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Syntheses of deuterium labelled atropine and scopolamine

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Easy preparative scale syntheses of deuterium labelled atropine and scopolamine are described. [$^2H_3$]-atropine and [$^2H_3$]-scopolamine are obtained in good yield and good isotopic purity in two steps starting from the unlabelled alkaloids.

Keywords: atropine; scopolamine; synthesis; labelled; deuterium

Introduction

The presence of atropine **1** and scopolamine **5** in biological samples is regulated by International Racing Rules and/or International Sports Authorities. In horse, atropine and scopolamine are prohibited because of their action on the nervous system.

In order to be used as internal standards both in qualitative screening and quantitative analysis in body fluids or foodstuff, labelled atropine and scopolamine should be chemically and isotopically pure, highly enriched, with none or trace amounts of unlabelled compound, and labelled on non-exchangeable positions.

The mass spectra of atropine and scopolamine exhibit two mains peaks: the first corresponding to molecular ion and the second corresponding to the tropane nucleus (from the scission of the tropic acid moiety), which is the most abundant.[1] The same fragmentation is observed with trimethylsilyl ether (TMS) derivatives. The mass spectra of labelled and unlabelled species should exhibit the least possible common fragment ions in order to reduce risk of interference during analyses of biological matrices. Therefore, those compounds should be labelled on the tropane ring. The most direct pathway to this labelling is a demethylation/trideuteromethylation sequence of the tropane nitrogen atom.

Results and discussion

[$^2H_3$]-atropine 4 (Figure 1)

Several syntheses of [^{14}C]-atropine, labelled at different positions of the tropane ring, have been published: via partial synthesis from nortropine[2] and via total synthesis.[3–7] [^{3}H]-atropine was prepared via exposition of atropine to $^3H_2$.[8] A synthesis of ^{13}C and ^{2}H atropine labelled on the tropic acid moiety has been recently published.[9] Unfortunately, these methods are not suitable for our purpose for the following reasons: long multistep syntheses [2–7] or labelling on the tropic acid moiety.[3,9]

Noratropine **3**, the first intermediate needed, has been prepared in various ways: partial synthesis from nortropine,[10] demethylation of atropine by reaction with trichloroethyl chloroformate followed by treatment with zinc dust in acetic acid,[11] oxidative demethylation of atropine with potassium permanganate with low yield,[12,13] photochemical demethylation of atropine[14,15] and iron salt mediated Polonovski demethylation of atropine *N*-oxide.[16]

We decided to prepare noratropine **3** by demethylation of atropine with α-chloroethyl chloroformate because this reagent is known to give high yields of demethylated products and the cleavage of the carbamate intermediate **2** is done by simply heating it in methanol.[17,18] The preparation of the carbamate intermediate was carried out by heating a mixture of atropine, α-chloroethyl chloroformate and sodium hydrogencarbonate. The latter compound was used to scavenge HCl produced by reaction of α-chloroethyl chloroformate with the primary alcohol of the tropic acid moiety. The carbamate and the carbonate were both cleanly cleaved by heating in methanol. Despite the sensitivity of the hydroxymethyl group to H_2O cleavage, this side reaction was not observed at any stage of this procedure. Noratropine **3** was obtained with quantitative yield as a colour less viscous oil, which solidified on standing. This is the most convenient procedure published to date.

[$^2H_3$]-atropine **4** was obtained by reacting noratropine **3** with CD_3I in the presence of potassium carbonate in acetonitrile. The yield was moderate (31.9%, not optimized), however no purification was needed to obtain pure [$^2H_3$]-atropine.

The disappearance of the 3H signal at 2.14 ppm indicates the complete labelling of the *N*-methyl group in good agreement with the published NMR data.[9] The mass spectrum of the [$^2H_3$]-atropine exhibits a 3 amu shift of all the characteristic fragments in good agreement with the published fragmentation pattern.[1] Most abundant fragments calculated ratio (*m-H*)/*z* 124/127 (atropine/[$^2H_3$]-atropine) = 0.0023.

[$^2H_3$]-scopolamine 7 (Figure 2)

Surprisingly, we did not found any description of deuterium labelled scopolamine synthesis in the literature. However,

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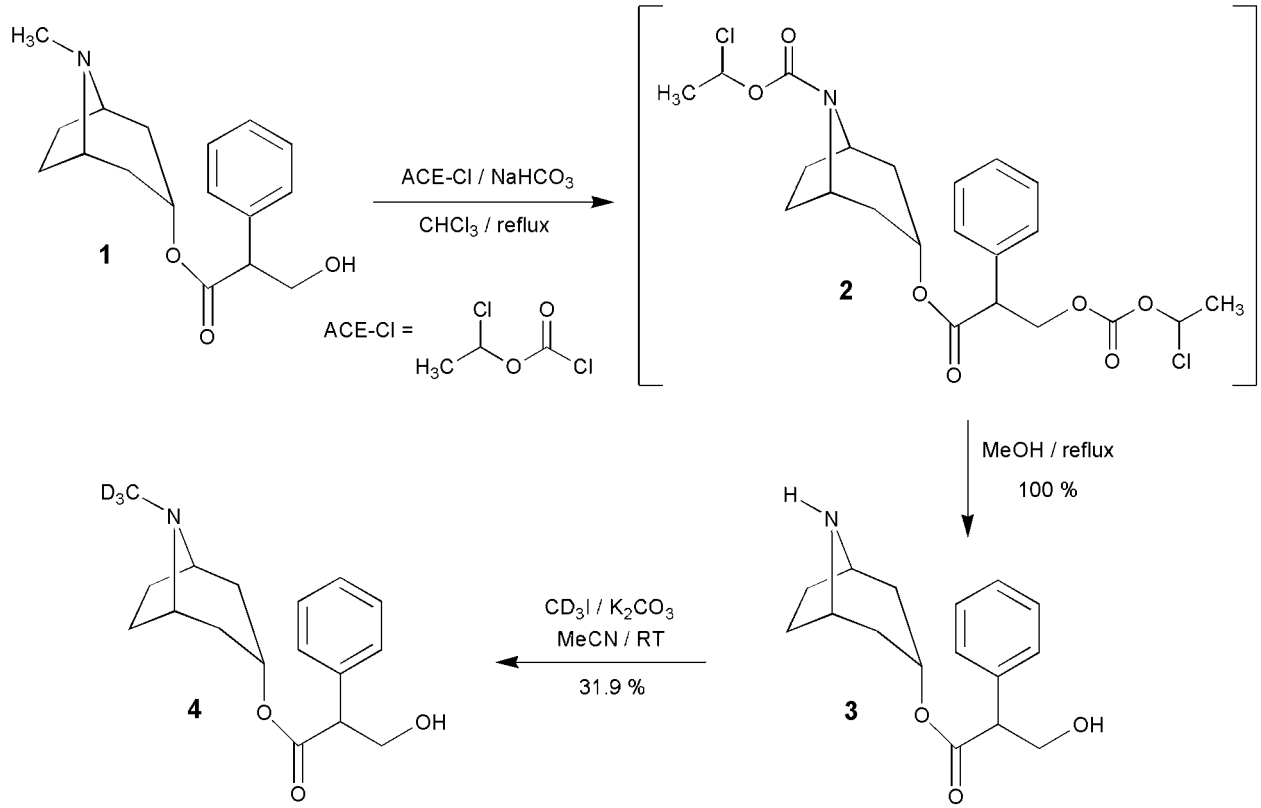

Figure 1. Synthesis of [$^2H_3$]-atropine.

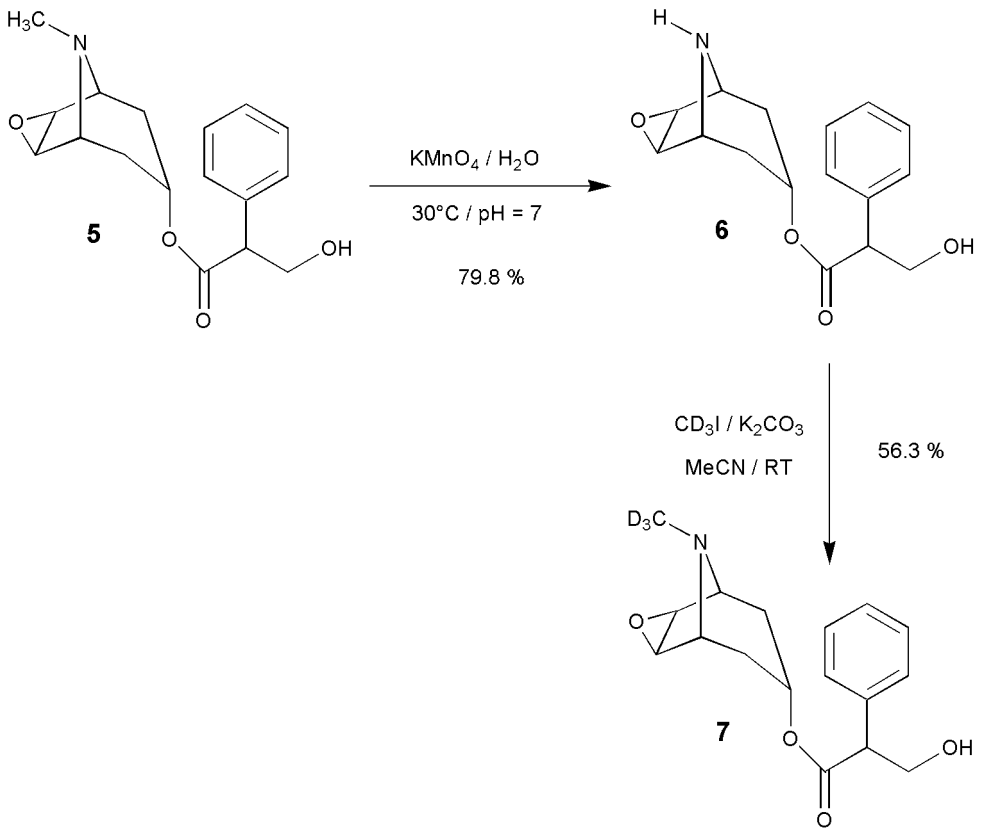

Figure 2. Synthesis of [$^2H_3$]-scopolamine.

several syntheses of labelled scopolamine have been published: with tritium via exposition of scopolamine to $^{3}H_{2}$[19] or methylation of norscopolamine with [^{3}H]CH_2I,[20] with ^{11}C from norscopolamine via reductive amination with labelled formaldehyde[21,22] and with ^{14}C via partial synthesis from norscopine[2] and from acetylnorscopolamine.[23]

Firstly, we tested the procedure used for the preparation of [$^{2}H_3$]-atropine. This method was very disappointing, the norscopolamine **6** obtained was contaminated by several by-products, probably due to some side reaction at the epoxide ring. Because we were unable to purify the norscopolamine with a satisfactory yield, we decided to re-examine the literature concerning scopolamine demethylation. Several methods have been published, by the action of phosgene on acetylscopolamine[24] and by the action of potassium permanganate on scopolamine (with low yield)[25] or on acetylscopolamine.[23] The latter method was then considerably improved, the alcohol protection by acetylation was shown to be unnecessary if the pH was maintained as close as possible to 7 during the course of the reaction.[26] This method allowed us to prepare pure norscopolamine directly from scopolamine with a yield of 79.8%. Direct methylation of norscopolamine with iodomethane in the presence of a base is known to give low yields because of rapid decomposition of the alkaloid; epoxide opening, ester hydrolysis and hydroxymethyl dehydration.[22] In order to overcome any decomposition, we carried out the methylation under very mild conditions: room temperature, weak base and non-aqueous aprotic solvent. This procedure allowed us to prepare [$^{2}H_3$]-scopolamine **7** with a satisfactory yield (57%). The [$^{2}H_3$]-scopolamine was easily purified as its hydrochloride salt. The disappearance of the 3H signal at 2.81 ppm indicates the complete labelling of the *N*-methyl group. The mass spectrum of the [$^{2}H_3$]-scopolamine exhibits a 3 amu shift of all the characteristic fragments in good agreement with the published fragmentation pattern.[1] Most abundant fragments calculated ratio (*m-H*)/*z* 138/141 (scopolamine/[$^{2}H_3$]-scopolamine) = 0.0018.

Experimental

All reagents were obtained from Sigma-Aldrich-Fluka (St Quentin Fallaviers, France) except CD_3I from Acros Organics (Halluin, France), potassium carbonate from Alfa Aesar (Bischheim, France) and were used without further purification. NMR spectra were recorded on a Bruker ARX250 instrument. Chemical shifts are quoted relative to residual MeOH or $CHCl_3$ in NMR solvent. GC/MS analyses were performed using Agilent GC5890/MSD5973, equipped with a 25 m DB-5MS column (J & W Scientific). Analytical samples were derivatized with *N*-methyl-*N*-trimethylsilyltrifluoroacetamide (Fluka) before GC/MS analyses. All reactions were performed under a dry nitrogen atmosphere.

Noratropine 3

α-chloroethyl chloroformate (3.4 mL, 31.5 mmol) was slowly added to a stirred mixture of sodium hydrogencarbonate (5.3 g, 63 mmol) and atropine **1** (1.8 g, 6.2 mmol) in chloroform (15 mL) at room temperature. After 4 h heating under reflux, the mixture was filtered and the inorganic salts were washed with chloroform (25 mL). The combined chloroformic solutions were distilled under vacuum to give **2** (light yellow oil), which was dissolved in methanol (50 mL) and heated under reflux for 3 h. After evaporation of methanol under vacuum, the oily residue was shaken with a mixture of cold water (5°C, 40 mL), HCl (37%, 1 mL) and chloroform (30 mL). The organic layer was discarded. The aqueous layer was alkalinized with cold ammonium hydroxide (30%, 5 mL) and extracted with chloroform (3 × 30 mL). The combined chloroformic solutions were dried with brine (50 mL), sodium sulphate and evaporated under high vacuum at 40°C yielding noratropine **3** (colourless oil, 1.71 g, 100%).

MS (EI), di-TMS derivative, *m/z* (%): 419 (11), 198 (8), 182 (100), 152 (8), 141 (17), 140 (18), 104 (5).

[$^{2}H_3$]-atropine 4

A mixture of noratropine **3** (3.9 g, 14.1 mmol), potassium carbonate (10.0 g, 72.4 mmol) and CD_3I (1.15 mL, 18.5 mmol) in acetonitrile (40 mL) was stirred for 2 days at room temperature in a sealed flask in the dark. The inorganic salts were removed by filtration and washed with chloroform (4 × 25 mL). The combined organic solutions were evaporated under vacuum at 60°C yielding a white solid. This solid was dissolved in cold HCl (0.35 N, 100 mL) and washed with chloroform (50 mL). The organic layer was discarded, the aqueous layer was alkalinized with cold ammonium hydroxide (30%, 10 mL) and the white precipitate was extracted with chloroform (2 × 50 mL). The combined chloroformic solutions were dried with brine (50 mL), sodium sulphate and evaporated under vacuum yielding a colourless oil. This oil was dissolved in acetone and evaporated under vacuum to give [$^{2}H_3$]-atropine **4** (white solid, 1.33 g, 31.9%).

^{1}H NMR ($CDCl_3$) ppm: 7.25 (m, 5H), 4.97 (d, *J* = 5, 1H), 4.11 (m, 1H), 3.73 (m, 2H), 3.08 (m, 1H), 2.98 (m, 1H), 2.86 (m, 1H), 1.99 (m, 2H), 1.80 (m, 1H), 1.69 (m, 2H), 1.43 (d, *J* = 12.5, 1H), 1.13 (m, 1H). MS (EI), m-TMS derivative, *m/z* (%): 364 (8), 143 (6), 127 (100), 97 (12), 85 (15).

Norscopolamine 6

A solution of potassium permanganate (4.3 g, 27.2 mmol) in water (100 mL) was slowly added (during 1 h) to a stirred solution of scopolamine **5** hydrobromide trihydrate (4.7 g, 10.7 mmol) in water (40 mL) thermostated at 30°C. The pH of the solution was maintained at 7 by periodic addition of 0.5 M sulphuric acid. This solution was stirred for 1 h at 30°C after the end of the addition and filtered through a Celite® pad. The manganese dioxide was washed with water (50 mL) and the combined aqueous solutions were alkalinized by addition of solid sodium carbonate (pH = 10). This solution was extracted with dichloromethane (2 × 50 mL), the organic layers were combined, dried with brine (100 mL), sodium sulphate and evaporated under vacuum at 50°C to give norscopolamine **6** (clear yellow gum, 2.4 g, 79.8%).

MS (EI), m-TMS derivative, *m/z* (%): 361 (7), 271 (3), 223 (3), 193 (6), 140 (7), 124 (94), 123 (65), 122 (100), 106 (20), 104 (22), 103 (19), 94 (31), 80 (47).

[$^{2}H_3$]-scopolamine 7

A mixture of norscopolamine **6** (2.4 g, 8.29 mmol), potassium carbonate (5.86 g, 42.4 mmol) and CD_3I (675 µL, 10.8 mmol) in acetonitrile (25 mL) was stirred for 24 h at room temperature in a sealed flask in the dark. The inorganic salts were removed by

filtration and washed with acetonitrile (2×25 mL) and the combined organic solutions were evaporated under vacuum at 50°C yielding a white solid. This solid was dissolved in cold water (50 mL). This aqueous solution was alkalinized with cold ammonium hydroxide (30%, 3 mL) and extracted with chloroform (2×50 mL). The combined chloroformic solutions were dried with brine (50 mL), sodium sulphate and evaporated under vacuum at 40°C yielding [$^2H_3$]-scopolamine **7** (colourless oil, 1.45 g, 57%).

[$^2H_3$]-scopolamine HCl

[$^2H_3$]-scopolamine (1.45 g) was dissolved in anhydrous diethylether (200 mL) and filtered. A solution of HCl (0.1 N in anhydrous Et_2O, 55 mL) was added slowly to the stirred solution of [$^2H_3$]-scopolamine leading to a white [$^2H_3$]-scopolamine hydrochloride precipitate. The diethylether was evaporated under vacuum to give the desired compound (white solid, 965 mg, 59.5%).

^{1}H NMR (MeOD) ppm: 7.33 (m, 5H), 5.00 (m, 1H), 4.11 (m, 1H), 3.77 (m, 2H), 3.26 (m, 2H), 2.41 (m, 2H), 2.03 (m, 1H), 1.82 (m, 2H). MS (EI), m-TMS derivative, *m/z* (%): 378 (19), 193 (4), 157 (32), 141 (100), 132 (10), 111 (30), 97 (61).

Conclusion

Rapid, simple and efficient preparations of deuterium labelled atropine and scopolamine have been performed. The main advantages of these procedures are excellent chemical and isotopic purities, good yields, facile processing and purification, low cost and possibility of labelling with other isotopes of hydrogen or carbon.

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