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SYNTHESIS OF TRITIUM LABELLED ROPIVACAINE - A NEW POTENTIAL LOCAL ANAESTHETIC.

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SUMMARY

A potential local anaesthetic, ropivacaine, is synthesized labelled with tritium. The label is introduced by reduction of a racemic tetrahydropyridine derivative with tritium gas. Resolution into the labelled enantiomers is accomplished by preferential crystallization with added carrier. The location of the tritium label is verified by ^{3}H NMR.

Key words; Ropivacaine, tritium labelled, local anaesthetic, fractional crystallization.

INTRODUCTION

It is well known that 1-alkylderivatives of (±)-N-(2,6-dimethylphenyl)piperidine-2-carboxamides exhibit local anaesthetic properties (1). The action of these derivatives has been found to vary in relation to their optical isomers (2). Therefore, current interest in pharmacological and metabolic evaluation of a potential local anaesthetic, N-(2,6-dimethylphenyl)-1-propylpiperidine-2-carboxamide hydrochloride, LEA 101 (Fig.), prompted the preparation of its (S)- and (R)-isomer labelled with tritium.

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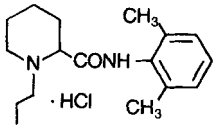


LEA 101

One of the simplest and most direct ways to label the compounds at sufficiently high specific activity would be to introduce the tritium into the aromatic portion of the molecule. This could be achieved either by aromatic halogenation followed by reductive dehalogenation or by hydrogen-tritium exchange in the presence of tritium gas and a metal catalyst. However, expected metabolic conversions rendered this label unsuitable and therefore the aliphatic portion of the molecule was chosen as the site for the labelling.

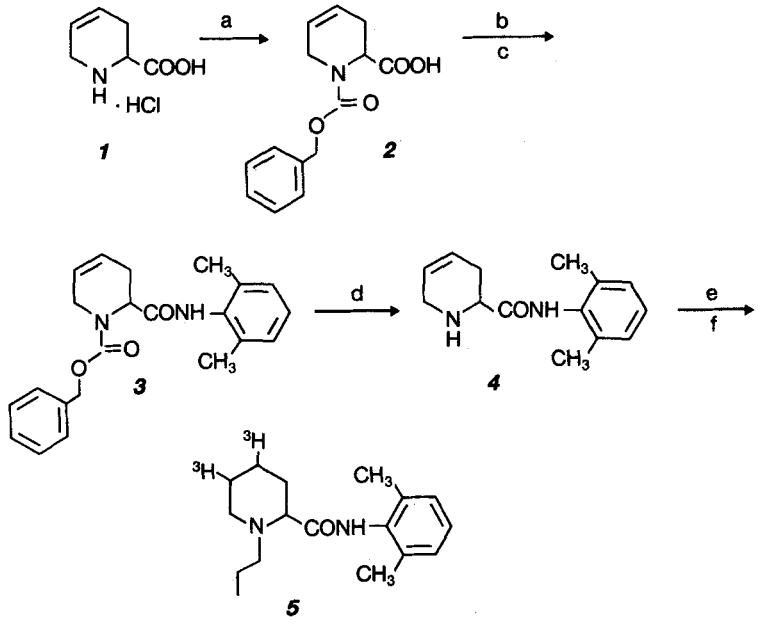
A tetrahydropyridine derivative should make a suitable substrate for the catalytic hydrogenation with tritium gas, although more synthetic work would be required in its preparation. It would allow the incorporation of two tritium atoms leading to a high specific activity and, if the double bond was in 3,4- or 4,5-position, the label would also elude the expected α-oxidation of amines (3). Therefore, a synthesis of N-(2,6- dimethylphenyl)-1,2,3,6-tetrahydropyridine-2-carboxamide (4) from 1,2,3,6-tetrahydropyridine-2-carboxylic acid (1) was elaborated (Scheme).

RESULTS AND DISCUSSION

An inviting strategy for the preparation of amide 4 was to use the (S)- and (R)-isomer of acid 1 as the starting material. The stereochemistry of the products should then be fixed beforehand and a resolution process should not be needed for the isolation of the two enantiomers. Unfortunately, only one of the isomers, the (S)-isomer, is known from the literature as a natural product, baikiain, isolated from Rhodesian teak (4). Consequently, with no known method for the preparation of the (R)-isomer, the synthetic racemic baikiain hydrochloride (1)(5) was chosen as starting material for the synthesis. The resolution of the labelled isomers of LEA 101 would then rely on the success of the preferential crystallization of added unlabelled (S)- or (R)-isomer.

Thus, racemic 1, prepared according to the procedure of Leete (6), was

Scheme



Reagents: a) $C_6H_5CH_2OCOCl$ b) 4-Dimethylaminopyridine/$SOCl_2$
c) 2,6-Xylidine d) $(CH_3)_3SiI$ e) $^3H_2$/Rh-Al_2O_3
f) $K_2CO_3/CH_3CH_2CH_2J$

treated with benzyl chloroformate under Schotten-Baumann conditions providing the N-protected acid (2).

The conditions for the coupling between acid 2 and 2,6-xylidine was established with the saturated, piperidine, analogue. When using the mixed anhydride procedure (7) with isobutyl chloroformate, no conversion of the initially formed anhydride could be effected with the xylidine. DCC-mediated coupling was also without success. However, when the method used by Palomo et al. (8) for the esterfication of carboxylic acids was applied, a good yield (74%) of amide was isolated.

The protected acid (2) was reacted at -20°C with 4-(N,N-dimethylamino)pyridinium chlorosulphite chloride to give the acid chloride which furnished carboxamide 3 upon in situ treatment with 2,6-xylidine.

Subsequent cleavage of the protecting group with iodotrimethyl silane gave the dehydropiperidine (4) in good yield.

The principle to incorporate the tritium label in the last step of the synthesis was not followed due to the instability of the starting material. The product, from the alkylation of 4 with 1-iodopropane, N-(2,6-dimethylphenyl)-1-propyl-1,2,3,6-tetrahydropyridine-2-carboxamide, decomposed both as the base and as the hydrochloride on contact

with air, leaving a dark brown residue. Consequently, in the penultimate step, the labelling was performed on compound 4 by hydrogenation with tritium gas in the presence of rhodium-on-alumina as catalyst. Alkylation of the crude labelled product with 1-iodopropane, followed by purification by filtering through a small alumina column afforded two fractions containing 373 mCi and 169 mCi of racemate 5. The amount of activity indicated that the compound was labelled at high specific activity and by stepwise addition of the two isomers of LEA 101, (S)-5 and (R)-5 were isolated by crystallization.

Thus a solution of unlabelled (S)-5 (9) was added to each of the two racemic fractions and allowed to crystallize. One recrystallization of the collected crystals furnished (S)-5 at a specific activity of 3.0 Ci/mmol and 1.8 Ci/mmol respectively, with a high radiochemical and enantiomeric purity. By the same procedure, employing unlabelled (R)-isomer and the combined mother liquors from the isolation of the (S)-isomer, two crops (866 mCi/mmol and 714 mCi/mmol) of (R)-5 were obtained.

Since the reaction conditions for the hydrogenation also might effect hydrogen-tritium exchange at the benzylic positions of 5, ^{3}H NMR was utilized to establish the positions of the tritium atoms. The spectrum of (R)-5 (26 mCi) showed only two signals at 1.40 ppm and 1.62 ppm of equal intensities, indicating exclusive tritium incorporation in the piperidine ring.

EXPERIMENTAL

Melting points were obtained on a Mettler FP 61 apparatus and are uncorrected. ^{1}H and ^{3}H NMR spectra were obtained on a Jeol FX 200 spectrometer with CD_3OD as solvent using Me_4Si as internal standard and ghost-reference (10). Mass spectra (EI, 70 eV) were recorded on an LKB 2091 mass spectrometer. Elemental analysis were performed by Analytische Laboratorien, Elbach, W. Germany. Tritium gas was purchased from Amersham International plc, Amersham, Bucks., England. Radiochemical purity was determined from TLC using a Berthold LB 283 Linear Analyzer. Enantiomeric purity was established by HPLC using a 4 x 100 mm LKB EnantioPac column eluted with 8 mM phosphate buffer pH 7.22/0.1 M NaCl/6% 2-propanol. The eluate was monitored for radioactivity by a Berthold LB 504 radioactivity monitor. Radioactivity was determined in a Packard Tri-Carb 460 C liquid scintillation spectrometer using Biofluor (New England Nuclear) as counting medium.

1-Benzyloxycarbonyl-1,2,3,6-tetrahydropyridine-2-carboxylic acid (2)

1,2,3,6-Tetrahydropyridine-2-carboxylic acid hydrochloride (1) (820 mg, 5 mmol) was dissolved in 2 M NaOH (5 ml). Benzyloxycarbonyl chloride (850 µl, 5.8 mmol) and 2 M NaOH solution (3 ml) were simultaneously added to the stirred solution at 4°C. During the addition, the pH of the reaction mixture was kept weakly basic (~8). When the addition was completed, the cooling bath was removed and the stirring was continued at room temperature for 2.5 hours. After dilution with H_2O (15 ml), unreacted acid chloride was removed by washing with diethyl ether (2 x 25 ml). The aqueous solution was made acidic (pH 2) with 2 M H_2SO_4 and extracted with diethyl ether (2 x 10 ml). The combined ethereal extracts were dried (Na_2SO_4) and evaporated in vacuo leaving an oil, 1.05 g (80%), which slowly crystallized providing 2. Mp 98-99°C. ^{1}H NMR (60 MHz, $CDCl_3$): δ 7.40 (s, 5H), 5.90-5.70 (m, 2H), 5.24 (s, 2H), 5.24-5.00 (m, 1H), 4.24-3.90 (m, 2H), 2.80-2.48 (m, 2H). Found: C 64.28; H 5.78; N 5.30; O 24.36; Calc. for $C_{14}H_{15}NO_4$: C 64.35; H 5.79; N 5.36; O 24.50.

1-Benzyloxycarbonyl-N-(2,6-dimethylphenyl)-1,2,3,6-tetrahydropyridine-2-carboxamide (3)

To a solution of 4-(N,N-dimethylamino)pyridine (487 mg, 4 mmol) in CH_2Cl_2 (25 ml), $SOCl_2$ (33 µl, 4.5 mmol) was added at -20°C. After stirring for 20 min, a precipitate had formed and acid 2 (900 mg, 3.4 mmol) dissolved in CH_2Cl_2 (3 ml) was added. The stirring was continued at -20°C while the precipitate slowly dissolved. After 30 minutes, a mixture of 2,6-xylidine (507 mg, 4.2 mmol) and 4-(N,N-dimethylamino)pyridine (500 mg, 4.1 mmol) in CH_2Cl_2 (5 ml) was slowly introduced and the solution left at room temperature for 18 hours. The mixture was successively washed with 1 M HCl, brine and saturated $NaHCO_3$. Drying (Na_2SO_4) and concentration in vacuo left an oil which was purified by column chromatography (SiO_2/CH_2Cl_2-EtOAc; gradient). Yield: 730 mg (59%) of 3. Mp 131-132°C ($CHCl_3$-petroleum ether 40-60). ^{1}H NMR (200 MHz, $CDCl_3$); δ 7.37-7.32 (m, 6H), 7.13-7.05 (m, 3H), 6.00-6.85 (m, 1H), 5.85-5.70 (m, 1H), 5.23 (s, 2H), 5.35-5.10 (m, 1H), 4.50-4.25 (m, 1H), 4.00-3.85 (m, 1H), 3.00-2.80 (m, 1H), 2.55-2.35 (m, 1H), 2.13 and 2.06 (2 s, 6H). Found: C 72.37; H 6.60: N 7.64; O 13.29. Calc. for $C_{22}H_{24}N_2O_3$: C 72.50; N 6.64; N 7.69; O 13.17.

N-(2,6-Dimethylphenyl)-1,2,3,6-tetrahydropyridine-2-carboxamide (4)

To a solution of carbamate 3 (640 mg, 1.75 mmol) in CH_2Cl_2 (10 ml), iodotrimethyl silane (630 mg, 3.15 mmol) was added at -20°C. The cooling bath was removed and, after 3.5 hours at room temperature, TLC

(SiO_2/CH_2Cl_2-EtOAc, 9:1) showed complete conversion of 3. The reaction mixture was cooled in an ice-water bath and ethanol (0.5 ml), followed by 2 M HCl (5 ml), was added. The aqueous layer was separated and the organic phase extracted with 2 M HCl (5 ml). The aqueous extracts were combined and made alkaline (pH 9) with 2 M NaOH. Extraction with CH_2Cl_2 (3 x 10 ml) and evaporation to dryness furnished amine 4 as a crystalline residue, 382 mg (95%). Mp. 121-22°C (EtOH-n-Hexane). ^1H NMR (200 MHz, $CDCl_3$); δ 8.33 (broad s, 1H), 7.12-7.00 (m, 3H), 5.89 (d, of q., J_1= 10.25 Hz, J_2 = 4.88 Hz, J_3 = 2.69 Hz, 1H), 5.78 (d of q. J1 = 10.25 Hz, J_2 = 4.88 Hz, J_3 = 2.69 Hz, 1H), 3.62 (dd, J_1 = 9.05 Hz, J_2 = 5.15 Hz, 1H), 3.46-3.51 (m, 2H), 2.11-2.58 (m, 2H), 2.22 (s, 6H), 1.69 (s, 1H). Found: C 73.02; H 7.79; N 12.18; O 7.04. Calc. for $C_{14}H_{18}N_2O$: C 73.01; H 7.88; N 12.17; O 6.95.

N-(2,6-Dimethylphenyl)-1-propyl-[4,5-^{3}H]piperidine-2-carboxamide (5)

A solution of 4 (7.0 mg, 30 μmol) in DMF (500 μl) was stirred at room temperature under carrier free tritium gas (10 Ci) in the presence of Rh/Al_2O_3 (9 mg). After 18 hours, the reaction mixture was filtered to remove the catalyst. To the filtrate, K_2CO_3 (3 mg) and 1-iodopropane (160 μl) were added. The mixture was left with stirring at room temperature. After 18 hours, TLC (Al_2O_3/diethyl ether-n-hexane, 1:1) of the reaction mixture showed the desired product (5) with a radiochemical purity of 86%. The total activity was 1.84 Ci (106%). The solvent was evaporated and the residue was taken up in diethyl ether-n-hexane (1:1) and filtered through a small alumina column (6 mm x 20 mm). Two fractions were collected containing 373 mCi and 169 mCi of 5 with a radiochemical purity of 99% and 94% respectively.

(S)-N-(2.6-Dimethylphenyl)-1-propyl-[4,5-^{3}H]piperidine-2-carboxamide (ropivacaine) hydrochloride monohydrate, ((S)-5)

The fraction obtained above, containing 169 mCi of racemic 5, was evaporated to dryness. Unlabelled (S)-enantiomer (15 mg) was dissolved in hot acetone-water (9:1, 300 μl) and added to the dried racemate. Small seeds of unlabelled (S)-5 were added to the solution and, after 18 hours at room temperature, the precipitated crystals were collected. Recrystallization from the same solvent (20 μl/mg) provided 7 mg (38 mCi) of (S)-5. The specific activity was 1.8 Ci/mmol and the radiochemical purity 98%. The enantiomeric purity was 100%. In the same way, when a part (330 mCi) of the remaining fraction was mixed with 19 mg of unlabelled (S)-isomer, 8 mg (73 mCi) of crystals was

obtained. The specific activity was 3.0 Ci/mmol and the radiochemical purity 97%. The crystals consisted of 97% (S)- and 3% (R)-isomer (i.e. 94% enantiomeric excess).

(R)-N-(2.6-Dimethylphenyl)-1-propyl-[4,5-^{3}H]piperidine-2-carboxamide hydrochloride monohydrate, ((R)-5)

The mother liquors from the preparation of (S)-5 were combined and by recrystallization of added unlabelled (R)-5, two crops were collected, 10 mg (26 mCi) consisting of 97% (R)-5 and 3% (S)-5 with a specific activity of 866 mCi/mmol and 14 mg (30 mCi) consisting of 94% (R)-5 and 6% (S)-5 with a specific activity of 714 mCi/mmol. The radiochemical purity was ≥99%.

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