SYNTHESIS OF SPECIFICALLY-DEUTERATED PROCAINE AND TETRACAINE

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SUMMARY

Local anesthetics procaine and tetracaine have been specifically-deuterated in two and three positions, respectively, for use in deuterium nuclear magnetic resonance experiments. The experimental procedure is either new or an adaptation of early synthetic methods.

Key Words: Anesthetic, Procaine, Tetracaine, Deuterium

Abbreviations: NMR, nuclear magnetic resonance; PRC, procaine; TTC, tetracaine.

INTRODUCTION

The interaction between local anesthetics and biological membranes has been extensively studied but no definite conclusion about their mechanism of action has been reached (1,2). In order to better characterize this interaction, deuterium nuclear magnetic resonance (^{2}H NMR) has been applied to a system composed of local anesthetics procaine (PRC) and tetracaine (TTC) in a phospholipid multilamellar dispersion (3-5). This technique allows the visualization of both the isotropic species dissolved in water and the bound species intercalated in the phospholipid multilamellar dispersions. Both the phospholipid and the local anesthetic can be specifically-deuterated and the extent of order experienced by each part of the molecules can be evaluated. We describe here the syntheses of specifically-deuterated procaine and tetracaine

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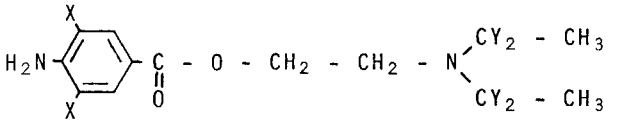
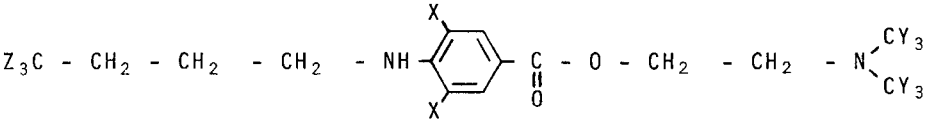
used in those experiments.

To our knowledge, no recent literature exists about the syntheses of procaine or tetracaine. The methods used in this work are either based on early procedures or are new ways to obtain those molecules. The amphiphilic character of these local anesthetics as well as the ionization of their aminogroups sometimes complicate the synthetic procedures. The structures of deuterated procaine and tetracaine are the following:



Procaine-d_2 (PRC-d_2), X = ^2H, Y = H

Procaine-d_4 (PRC-d_4), X = H, Y = ^2H



Tetracaine-d_2 (TTC-d_2), X = ^2H, Y = H, Z = H

Tetracaine-d_3 (TTC-d_3), X = H, Y = H, Z = ^2H

Tetracaine-d_6 (TTC-d_6), X = H, Y = ^2H, Z = H

EXPERIMENTAL

Procaine hydrochloride and tetracaine hydrochloride were purchased from Sigma Chemical Company. Dimethylamine-d_6 (99% d) and deuterium oxide (99.7% d) were obtained from Merck, Sharp and Dohme. p-Nitrobenzoylchloride and dimethylaminoethanol were purchased from Eastman. All other products were analytical reagent grade.

^1H and ^{13}C NMR spectra were run on a Varian EM-360 and

on a Varian CFT-20, respectively. Mass analysis was performed on a Finnigan 31000 mass spectrometer.

Procaine-d_2 and Tetracaine-d_2

Procaine or tetracaine (5 g) were refluxed in D_2O (100 ml) with 3-4 drops of DCl for periods ranging from 48 to 96 hours for the former, and of 24 hours for the latter. The reaction was followed by ^1H NMR until most of the signal due to the aromatic protons ortho to the nitrogen had disappeared. Water was evaporated under vacuum and the residue recrystallized from absolute ethanol: m.p., PRC-d_2·HCl 157°C, TTC-d_2·HCl 146-9°C; analysis: TTC-d_2·HCl cald., C 59.49, H 8.38, N 9.25, Cl 11.70, found, C 58.99, H 8.48, N 9.12, Cl 11.88; PRC-d_2·HCl cald., C 57.03, H 8.10, N 10.23, Cl 12.95; found, C 56.61, H 7.93, N 10.09, Cl 12.72; both compounds greater than 90% d from ^{13}C NMR.

Procaine-d_4

Diacetamide was first prepared by adding acetyl chloride (78.5 g; 1.0 mole) to acetamide (60 g; 1.0 mole) in acetic anhydride (100 g; 1.0 mole), while stirring and heating under a reflux condenser. A white solid separated slowly from the solution and hydrogen chloride was evolved. After three hours, the reaction mixture was cooled slightly and benzene (300 ml) was added. The yellow hygroscopic solid was removed by filtration. Benzene was removed by distillation on a steam bath and acetic acid by pumping on a vacuum line, to yield a crystalline product. The crude diacetamide, (60 g; m.p. 77-78°C) was purified by vacuum sublimation under 0.02 mm Hg from a bath heated to 65°C. The hard white crystals melted at 80-81°C.

The diacetamide was reduced to the labelled amine with lithium aluminum deuteride in ether. Twenty-five grams (0.25 mole) of sublimed diacetamide in ether were added dropwise (100 ml) to a suspension of lithium aluminum deuteride (10.0 g) in 450 ml of

absolute ether and the reaction mixture was stirred for 10 hours. The complex salt and excess deuteride were decomposed by careful addition of 3 N hydrochloric acid until the reaction mixture was acidic. The ether was then evaporated, and the residue made alkaline by addition of 50% sodium hydroxide before distillation with steam. The distillate was collected in 20 ml concentrated hydrochloric acid diluted with 60 ml of water. The distillate was freed of water and excess acid in the rotary evaporator under reduced pressure. The residue was dissolved in 300 ml chloroform and distilled for azeotropic elimination of the residual solvent. Suspended ammonium chloride was removed by filtration and the residual chloroform evaporated. The yield of light brown diethylamine-1,1,1',1'-d_4 hydrochloride was 22.5 g.

The free base was obtained by adding 50% sodium hydroxide to the salt in an evacuated system and condensing the liberated base in a trap cooled in dry ice. The diethylamine-1,1,1',1'-d_4 was dried over a few pellets of potassium hydroxide and redistilled into another trap. The yield was 10.0 ml (mass analysis: 85% d_4, 6.5% d_3, minor amounts of d_2 and d_1).

Diethylamine-1,1,1',1'-d_4 (10 ml, 0.10 mole) was reacted for one hour with an equimolar amount (5 ml, 0.10 mole) of ethylene oxide containing one drop of water in a round bottom flask, at dry ice temperature (6). The flask was then heated in a water bath (50-60°C) for 20 hours and finally the diethyl-1,1,1',1'-d_4-aminoethanol was distilled on a vacuum line.

Diethyl-1,1,1',1'-d_4-aminoethanol (7 g, 0.06 mole) was dissolved in dry benzene (100 ml) to which was added p-nitrobenzoyl chloride crystals (15 g, 0.08 mole) followed by reflux for one hour. After cooling, the crystals were filtered and washed with benzene.

The resulting 2-diethyl-1,1,1',1'-d_4-aminoethyl-4-nitrobenzoate was dissolved in methanol, the solution placed in a

reducing apparatus with 0.2 g platinum oxide and shaken under 25 p.s.i. of hydrogen for ca. 15 hours. The platinum was removed by filtration, the methanol evaporated under reduced pressure, and procaine-d_4 recrystallized from absolute ethanol (m.p. 153-5°C, 85% d from ^{1}H NMR; analysis: C 56.89, H 8.02, N 10.36, Cl 12.72).

Tetracaine-d_3

A solution of 3-chloropropene (60 g, 0.8 mole) and dibenzoylperoxide (2.0 g) in bromotrichloromethane (300 ml) was heated under reflux for six hours. Excess bromotrichloromethane was then removed under reduced pressure (6.0 mm Hg) in a rotary evaporator. The residue was fractionated in a Vigreux column with partial take-off stillhead under 0.1-0.2 mm Hg and collected in a receiver cooled in ice-water. The yield of 3-bromo-1,1,1,4-tetrachlorobutane was 188 g (b.p. 46°C/0.1 mm Hg).

Acetic anhydride (100 ml) was shaken in a separatory funnel with deuterium oxide (10.0 ml) to remove traces of acetic acid. It was then stirred in a 250 ml round bottomed flask with deuterium oxide (21 ml) to which a drop of acetyl chloride had been added. The solution was slowly heated to boiling to generate acetic acid-d_1.

In a 500 ml round-bottomed flask fitted with a thermometer, a separatory funnel, and reflux condenser to which a spiral trap and a drying tube were connected, were placed zinc dust (50.0 g) and acetic acid-d_1 (75 ml). The spiral trap was immersed in a Dewar cooler with dry ice. 1,1,1,4-tetrachloro-3-bromobutane (60.0g, 0.22 mole) was added dropwise to the stirred zinc dust and acetic acid-d_1. An exothermic reaction occured with evolution of deuterated butene. The rate of addition of halide was adjusted to keep the reaction mixture at 80-90°C. When the addition of halide was completed, the apparatus was swept with a current of dry nitrogen or argon. The yield of liquid 1-butene-4,4,4-d_3 in the trap was 10.8 g.

Dichloromethane (50 ml) and dibenzoyl peroxide (2.0 g)

were placed in a 300 ml glass tube and attached to a vacuum line through a U-shaped trap. The contents of the tube were frozen in liquid nitrogen and evacuated. 1-Butene-4,4,4-d_3 (25.0 ml) was distilled into the tube from the trap. The tube was allowed to come to -10 - 0°C and addition of hydrogen bromide from a cylinder was started while stirring and cooling in ice-water. The pressure in the system was carefully monitored with a manometer. After three hours, no more hydrogen bromide was absorbed and the reaction mixture turned pale yellow (free bromide).

The reaction mixture was stirred for one hour, washed with water, dilute sodium bisulfite, and water, and dried over anhydrous potassium carbonate. The solvent was distilled in a suitable column (spinning band or concentric tube type). After collecting 1.0-1.5 ml of a mixture on n- and iso-butane, pure 1-bromobutane-4,4,4-d_3 was obtained at 99-100°C/760 mm Hg (yield, 26.5 g).

p-Aminobenzoic acid (8 g, 0.06 mole) was neutralized with potassium bicarbonate in water until dissolution was complete. 1-Bromobutane-4,4,4-d_3 (7 g, 0.05 mole) was added and the solution refluxed for about 5 hours. Two products were obtained, N-butyl-4,4,4-d_3-p-aminobenzoic acid and N,N-dibutyl-4,4,4,4',4',4'-d_6-p-aminobenzoic acid. The solution was made acidic and the precipitate was filtered and washed with methylene chloride to remove the N,N-dibutyl-4,4,4,4',4',4'-d_6-p-aminobenzoic acid.

The residue (7.3 g) which contained unreacted p-aminobenzoic acid and N-butyl-4,4,4-d_3-p-aminobenzoic acid was put in a reflux apparatus with an excess of ethanol (100 ml). The solution was saturated with HCl gas (2 hours) and refluxed overnight under a slow stream of HCl. Ethanol was evaporated and the residue dissolved in water. Sodium acetate was added to neutralize the solution and to precipitate ethyl N-butyl-4,4,4-d_3-p-aminobenzoate.

When the first traces of red appeared (due to ethyl p-aminobenzoate), the solution was filtered and the product dried at room temperature.

The ethyl N-butyl-4,4,4-d_3-p-aminobenzoate was transesterified with an excess of dimethylaminoethanol (100 ml) and sodium ethoxide as catalyst for 10 hours. Ethanol was evaporated under reduced pressure and the excess dimethylaminoethanol was removed on a vacuum line. The residue was dissolved in ether and extracted with 20 ml fractions of an aqueous solution of 0.1 N HCl. When the pH became acidic, the extracts were combined, the water evaporated, and the tetracaine-d_3 recrystallized from ethanol (m.p. 147-148°C; analysis: C 59.04, H 8.41, N 9.33, Cl 11.52).

Tetracaine-d_6

Dimethylamine-d_6 (5 g, 0.10 mole) was reacted with ethylene oxide (4.9 ml, 0.10 mole) in the same manner as diethylamine-1,1,1',1'-d_4 (see procaine-d_4) to yield 3.5 g of dimethyl-d_6-aminoethanol (b.p. 134°C).

Dimethyl-d_6-aminoethanol (3.5 g, 0.037 mole) was dissolved in dry benzene and p-nitrobenzoyl chloride added (10 g, 0.54 mole). The mixture was refluxed for one hour, cooled, filtered and washed with benzene. The product was dried and dissolved in water. The aqueous solution was filtered to remove p-nitrobenzoic acid, made alkaline with potassium bicarbonate, and extracted with ether. The ether solution was dried over potassium carbonate and the solvent evaporated.

The 2-dimethyl-d_6-aminoethyl p-nitrobenzoate (6.3 g, 0.026 mole) was dissolved in ethanol with 0.3 g of platinum oxide and the hydrogenation was carried out (25 p.s.i. *ca*. 16 hours). After a decrease of 7 p.s.i. in pressure, butyraldehyde (5.6 g, 0.078 mole) was added and the reduction continued for 24 hours. The residue was dissolved in ether, extracted with aqueous 0.1 N HCl solution and the resultant tetracaine-d_6 was recrystallized from ethanol

(m.p. 147-8°C; analysis: C 58.24, H 8.40, N 9.14, Cl 11.17).

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