THE SYNTHESIS OF DEUTERIUM-LABELLED PROPANTHELINE BROMIDE

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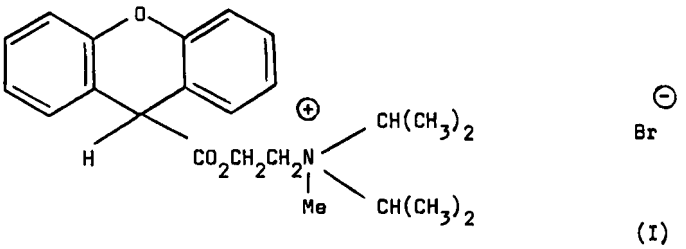
SUMMARY

The synthesis of propantheline bromide -d_3 and propantheline bromide -d_{14} for use in bioavailability studies, is described. The identity of the compounds was established by mass spectrometry and other analytical methods.

Key Words: Propantheline bromide, deuterium, synthesis, purity

INTRODUCTION

2-(N,N-diisopropylamino) ethyl xanthen-9-carboxylate methobromide hereafter called propantheline bromide (I) (Pro-Banthine (R), G.D. Searle & Co. Ltd) is a parasympatholytic agent used in the treatment of peptic and duodenal ulcer and renal colic.



(I)

This compound has proved difficult to measure in biological fluids because of the lack of a sensitive method to measure low levels of drug in urine and plasma.

Although a spectrophotometric method[1] and a fluorometric method[2] have

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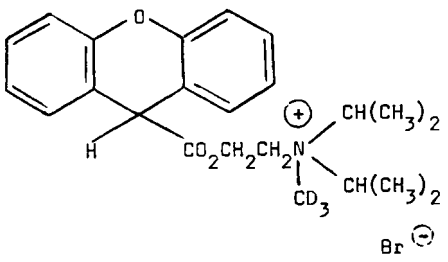
been described these methods had limited sensitivity for studies of the pharmacokinetics and bioavailability of the compound after oral administration of single low doses of the drug. The development of a sensitive and specific stable isotope dilution technique to measure propantheline bromide in urine and plasma[3] required the synthesis of deuterium labelled analogues of the drug for use as an internal standard in the gas chromatography - mass spectrometric assay.

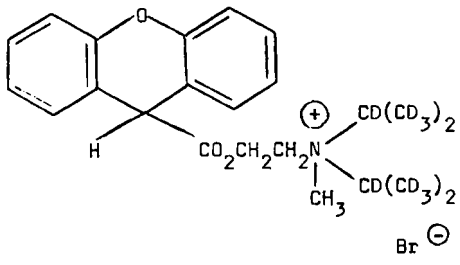
It has been established that standards with around 3-4 deuterium atoms per molecule are ideal for quantification in selected ion-monitoring.[4]

Propantheline bromide (I) N-deisopropylates when thermally degraded in the injection block of the gas chromatograph. The product formed gave a strong pseudo-molecular ion $(M + 1)^+$, and using ammonia as the reactant gas this ion can be selectively monitored. Propantheline bromide -d_3 (II) was found to behave similarly to unlabelled propantheline bromide. For use in double-labelling bioavailability studies another labelled standard had to be prepared which contained 3-4 deuterium atoms more than propantheline bromide -d_3 in order to prevent any interference from other ions. Therefore propantheline bromide with fourteen deuterium atoms (III) was prepared because due to the N-deiso-propylation the ion that one is in fact selectively monitoring contains only seven deuterium atoms.

We describe here the synthesis of propantheline bromide -d_3 (II) and propantheline bromide -d_{14} (III).



II

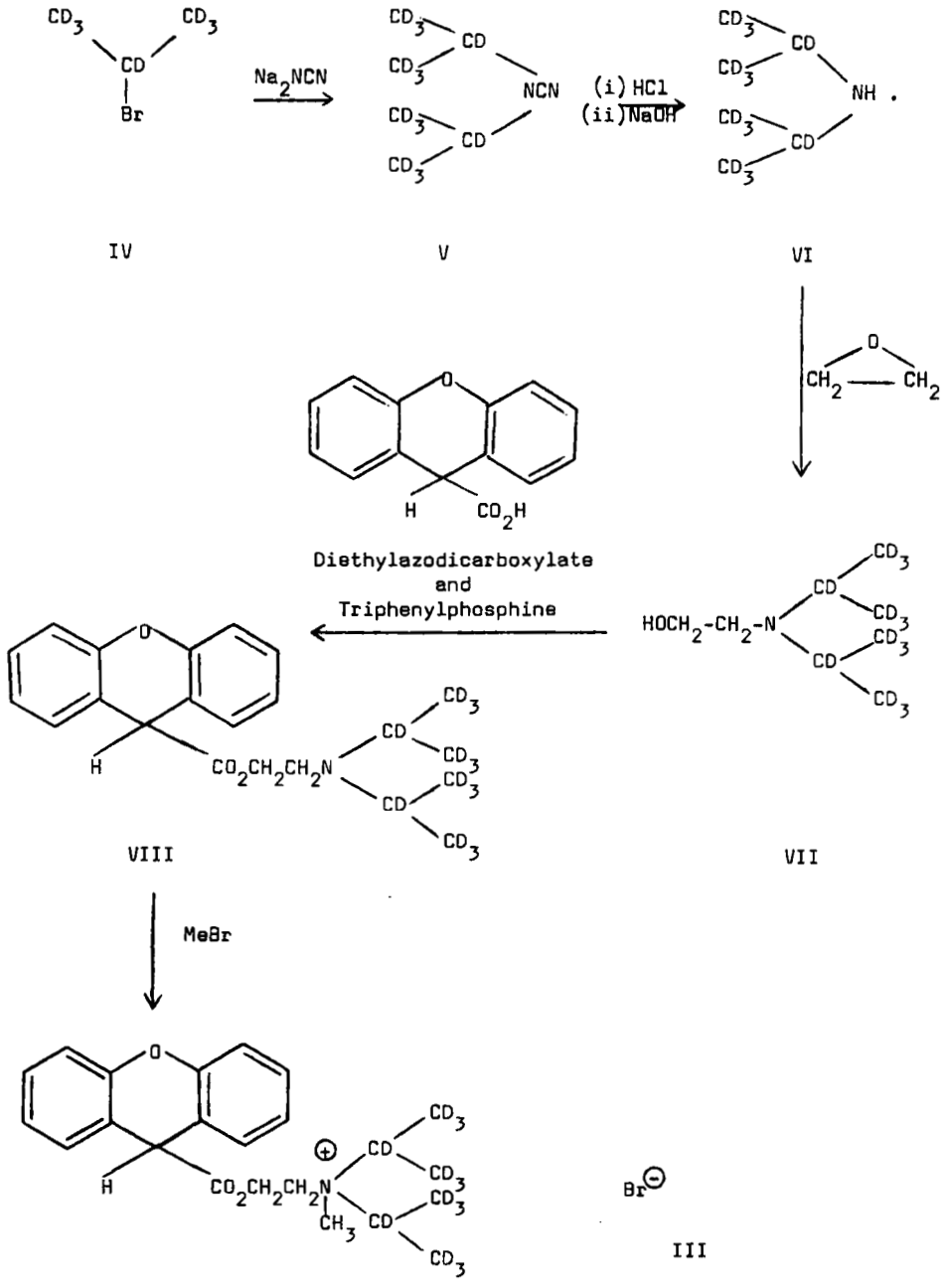


III

Propantheline bromide -d_3 was prepared by the condensation of xanthen-9-carboxylic acid with 2-(N,N-diisopropylamino) ethyl chloride to form

2-(N,N-diisopropylamino) ethyl xanthen-9-carboxylate. Reaction of the ester with methyl bromide -d_3 under pressure formed propantheline bromide -d_3.

The preparation of propantheline bromide -d_{14} is less straightforward. The following synthetic scheme was devised.



Isopropyl alcohol-d_8 was converted into 2-bromopropane-d_7 (IV) using phosphorus tribromide with xylene as solvent. Reaction of (IV) with sodium cyanamide in hexamethylphosphoramide, gave diisopropyl cyanamide-d_{14} (V) by a modification of a literature method.[5] The cyanamide (V) was hydrolysed[6] with 7M hydrochloric acid to give diisopropylamine hydrochloride (VI). The advantage of using cyanamides as intermediates in the preparation of secondary amines lies in the fact that the amines prepared are quite free from primary and tertiary amines which often prove troublesome impurities in secondary amines prepared by alternative synthetic methods.

The amine hydrochloride (VI) was converted into the free amine and this was then allowed to react with ethylene oxide[7] in a Carius tube to give 2-(N,N-diisopropylamino) ethanol-d_{14} (VII). Condensation of (VII) with xanthen-9-carboxylic acid using diethyl azodicarboxylate[8] and triphenylphosphine gave 2-(N,N-diisopropylamino) ethyl xanthen-9-carboxylate-d_{14} (VIII). This ester (VIII) is a rather unstable compound but it was synthesised in good yield on this small scale of operation using the reagents diethyl azodicarboxylate and triphenylphosphine and the quality of the product as determined by thin layer chromatography was such that no further purification was required. Quaternisation of (VIII) with methyl bromide in chloroform as solvent under pressure gave propantheline bromide-d_{14} (III).

EXPERIMENTAL

In general organic extracts were dried over sodium sulphate and solvents were removed on a rotary evaporator under vacuum. The products were characterised by their melting point or boiling point, spectral and chromatographic properties. Mass spectral analysis was performed on a Finnigan 3200E gas chromatograph mass spectrometer with a c.i. source using ammonia as reactant gas. (Finnigan Instruments, Sunnyvale, California). Data was analysed by an on-line Finnigan 6000 data system. N.M.R. spectra were recorded on a Varian EM.360

2-(N,N-diisopropylamino) ethyl xanthen-9-carboxylate

2-(N,N-diisopropylamino) ethyl chloride hydrochloride (10.85 g, 54 mmol) was dissolved in water (18 ml) and chloroform (52 ml). To this solution was added 0.88 ammonia (10 ml) and the mixture stirred for five minutes. The pH of the aqueous phase was brought to pH 10 by addition of further 0.88 ammonia (4 ml). The mixture was stirred for a further 10 minutes, allowed to stand and the layers separated. The aqueous layer was re-extracted with chloroform twice (11 and 4 ml). The combined chloroform extracts were filtered through Hyflo, and added to xanthen-9-carboxylic acid, (11.0 g, 48 mmol) and anhydrous sodium carbonate (4.7 g, 44 mmol) and the mixture refluxed overnight. On cooling the reaction mixture was washed twice with water and the organic extract evaporated to give a clear brown oil which was used without purification. This compound was characterised by the preparation of its hydrochloride, mp 108.5 - 109.5°C (lit[9] mp 110 - 2°C). The free base can be obtained as a white solid, mp 37 - 39.5°C but is unstable.

Propantheline bromide-d_3 (II)

The clear brown oil was taken up in chloroform (20 ml) and added to methyl bromide-d_3, (purchased from Prochem B.O.C. Ltd. 99% isotopic purity), (10 g, 105 mmol) in a Carius tube (14 x 3.5 cm). The tube was attached to a vacuum manifold, frozen down under liquid nitrogen, evacuated and sealed and kept at 60°C overnight. The cooled crude mixture was stirred for 30 minutes with water (6 ml) and charcoal (300 mg) then filtered through Hyflo. The chloroform layer was separated and the aqueous phase was back extracted with chloroform (2 x 4 ml). The combined chloroform extracts were evaporated to give a pale-brown oil. Methyl ethyl ketone (86 ml) was added to the oil at 50°C and the solution was distilled down under reduced pressure until approximately 50 ml of solution remained. The product precipitated as a white solid, 8.2 g, (18% based on methyl bromide-d_3) mp 161.5 - 162.5°C (lit[9] mp 156 - 7°C for unlabelled material). Mass spectral analysis:- unlabelled propantheline bromide-d_0 m/e (%) 354 (24.2), 326 (100) 181 (2.3), 128 (0.3), 100 (0.8); propantheline bromide-d_3 m/e (%) 354 (10.2), 329 (100.0), 181 (8.0), 128 (2.1), 103 (6.2). NMR:- unlabelled propantheline bromide-d_0

($CDCl_3$), δ 7.1 (m, 8, ArH), 5.1 (s, 1, benzylic H), 4.4 (m, 2, $-CH_2$), 4.1 - 3.5 (m, 4, $-CH_2$ and isopropyl -CH), 2.7 (s, 3, $N-CH_3$), 1.32 and 1.2 (d, 12, isopropyl $-CH_3$); propantheline bromide-d_3 ($CDCl_3$) δ 7.1 (m, 8, ArH), 5.1 (s, 1, benzylic H), 4.4 (m, 2, $-CH_2$), 4.1 - 3.5 (m, 4, $-CH_2$ and isopropyl -CH), 1.32 and 1.2 (d, 12, isopropyl $-CH_3$).

Preparation of 2-bromopropane-d_7 (IV)

To a solution of isopropyl alcohol-d_8 (purchased from Merck, Sharp and Dohme, Canada Limited), (50 g, 735.3 mmol) in xylene (50 ml), kept stirring at -10°C, was added phosphorus tribromide (25 ml, 262.9 mmol) so that the temperature did not rise above 0°C. The addition took approximately 1 hour. The reaction mixture was allowed to stand at room temperature for three days then distilled at atmospheric pressure using a fractionating column. The distillate with a boiling point below 70°C was collected and washed with saturated sodium bicarbonate solution. Redistillation yielded an oil 81.6 g (80%), bp 59°C (lit[10] bp 59°C unlabelled material).

Preparation of diisopropylcyanamide-d_{14} (V)

To sodium cyanamide (36.3 g, 422 mmol) in hexamethylphosphoramide (350 ml) was added in one portion 2-bromopropane-d_7 (81.6 g, 680 mmol) through the top of an extended length condenser to the stirred solution below and the resulting suspension was stirred at 80°C for 12 hours. The cooled solution was poured into a vast excess of water and extracted into ether. The organic extract was washed with brine and evaporated to give a pale-yellow oil, 33.3 g (70%), bp 90 - 92°C (2 mm) (lit[11] bp 115-116°C (10 mm)).

Preparation of diisopropylamine-d_{14} (VI)

Diisopropylcyanamide-d_{14} (33.3 g, 237.8 mmol) was refluxed with hydrochloric acid 7M (50 ml) for 6 hours. After cooling the reaction mixture was treated carefully with 50% (w/v) sodium hydroxide until pH 13-14 and the mixture fractionally distilled at atmospheric pressure using an ice cooled receiver. All distillate below 100°C was collected and after drying over sodium hydroxide pellets, redistillation gave the amine, 22.7 g (84%), bp 86-88°C (lit[12] bp 84°C unlabelled material).

Preparation of 2-(N,N-diisopropylamino) ethanol-d_{14} (VII)

Diisopropylamine-d_{14} (22.7 g, 197.4 mmol) was added to a Carius tube containing ethylene oxide (10.2 g, 227.2 mmol), water (4 ml), and concentrated hydrochloric acid (0.5 ml). The tube was attached to a vacuum manifold, frozen down under liquid nitrogen, evacuated, sealed and kept at 110°C for 18 hours. The cooled mixture was transferred to a separating funnel and the organic layer was dried over anhydrous potassium carbonate and distilled at atmospheric pressure to give a clear mobile oil, 22.5 g (72%), bp 187 - 195°C (lit[7] bp 183 - 7°C for unlabelled material).

Preparation of 2-(N,N-diisopropylamino) ethyl xanthen-9-carboxylate-d_{14} (VIII)

2-(N,N-diisopropylamino) ethanol-d_{14} (2.5 g, 15.7 mmol), xanthen-9-carboxylic acid, (7.92 g, 35.0 mmol), triphenylphosphine (8.91 g, 32.75 mmol), were dissolved in dry tetrahydrofuran (50 ml). Diethyl azodicarboxylate (5.95 g, 28.6 mmol) dissolved in dry tetrahydrofuran (10 ml) was added slowly over a period of 0.5 hours. A mild exothermic reaction took place and after 1 hour T.L.C. indicated that no 2-(N,N-diisopropylamino) ethanol remained. After evaporation of the tetrahydrofuran the residue was taken up in ether (100 ml) and extracted with 2M hydrochloric acid. The acid extract was washed with ether and then neutralised carefully with sodium hydroxide (50% w/v). The resulting precipitate was extracted into ether and the organic extract was washed with sodium bicarbonate, water and brine. Evaporation gave a clear colourless oil. The product was unstable and was therefore not purified further. T.L.C. (eluant: $(CH_2)_2Cl_2$(150)/MeOH(50)/H_2O(5)/HCO_2H(2)) revealed that the product showed as one spot corresponding in Rf to authentic unlabelled material.

Preparation of propantheline bromide-d_{14} (III)

The 2-(N,N-diisopropylamino) ethyl xanthen-9-carboxylate-d_{14} was taken up in chloroform (10 ml) and placed in a Carius tube. Methyl bromide (5 g, 52.6 mmol) was distilled into the tube on a vacuum manifold. The tube was frozen down under liquid nitrogen, evacuated, sealed, and allowed to stand for 5 days at room temperature. The contents of the tube were transferred to a 100 ml conical flask and the chloroform was boiled off and methyl ethyl ketone added keeping the volume constant. When the solution began to go cloudy the

heating was stopped and on cooling the product was obtained as a white solid, 3.7 g (51% based on 2-(N,N-diisopropylamino) ethanol). mp 161.5 - 162.5°C (lit[9] mp 156 - 7°C for unlabelled material). Mass spectral analysis: propantheline bromide-d_{14} m/e (%) 368 (9.5), 333 (100), 181 (1.3), 142 (0.3), 107 (0.7). NMR:- propantheline bromide-d_{14} ($CDCl_3$)δ 7.1 (m, 8, ArH), 5.1 (s, 1H, benzylic H), 4.4 (m, 2, $-CH_2$), 3.8 - 3.5 (m, 2, $-CH_2$), 2.7 (s, 3, N $-CH_3$).

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