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DUAL LABELLING OF LOBUPROFEN WITH TRITIUM AND CARBON-14

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SUMMARY

Dual labelling of 2-[4-(3-chlorophenyl)-1-piperazinyl]ethyl 2-(4-isobutylphenyl) propionate (Lobuprofen) with tritium and carbon-14 was performed. The synthesis between 2-(4-isobutylphenyl)propionic acid (Ibuprofen), randomly labelled with tritium, and 2-[4-(3-chlorophenyl)-1-piperazinyl]ethanol (Cl-Alkanol) labelled with carbon-14 in the piperazine ring was achieved. Prior to this synthesis, the [^{14}C]Cl-Alkanol was obtained using 2-amino-[2-^{14}C]ethanol as a precursor.

Purification was accomplished by thin layer chromatography (TLC). Concentration, purity and specific activity of the compound were determined by UV spectrophotometry, high performance liquid chromatography (HPLC) and liquid scintillation techniques.

Key words: Dual labelling. Carbon-14/tritium. Diethanolamine. 4-(3-Chloropehnyl)-piperazine. 2-[4-(3-chlorophenyl)-1-piperazinyl]ethanol. Ibuprofen-ester.

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1. INTRODUCTION

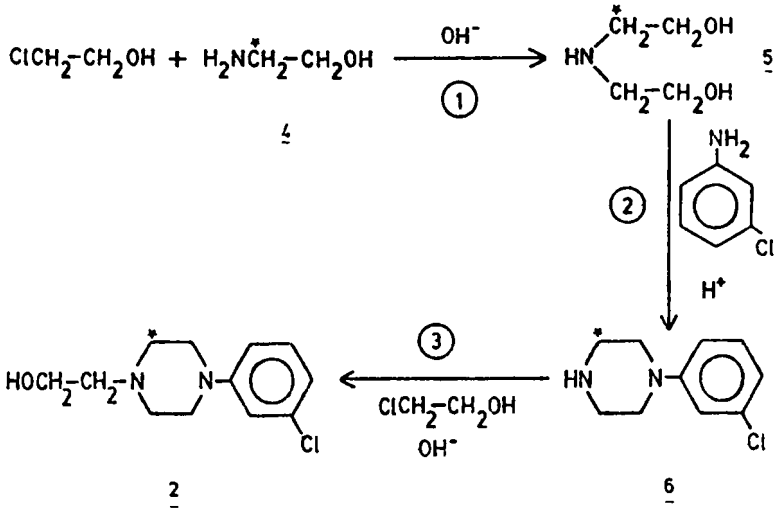
The analgesic activity of some arylpropionic acids can be increased by esterification with some phenylpiperazinylalkanols. In this way we synthesized the 2-[4-(3-chlorophenyl)-1-piperazinyl]ethyl 2-(4-isobutylphenyl)propionate, 1, (Lobuprofen) from 2-[4-(3-chlorophenyl)-1-piperazinyl]ethanol, 2, (Cl-Alkanol) and 2-(4-isobutylphenyl)propionic acid, 3, (Ibuprofen). We expected this ester to have analgesic activity and reduced antiinflammatory activity. We also espected the ester to be less ulcerogenic and better absorbed because of its increased liposolubility (1,2). Considering the structure of this drug and the hypothesis of its hydrolysis "in vivo", it was important to have the labelled compound with two different isotopes in the molecular estructure in order to study simultaneously the two hydrolysis products: acidic and alcoholic metabolites.

Therefore we synthesized the dual labelled Lobuprofen with tritium and carbon-14 from the Ibuprofen labelled unspecifically with tritium, and the Cl-Alkanol labelled with carbon-14 in the piperazine ring.

2. MATERIALS AND METHODS

2.1. ^{14}C-Cl-ALKANOL SYNTHESIS

The synthetic route for the preparation of ^{14}C-labelled 2-[4-(3-Chlorophenyl)-1-piperazinyl]ethanol, 2, (Cl-Alkanol) is shown in scheme 1.



Scheme 1

2.1.1. 2-(2-Hydroxyethylamino)-[2-^{14}C]ethanol, 5, (^{14}C-diethanolamine)

The radiolabelled starting material was 2.5 mCi (92.5 MBq) of 2-amino-[2-^{14}C] ethanol hydrochloride, 4, purchased from Amersham International, with a specific activity of 49 mCi/mmol (1.81 GBq/mmol) and a radiochemical purity of 98 % (as determined by TLC).

To 12.5 ml of the aqueous solution of 2-amino-[2-^{14}C]ethanol hydrochloride, 4, (0.05 mmoles) the following were added: 100 µl of freshly distilled ethanolamine (b.p.: 169-170ºC) (1.67 mmoles), 200 µl of a 50 % aqueous sodium hydroxide (3.28 mmoles) and 300 µl of 2-chloroethanol (4.48 mmoles). The mixture was magnetically stirred at room temperature for 4 hours.

The reaction mixture was analyzed by thin layer chromatogrphy (TLC). An aliquot of the crude product was chromatographed on a 0.25 mm silica gel plate using as solvent a mixture of methanol/ammonium hydroxide/water (75/5/20) (V/V). Cold ethanolamine and diethanolamine were used as reference compounds. By ^{14}C-scanning (Berthold Dünnschicht-Scanner, Mod. LB-2723) 3 peaks were shown. This indicated that the reaction yielded an unknown product (probably triethanolamine), diethanolamine, 5, and unreacted ethanolamine, 4.

After vacuum evaporation, the residue was dissolved in 2 ml of ethanol, and transferred into a glass ampoule. The solvent was removed under nitrogen stream at room temperature.

2.1.2. 4-(3-Chlorophenyl)-[2-^{14}C]piperazine, 6

To the glass ampoule containing the [^{14}C]diethanolamine, 5, 172 µl of 3-chloroaniline (1.64 mmoles) and 189 µl of 47 % hydrobromic acid (3.50 mmoles) were added. After sealing the ampoule, it was maintained at 200ºC for 5 hours. The reaction mixture was made alkaline with a 50 % aqueous sodium hydroxide and the 4-(3-chlorophenyl)-[2-^{14}C]piperazine, 6, was extracted with diethyl ether.

The piperazine, 6, was purified by preparative TLC on 2 mm silica gel plates developed with methanol/ammonium hydroxide/water (75/5/20) (V/V). The piperazine was eluted from silica gel with methanol. An analytical TLC was made in the same way as for the preparative TLC, and TLC-radioscan indicated a pure product. Chemical concentration was determined by UV spectrometry at 254 nm (Hewlett-Packard spectrophotometer, Mod. 8450 UV/VIS).

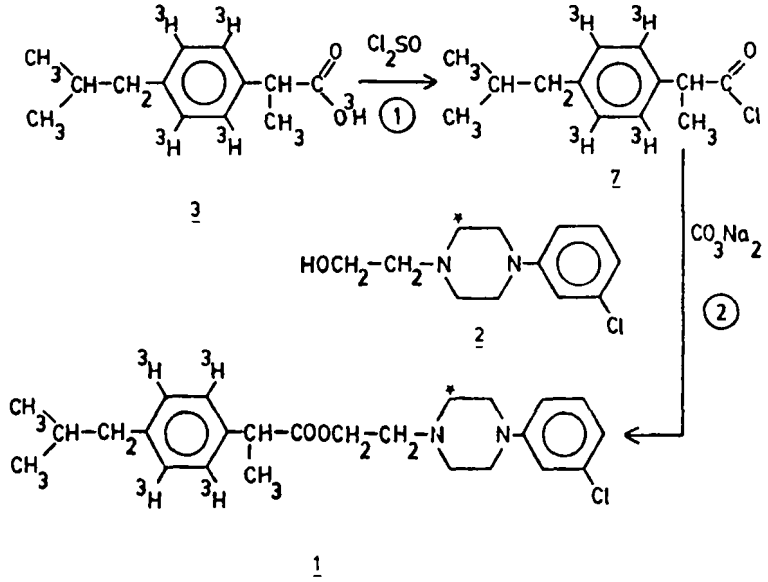
2.1.3. 2-[4-(3-Chlorophenyl)-1-[2-^{14}C]-piperazinyl]ethanol, 2, (^{14}C-Cl-Alkanol)

To a solution of 4-(3-chlorophenyl)-[2-^{14}C]piperazine, 6, (15 mg; 0.08 mmoles) in ethanol (3 ml), 16 µl of a 50 % aqueous sodium hydroxide (0.20 mmoles) and 17

µl of 2-chloroethanol (0.25 mmoles) were added. The reaction mixture was magnetically stirred at room temperature for 48 hours. pH was maintained basic by adding sodium hydroxide and 2-chloroethanol at different time intervals after the reaction had started. The crude product was filtered (Millex-SR, 0.5 µm from Millipore) in order to remove sodium chloride formed and it was chromatographed on a 0.25 mm silica gel plate with methanol. The radiochromatogram showed a peak (Rf = 0.66) after elution of the required product, 2, from the silica gel, corresponding to 96 % of radiochemical purity. Chemical concentration was calculated by measuring the [^{14}C]Cl-Alkanol, 2, absorbance at 255 nm, obtaining 17 mg (0.07 mmol) of the product. Specific activity of the compound (0.85 mCi/mmol; 31.45 MBq/mmol) was determined by liquid scintillation counting (Packard, Mod. Tri-Carb 460C). A yield of 2.4 %, after the three first steps of the reaction, was obtained.

2.2. [^{3}H/^{14}C]LOBUPROFEN SYNTHESIS

The synthetic route for the preparation of dual labelled Lobuprofen with tritium and carbon-14 is shown in scheme 2, and it is based on the general procedure of Martín et al. (3).



Scheme 2

2.2.1. [^{3}H] Ibuprofen chloride, 7

A mixture of 150 mg of [^{3}H]Ibuprofen, 3, (4) (0.73 mmoles; 2.5 mCi; 92.5 MBq)

and 110 µl of thionyl chloride (1.48 mmoles) was magnetically stirred at room temperature for 3 hours. The thionyl chloride excess was removed under nitrogen stream at room temperature, and the crude product was used without further purification.

2.2.2. [$^{3}H/^{14}C$]Lobuprofen, 1

In a 2 ml flask equipped with a magnetical stirrer, 100 mg of cold product plus 17 mg of crude [^{14}C]Cl-Alkanol, 2, (0.49 mmoles; 60 µCi; 2.22 MBq), 106 mg of sodium carbonate (1.0 mmol) and 0.5 ml of toluene were added. The flask was cooled at 0-5ºC and the [^{3}H]Ibuprofen chloride, 7, dissolved in 0.5 ml of toluene, was added. After addition, the reaction mixture was kept at room temperature for 17 hours.

Purification of [$^{3}H/^{14}C$]Lobuprofen, 1, in order to remove the residual [^{3}H]Ibuprofen and [^{14}C]Cl-Alkanol, was achieved by preparative TLC on 2 mm silica gel plates developed in n-butanol/acetic acid/water (9/1/3, by vol). [$^{3}H/^{14}C$]Lobuprofen, 1, was eluted with diethyl ether from silica gel.

A new analytical TLC was made in the same conditions as above. TLC-radioscan indicated a product (Rf = 0.57) with a radiochemical purity of 98.5 %.

Another TLC was made on 0.25 mm silica gel plate using as solvent a mixture of benzene/methanol/acetic acid (30/70/0.4) (V/V). In this way, the radiochemical purity of [$^{3}H/^{14}C$]Lobuprofen was shown to be 98 %.

Chemical purity was determined by high performance liquid chromatography (HPLC). HPLC analysis was performed using a Mod. 45 pump, U6K injector and Mod. 440 absorbance detector manufactured by Waters Associates. The detector was operated at 254 nm. The column used was a Radial-PAK cartridge (10 cm x 8 mm i.d.) packed with 10 µm permanently bonded octadecylsilane reverse phase. The eluting solvent was a mixture of 73 % acetonitrile and 23 % sodium acetate (0.1 M) and the flow rate was 4.0 ml/min. The HPLC chromatogram (t_R = 12 min) indicated a 99.5 % chemical purity of the final product.

Chemical concentration was calculated by ultraviolet technique by measuring the [$^{3}H/^{14}C$]Lobuprofen absorbance at 252 nm. Specific activity was determined by liquid scintillation counting.

3. RESULTS

Dual labelled [$^{3}H/^{14}C$]Lobuprofen, 1, showed the following characteristics:

Amount: 82 mg (40 % yield of esterification).
Total activity: ^{3}H: 728 μCi (26.94 MBq); ^{14}C: 23.4 μCi (0.87 MBq).
Specific activity: ^{3}H: 4.13 mCi/mmol (152.81 MBq/mmol); ^{14}C: 132.70 μCi/mmol (4.91 MBq/mmol).

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