deprotection sequence.[13] In the context of these alternative approaches the current method offers a direct synthetic approach, and is amenable to the introduction of isotopes at the 3′-site of hyoscyamine.

The isotopically labelled hyoscyamines (**2a**) and (**2b**) were incubated at a final concentration of 0.25 mM with transformed root cultures of *D. stramonium* over 21 days, following established protocols.[10,14] Aliquots were extracted for analysis at five day intervals between days 11 to 21, for GC-MS analysis. In the event there was no indication of any washout of deuterium from the C-3′position of hyoscyamine (**2**) even over these extended time periods and thus there is no evidence that hyoscyamine (**2**) is involved in an *in vivo* reversible oxidation to aldehyde (**3**). These and other related results will be reported elsewhere in a more detailed analysis of the mechanism of the conversion of littorine (**1**) to hyoscyamine (**2**).

Experimental

All isotopically labelled compounds were purchased from The Aldrich Chemical Co. and used without further purification. THF was dried over sodium wire and distilled prior to use. NMR spectra were recorded on a Brucker Avance 300 MHz (^{1}H at 300.06 MHz, ^{13}C at 75.45 MHz) and Varian Unity Plus 300 MHz (^{1}H at 299.99 MHz, ^{13}C at 75.43 MHz) spectrometers. Chemical shifts are quoted relative to $CHCl_3$ in $CDCl_3$ (δ_H 7.26 or δ_C 77.0 ppm), and coupling constants are given in Hertz.

GCMS analyses were conducted using an Agilent 5890 plus gas chromatograph equipped with a 5973 N mass selective detector and 7683 series injector. The carrier gas was helium. Chromatographic separations were performed using a chiral SGE column (fused silica, cydex-B, 25 mm × 0.22 mm with a film thickness of 0.25 μm).

Preparative t.l.c. were performed on Whatman K6F silica gel 60 Å, (0.25 mm) plates with fluorescent indicator.

*Phenylacetyl tropine (**4**)*

A solid phase mixture of phenylacetic acid (408 mg, 3.0 mmol) and tropine (424 mg, 3.0 mmol) was heated to 145°C under a stream of dry nitrogen. A stream of dry hydrochloric acid gas was then passed over the mixture for 3 h with stirring. After cooling the reaction mixture was dissolved in sulfuric acid (50 mM, 20 ml), and it was then basified with ammonia solution (35%) and the product was extracted into chloroform (4×30 ml). The solvent was removed under reduced pressure to yield the product as a clear oil (620 mg, 80%).

^{1}H-NMR (300 MHz) ($CDCl_3$): 7.32–7.18 (5H, m, aromatics), 4.93 (1H, m, C3-H), 3.55 (2H, s, C2′-H_2), 2.96 (2H, m, C1-H, C5-H), 2.18 (3H, s, NCH_3), 2.03 (2H, dt, $^2J_{HH} = 15.0$, $^3J_{HH} = 4.3$, C2-Hα, C4-Hα), 1.82 (2H, m, C6-Hα, C7-Hα), 1.57 (4H, m C2-H*β*, C4-H*β*, C6-H*β*, C7-H*β*).

^{13}C-NMR (75 MHz) ($CDCl_3$): 170.4 (C1′), 133.9, 129.1, 128.4, 126.8 (aromatics), 67.8 (C3), 59.5 (C1, C5), 42.1 (C2′), 40.2 (NCH_3), 36.3 (C2, C4), 25.3 (C6, C7).

GCMS (EI): 259 (M^+, 29%), 140 (9.5%), 124 (100%), 94 (19%), 91 (18%), 82 (21%), 67 (9.5%).

*[1′-^{13}C]-Phenylacetyl tropine (**4a**)*

The title compound was prepared following the method for phenylacetyl tropine using [1-^{13}C]-phenylacetic acid (411 mg, 3.0 mmol). Yield (620 mg, 79%).

^{1}H-NMR (300 MHz) ($CDCl_3$): 7.32–7.20 (5H, m, aromatics), 4.95 (1H, m, C3-H), 3.56 (2 H, d, $^2J_{CH} = 7.8$, C2′-H_2), 2.98 (2H, m, C1-H, C5-H), 2.19 (3H, s, NCH_3), 2.05 (2H, dt, $^2J_{HH} = 15.0$, $^3J_{HH} = 4.3$, C2-Hα, C4-Hα), 1.83 (2H, m, C6-Hα, C7-Hα), 1.59 (4H, m, C2-H*β*,C4-H*β*, C6-H*β*, C7-H*β*).

^{13}C-NMR (75 MHz) ($CDCl_3$): 170.4 (C1′), 133.9, 129.1 128.3, 126.8 (aromatics), 67.8 (C3), 59.5 (C1/C5), 42.1 (C2′, d, $^1J_{CC} = 57.6$), 40.2 (NCH_3), 36.3 (C2/C4), 25.3 (C6/C7).

GCMS (EI): 260 (M^+, 16%), 140 (8%), 124 (100%), 94 (22%), 91 (24%), 82 (26%), 67 (11%).

*(RS)-Hyoscyamine (**2**)*

n-BuLi (1.6 M in hexanes; 1.9 ml, 3.0 mmol) was added to diisopropylamine (3 mmol, 420 µl) in THF (10 ml) at 0°C, and the reaction was

stirred for 30 min. The reaction was then cooled to –78°C and phenylacetyl tropine (518 mg, 2 mmol) in THF (5 ml) was added slowly over 5 min and then the reaction was stirred for a further 30 min. The reaction mixture was warmed to −20°C and formaldehyde gas, generated by heating paraformaldehyde (300 mg, 10.0 mmol) to 150°C, was bubbled through the mixture with a stream of dry nitrogen, until all of the paraformaldehyde had sublimed. After warming to ambient temperature the reaction was quenched by the addition of water (10 ml). The aqueous layer was separated and extracted into diethylether (4 × 30 ml). The organic extracts were combined and evaporated under reduced pressure to yield a pale orange gum (480 mg) which was purified over silica gel ($CHCl_3$: MeOH : NEt_3, 75 : 25 : 1) to yield hyoscyamine (121 mg, 21%) and recovered phenylacetyl tropine (164 mg). For GCMS analysis the TMS derivative was prepared using MSTFA.[15]

^{1}H-NMR (300 MHz) ($CDCl_3$): 7.39–7.22 (5H, m, aromatics), 4.99 (1H, t, $^3J_{HH} = 5.2$, C3-H), 4.16 (1H, m, C3′-Hα), 3.79 (2H, m, C3′-Hβ, C2′-H), 3.02 (1H, m, C1/C5-H), 2.90 (1H, m, C1/C5-H), 2.18 (3H, s, NCH_3), 2.05 (2H, m, C2-Hα, C4-Hα), 1.72 (3H, m, C6-Hα, C7-Hα, C6/C7-Hβ), 1.59 (1H, m, C2/C4-Hβ), 1.54 (1H, m, C2/C4-Hβ), 1.18 (1H, m, C6/C7-Hβ).

^{13}C-NMR (75 MHz) ($CDCl_3$): 172.3 (C1′), 135.6, 128.8, 128.1, 127.7 (aromatics), 68.1 (C3), 64.1 (C3′), 59.6 (C1/C5), 59.5 (C1/C5), 54.4 (C2′), 40.2 (NCH_3), 36.3 (C2/C4), 36.1 (C2/C4), 25.4 (C6/C7), 24.8 (C6/C7).

NB where two carbon numbers are given for diastereomeric carbon e.g. C6/C7 the assignment is uncertain.

GCMS (EI): 361 (M^+, 17%), 140 (7%), 124 (100%), 104 (7%), 94 (11%), 82 (13%), 73 (10%), 67 (7%).

*(RS)-[3′, 3′-$^2H_2$]-Hyoscyamine (**2a**)*

The title compound was prepared as (*RS*)-hyoscyamine using [$^2H_2$]-formaldehyde gas (538 mg, 16.8 mmol). The reaction yielded a pale orange oil (520 mg) which was estimated to be 38% hyoscyamine by GC analysis. Aliquots were purified by preparative t.l.c. ($CHCl_3$: MeOH : Net_3, 75 : 25 : 0.5), and the silica extracted into chloroform/methanol (10 mg of product was recovered from 30 mg of the oil).

^{1}H-NMR (300 MHz) ($CDCl_3$): 7.38–7.24 (5 H, m, aromatics), 5.03 (1 H, t, $^3J_{HH} = 5.4$, C3-H), 3.78 (1H, s, C2′-H), 3.04 (1H, m, C1/C5-H),

2.92 (1H, m, C1/C5-H), 2.20 (3H, s, NCH$_3$), 2.07 (2H, m, C2-Hα, C4-Hα), 1.75 (3H, m, C6-Hα, C7-Hα, C6/C7-H*β*), 1.60 (1H, m, C2/C4-H*β*), 1.55 (1H, m, C2/C4-H*β*), 1.15 (1H, m, C6/C7-H*β*).

^{13}C-NMR (75 MHz) ($CDCl_3$): 172.0 (C1′), 135.7, 128.7, 128.0, 127.6 (aromatics), 67.4 (C3), 59.8 (C1/C5), 59.7 (C1/C5), 54.5 (C2′), 39.8 (NCH$_3$), 35.6 (C2/C4), 35.8 (C2/C4), 25.1 (C6/C7) 24.6 (C6/C7). NB The signal for C3′ was below the detectable threshold due to $^1J_{CD}$ coupling.

GCMS (EI): 363 (M^+, 9%), 140 (7%), 124 (100%), 106 (8%), 94 (13%), 82 (15%), 73 (13%), 67 (8%).

Mass abundances: 361 (M, 0.14%), 362 (M + 1, 0.58%), 363 (M + 2, 77.26%), 364 (M + 3, 22.02%).

*(RS)-[1′-^{13}C, 3′, 3′-$^2H_2$]-Hyoscyamine (***2b***)*

The title compound was prepared as (*RS*)-hyoscyamine using [1-^{13}C]-phenylacetyl tropine (620 mg, 2.38 mmol) and [$^2H_2$]-formaldehyde gas (382 mg, 11.9 mmol). The reaction yielded a pale orange gum (506 mg) which was estimated to be 56% hyoscyamine by GC analysis. An aliquot was purified by preparative t.l.c. ($CHCl_3$: MeOH : NEt_3, 75: 2 5 : 0.5), and the silica extracted with chloroform/methanol (11 mg of product was recovered from 30 mg of the oil).

^{1}H-NMR (300 MHz) ($CDCl_3$): 7.36-7.23 (5 H, m, aromatics), 5.01 (1 H, m, C3-H), 3.76 (1 H, d, $^2J_{CH} = 7.4$, C2′-H), 3.02 (1H, m, C1/C5-H), 2.90 (1H, m, C1/C5-H), 2.18 (3H, s, NCH$_3$), 2.05 (2H, m, C2-Hα, C4-Hα), 1.72 (3H, m, C6-Hα, C7-Hα, C6/C7-H*β*), 1.59 (1H, m, C2/C4-H*β*), 1.54 (1 H, m, C2/C4-Hβ), 1.18 (1 H, m, C6/C7-Hβ).

^{13}C-NMR (75 MHz) ($CDCl_3$): 172.2 (C1′), 135.3, 128.8, 128.1, 127.7 (aromatics), 68.1 (C3), 59.6, 59.5 (C1/C5), 54.2 (d, $^1J_{CC} = 56$, C2′), 40.3 (NCH$_3$), 36.4, 36.1 (C2/C4), 25.4, 24.9 (C6/C7). NB The signal for C3′ was below the detectable threshold due to $^1J_{CD}$ coupling.

GCMS (EI): 364 (M^+, 10%), 140 (6%), 124 (100%), 106 (8%), 94 (12%), 82 (14%), 67 (8%).

Mass abundances: 361 (M, 0.06%), 362 (M + 1, 0.14%), 363 (M + 2, 1.16%), 364 (M + 3, 74.78%), 365 (M + 4, 23.86%).

Acknowledgements

We thank the EPSRC for a studentship (S.P.).

References

1. Humphrey AJ, O'Hagan D. *Nat Prod Rep* 2001; **18**: 494–502.
2. Leete N, Kowanko N, Newmark RA. *J Am Chem Soc* 1975; **97**: 6826–6830.
3. Ansarin M, Woolley JG. *Phytochemistry* 1994; **35**: 935–939.
4. Chesters NCJE, O'Hagan D, Robins RJ, Kastelle A, Floss HG. *J Chem Soc, Chem Comm* 1995; 129–130.
5. Chesters NCJE, Walker K, O'Hagan D, Floss HG. *J Am Chem Soc* 1996; **118**: 925–926.
6. Robins RJ, Bachmann P, Woolley JG. *J Chem Soc Perkin Trans 1* 1994; 615–619.
7. Leete E. *Can J Chem* 1987; **65**: 226–228.
8. Ollagnier S, Kervio E, Rétey J. *FEBS Lett* 1998; **437**: 309–312.
9. O'Hagan D, Robins RJ. *Chem Soc Rev* 1998; **27**: 207–212.
10. Wong CW, Hamilton JTG, O'Hagan D. Robins RJ. *Chem Comm* 1998; 1045–1046.
11. Majetich G, Song JS, Leigh AJ, Condon SM. *J Org Chem* 1993; **58**: 1030–1037.
12. Liebermann C, Limpach L. *Chem Ber* 1892; **25**: 927–939.
13. Dei S, Bartolini A, Bellucci C, *et al. Eur J Med Chem Chim Ther* 1997; **32**: 595–605.
14. Zabetakis I, Edwards R, Hamilton JTG, O'Hagan D. *Plant Cell Reports* 1998; **18**: 341–345.
15. Bakolachristianopoulou MN, Papageorgiou VP, Apazidou KK. *Phosphorus Sulfur Silicon Relat Elem* 1994; **88**: 53–65.

