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SYNTHESIS OF DOPEXAMINE HYDROCHLORIDE LABELLED WITH TRITIUM, DEUTERIUM AND CARBON-14

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SUMMARY

Dopexamine hydrochloride labelled with carbon-14 has been prepared by a mixed anhydride reaction between 6-[[2-(3,4-dimethoxyphenyl)ethyl]amino]-6-oxohexanoic acid, ethyl carbonochloridate and 2-phenyl-[1-^{14}C]ethylamine. Deuterium isotopomers have been prepared by exchange, reduction of an unsaturated precursor, and by reductive dehalogenation of a chloro-derivative. Tritium-labelled compound was obtained by acid-catalysed tritium exchange.

Key words: Dopexamine hydrochloride, carbon-14, tritium, deuterium, Dopacard(R), 4-[2-[[6-[(2-phenylethyl)amino]hexyl]-amino]ethyl]-1,2-benzenediol dihydrochloride.

INTRODUCTION

Dopexamine hydrochloride (4-[2-[[6-[(2-phenylethyl)amino]hexyl]-amino]ethyl]-1,2-benzenediol dihydrochloride, 1, figure 1) is a catechol containing compound synthesised by Fisons (1,2) and possessing novel pharmacological properties (3,4,5). A formulation of the compound, Dopacard(R), is currently undergoing clinical trials for use as an afterload reducing agent in the treatment of acute cardiovascular disorders involving cardiac decompensation. The requirement for suitably labelled derivatives to allow metabolic, pharmacokinetic and receptor-binding studies led us to synthesise various isotopomeric forms of the drug.

To allow initial metabolism studies and to support receptor-binding studies, dopexamine labelled with tritium in the catechol ring was

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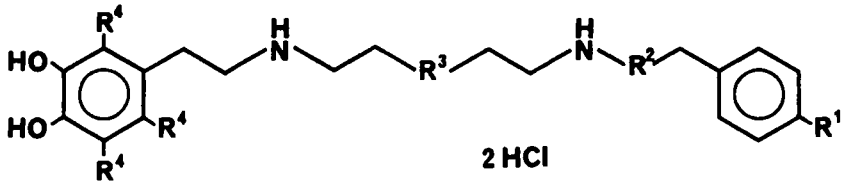
synthesised, at high specific activity, by acid-catalysed exchange with tritium oxide. Additionally, dopexamine labelled with deuterium at various positions in the molecule was also synthesised, by various routes, to support spectroscopic assignments and to investigate the quantitation of the drug via isotopic dilution techniques. For detailed metabolic and pharmacokinetic studies ^{14}C-labelled dopexamine (4) was prepared. The simplest and most cost-effective route to a ^{14}C-isotopomer employed commercially available 2-phenyl-[1-^{14}C]ethylamine as a labelled precursor. This paper describes the detailed procedures used for the synthesis of all the above dopexamine isotopomers.

RESULTS AND DISCUSSION

Since any N-dealkylative metabolism of dopexamine would lead to fragmentation of the molecule, the suitability of the proposed ^{14}C-labelled compound (4) for metabolic work required demonstration. The synthesis of ^{3}H-labelled dopexamine (2), in which tritium was introduced into the catechol ring via acid-catalysed exchange, was therefore undertaken to allow an evaluation of the comparative metabolic fate of the two isotopomers.



1; R^1=H, R^2=CH_2, R^3=$(CH_2)_2$, R^4=H

2; R^1=H, R^2=CH_2, R^3=$(CH_2)_2$, R^4=^{3}H

3; R^1=H, R^2=CH_2, R^3=$(CH_2)_2$, R^4=^{2}H

4; R^1=H, R^2=$^{14}CH_2$, R^3=$(CH_2)_2$, R^4=H

5; R^1=Cl, R^2=CH_2, R^3=$(CH_2)_2$, R^4=H

6; R^1=^{2}H, R^2=CH_2, R^3=$(CH_2)_2$, R^4=H

7; R^1=H, R^2=CH_2, R^3=*t*-CH=CH, R^4=H

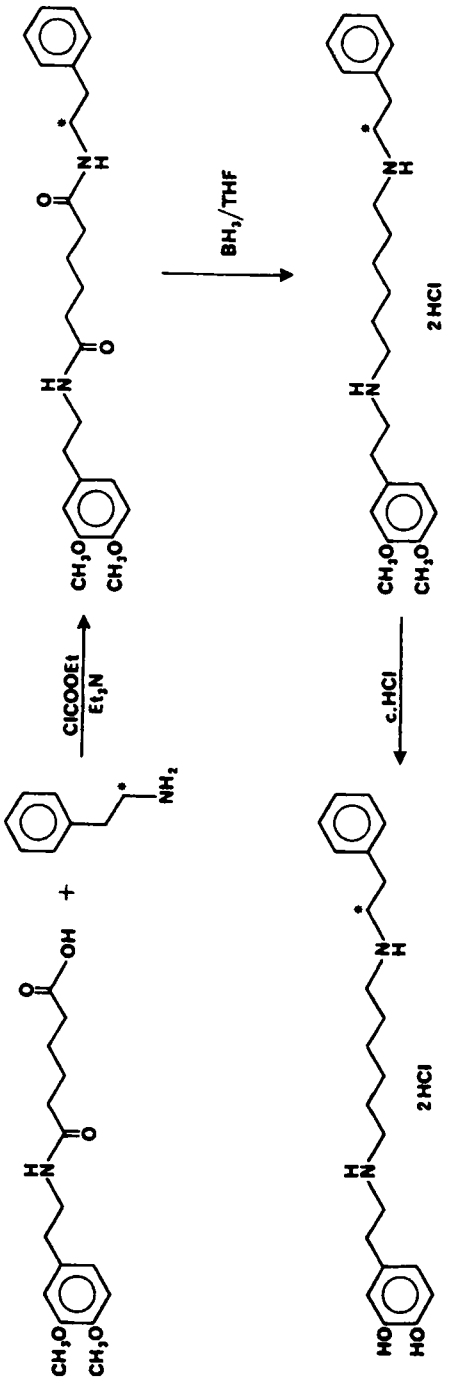
8; R^1=H, R^2=CH_2, R^3=$(C^2H_2)_2$, R^4=H

FIGURE 1

Suprisingly, however, the electron-impact (EI) mass spectrum of the corresponding exchange-deuterated analogue (3) included fragment ions suggesting the presence of isotope in the phenylethylamine group and also in the central aliphatic portion of the molecule. This was contrary to expectations and cast doubts upon the regioselectivity of tritium labelling in 2. Fortunately, subsequent studies using ^{2}H-nmr demonstrated that the label was indeed exclusively associated with the catechol ring. This result was supported by fast atom bombardment (FAB) mass spectrometry, which demonstrated the absence of isotope elsewhere in the molecule and thus confirmed that the apparent extensive distribution of isotope seen in the electron impact mass spectrum arose from scrambling in the spectrometer. Having resolved this ambiguity, the integrity of the catechol-labelling in 3 and hence in 2 was assured. Subsequent metabolic comparison between the ^{14}C and ^{3}H isotopomers (4 and 2) demonstrated essentially identical metabolic profiles in the rat allowing the ^{14}C-labelled isotopomer (4) to be selected for use in extensive metabolic and pharmacokinetic investigations.

The synthesis of the ^{14}C-labelled dopexamine hydrochloride (4) described above was carried out according to the route in Figure 2. Formation of a mixed anhydride between ethyl carbonochloridate and 6-[[2-(3,4-dimethoxyphenyl)ethyl]amino]-6-oxohexanoic acid and subsequent reaction with 2-phenyl-[1-^{14}C]ethylamine yielded the diamide in good yield and with suitable radiochemical purity. Reduction of the diamide, using borane/tetrahydrofuran, followed by demethylation with concentrated hydrochloric acid yielded the desired product (4), which after a single recrystallisation was of adequate radiochemical purity for the intended use of the material; the overall radiochemical yield was 32.4% from the labelled phenylethylamine.

In addition to the above isotopomers, dopexamine labelled with deuterium in the phenyl ring and in the central hexamethylene chain were



* Indicates position of ^{14}C-label

FIGURE 2

synthesised for proposed isotopic dilution analyses, and to assist the mass spectrometric assignment of label distribution in the exchange deuterated compound (3). Dopexamine labelled with deuterium in the para position of the phenyl ring was obtained by deuterodehalogenation of the corresponding chloro-derivative (5). Reduction of pre-exchanged 5 in [O-^{2}H]methanol, with deuterium gas in the presence of 5% palladium on carbon and triethylamine yielded 6 in which the atom % abundance of deuterium at the para position was > 96%. If reductive dehalogenation was performed in unlabelled methanol the atom percentage abundance dropped to 50% reflecting isotopic scrambling between the solvent and the deuterium gas. No exchange-labelling of the catechol residue occurred during the reduction as evidenced by ^{2}H-nmr and mass spectrometry (FAB and EI).

Reduction of the unsaturated derivative 7 with deuterium gas in the presence of a 5% rhodium on carbon catalyst in [O-^{2}H]methanol yielded a deuterated dopexamine isotopomer 8 in which extensive exchange labelling(6) of the olefinic and allylic protons of 7 had occurred prior to reduction. Overall, 3.6 atoms of deuterium were introduced into the molecule by reduction. Labelling was, however, restricted to the original olefinic and allylic sites.

No inconsistencies were observed between the FAB and EI mass spectra of either of the above isotopomers indicating that isotopic scrambling was important only for the catechol deuterated isotopomer.

EXPERIMENTAL

Authentic samples of dopexamine hydrochloride, 6-[[2-(3,4-dimethoxyphenyl)ethyl]amino]-6-oxohexanoic acid, 4-[2-[[6-[[2-(4-chlorophenyl)ethyl]amino]hexyl]amino]ethyl]-1,2-benzenediol dihydrochloride (5) and 4-[2-[[6-[(2-phenylethyl)amino]-3-hexenyl]amino]ethyl]-1,2-benzenediol dihydrochloride (7) were obtained from the Departments of Medicinal Chemistry and Chemical Process Research and Development, Fisons

plc, Pharmaceutical Division, Loughborough, Leics, UK. 2-Phenyl-[1-^{14}C]-ethylamine was obtained from ICI Physics and Radioisotope Services, Labelled Compounds Section, Billingham, Cleveland, UK. The tritiation was performed by Amersham International plc, Amersham, Bucks, UK via the TR8 tritium labelling service. Deuterium gas (99.5 + % atom ^{2}H) was obtained from MSD Isotopes, distributed by Cambrian Gases, Croydon, Surrey, UK. Palladium on carbon (Pd content 5%) and rhodium on carbon (Rh content 5%) catalysts were both obtained from the Aldrich Chemical Co Ltd, Gillingham, Dorset, UK. All other chemicals were of reagent quality.

N-[2-(3,4-Dimethoxyphenyl)ethyl]-N'-(2-phenyl-[1-^{14}C]ethyl)hexanediamide

6-[[2-(3,4-Dimethoxyphenyl)ethyl]amino]-6-oxohexanoic acid (292 mg, 0.944 mmol) and triethylamine (145 mm^{3}, 0.944 mmol) were dissolved in dichloromethane (20 cm^{3}), with the aid of stirring, and cooled to 0°. Ethyl carbonochloridate (90.2 mm^{3}, 0.944 mmol) was added and stirring continued for a further 20 minutes at 0°. 2-Phenyl-[1-^{14}C]ethylamine (67.2 mg, 0.556 mmol; 925 MBq) and 2-phenylethylamine (48.8 mm^{3}, 0.389 mmol) dissolved in dichloromethane (5 cm^{3}) were added to the stirred solution over a 20 minute period; stirring was continued for a further one hour at 0°. The precipitated diamide was filtered, washed with ice-cold ethanol (ca 10 cm^{3}) and dried under vacuum to yield N-[2-(3,4-dimethoxyphenyl)ethyl]-N'-(2-phenyl-[1-^{14}C]ethyl)hexanediamide (285 mg; 2.46 MBq mg^{-1}, 701 MBq).

The synthesis was repeated in exactly the same way, using a second batch of 2-phenyl-[1-^{14}C]ethylamine (925 MBq) to yield a further batch of the ^{14}C-labelled hexanediamide (292.3 mg; 2.53 MBq mg^{-1}, 739 MBq). The two batches were combined for use in the next step of the synthesis.

N-[2-(3,4-Dimethoxyphenyl)ethyl]-N'-(2-phenyl-[1-^{14}C]ethyl)-1,6-hexanediamine dihydrochloride

N-[2-(3,4-Dimethoxyphenyl)ethyl]-N'-(2-phenyl-[1-^{14}C]ethyl)-hexanediamide (577 mg, 1.40 mmol, 1.44 GBq) was placed in a dry three-necked round-bottomed flask equipped with two condensers. The flask was purged

with a stream of dry nitrogen, prior to the addition of dry tetrahydrofuran (60 cm^3), followed by a solution of borane in tetrahydrofuran (1 mol dm^{-3}, 25.2 cm^3, 25.2 mmol). The solution was brought to reflux, under a constant, slow stream of dry nitrogen and reflux was continued for 24 hours. The reaction mixture was allowed to cool and methanol added to destroy the excess borane. The solvent was removed under reduced pressure to leave an oil which was dissolved in dichloromethane (200 cm^3) and washed with water (200 cm^3). The dichloromethane layer was separated, dried over anhydrous magnesium sulphate, filtered and the solvent removed under reduced pressure. The resulting oil was dissolved in methanol (50 cm^3) containing concentrated hydrochloric acid (0.5 cm^3) and refluxed for 2.5 hours. After cooling, the volume of the solution was reduced by a half using a rotary evaporator and the hexanediamine dihydrochloride salt allowed to crystallise overnight at 4°. The resulting white crystals were filtered and dried under vacuum at 75° to yield N-[2-(3,4-dimethoxyphenyl)ethyl]-N'-(2-phenyl-[1-^{14}C]ethyl)-1,6-hexanediamine dihydrochloride (416.6 mg; 2.48 MBq mg^{-1}, 1.036 MBq).

4-[2-[[6-[(2-Phenyl-[1-^{14}C]ethyl)amino]hexyl]amino]ethyl]-1,2-benzenediol dihydrochloride; [^{14}C]dopexamine hydrochloride (4)

N-[2-(3,4-Dimethoxyphenyl)ethyl]-N'-(2-phenyl-[1-^{14}C]ethyl)-1,6-hexanediamine dihydrochloride (300 mg, 0.656 mmol; 744 MBq) was divided equally, between two 5 cm^3 thick-walled screw-topped vials and concentrated hydrochloric acid (2.6 cm^3; previously degassed with nitrogen) was added to each vial. Before sealing the vials, the head space was purged with nitrogen; the vials were then heated at 105° for 24 hours. The vials were allowed to cool to room temperature and then further cooled in an ice-bath. To effect crystallisation, a very small quantity of crystalline unlabelled dopexamine hydrochloride was added to each vial, and the vials allowed to stand at 4° for a further two hours. The crystalline [^{14}C]dopexamine hydrochloride was filtered and washed with ice-cold methanol (*ca* 2 cm^3). The material was further purified by recrystallisation from methanol. The whole of [^{14}C]dopexamine hydrochloride (236 mg) was dissolved in hot

methanol (ca 6 cm^3), treated with charcoal and filtered whilst hot; after seeding with a very small quantity of unlabelled crystalline dopexamine hydrochloride the solution was allowed to stand overnight at 4°. The ^{14}C-labelled material was filtered, washed with ice-cold methanol (ca 2 cm^3) and dried under vacuum to yield [^{14}C]dopexamine hydrochloride (189 mg; 2.51 MBq mg^{-1}, 474 MBq). The radiochemical purity was assessed by hplc/fraction collecting/liquid scintillation counting and found to be 97.9%.

4-[2-[[6-[(2-Phenylethyl)amino]hexyl]amino]ethyl]-1,2-[3,5,6-^2H]benzenediol dihydrochloride (3) and 4-[2-[[6-[(2-phenylethyl)amino]hexyl]amino]ethyl]-1,2-[(n)3,5,6-^3H)benzenediol dihydrochloride (2)

Dopexamine hydrochloride (1, 100 mg) was dissolved in a solution of deuterium chloride in deuterium oxide (20% weight per volume ^2HCl, 1.5 cm^3) and the solution degassed with helium. The solution was heated under nitrogen at 116° for eighteen hours, cooled, and the solvent removed under reduced pressure. The resulting solid was dissolved in water (5 cm^3) and the water removed under reduced pressure. Crystallisation from methanol yielded 3 (56 mg); λmax (EtOH) 282 nm; νmax (KBr) 3300, 2950, 2780, 2480, 1590, 1460, 770, 710 cm^{-1}; ^1H-nmr (δ,360 MHz, ^2H_2O) 1.34(b,4H), 1.64(b,4H), 2.90 to 3.40 (multiplets, 12H), 6.70 to 6.90 (residual traces of catechol protons), 7.40 (m,5H) ppm; ^2H-nmr (δ,55 MHz, H_2O) 6.85 (bs) ppm, no other resonances; m/z (EI) 360, 359, 358, 357, 356 (unlabelled = 356), 270, 269, 268, 267, 266, 265 (unlabelled = 265), 234, 233 (unlabelled = 233), 113, 112 (unlabelled = 112); m/z (FAB) 360 (unlabelled = 357), 233 (unlabelled = 233), 112 (unlabelled = 112), 105 (unlabelled = 105).

A similar reaction was performed by Amersham International plc, in which dopexamine hydrochloride (1, 10 mg) was heated with hydrochloric acid (36% weight per volume, 9 mm^3), DMF (9 mm^3), and tritium oxide (> 90 atom % ^3H, 925 GBq) at 120° for eighteen hours. After removal of volatile tritium by freeze-drying, 10% of the crude tritiated dopexamine was purified, in a single injection, by high performance liquid chromatography using a

250 x 8 mm Spherisorb 5ODS column. The chromatographic mobile phase was prepared as follows: sodium 1-heptanesulphonate (16.2 g) was dissolved in orthophosphoric acid solution (5% by volume, 840 cm^3) and diprop-2-ylamine (90 cm^3) added slowly. After cooling, the solution was made up to 1 dm^3 with water. This aqueous solution was mixed with methanol to yield a final methanol content of 33% by volume. The retention volume of dopexamine using these chromatographic conditions and a flow rate of 3 cm^3 min^{-1} was 81 cm^3. The purification yielded tritiated dopexamine (2, 0.41 mg, 1.2 TBq $mmol^{-1}$) with a radiochemical purity > 98%.

4-[2-[[6-[(2-[4-^{2}H]Phenylethyl)amino]hexyl]amino]ethyl]-1,2-benzenediol dihydrochloride (6)

The chloro-precursor (5, 250 mg) was dissolved in [O-^{2}H]methanol (4 cm^3) and the solvent removed under reduced pressure. After a further such cycle the labile protons were completely exchanged for ^{2}H. After dissolution of the residue in [O-^{2}H]methanol (4 cm^3) and triethylamine (250 mm^3) the solution was hydrogenated with deuterium gas over a 5% palladium on carbon catalyst until gas uptake ceased. The suspension was filtered free of catalyst, evaporated to dryness, dissolved in methanol (15 cm^3) and hydrochloric acid (0.5 cm^3, 2 mol dm^{-3}) and evaporated under reduced pressure. Recrystallisation from hot methanol (3 cm^3) yielded 6 (125 mg); λmax (EtOH) 282 nm; νmax (KBr) 3300, 2950, 2780, 2490, 1595, 1505, 1460, 640 cm^{-1}; ^{1}H-nmr (δ,360 MHz; $^{2}H_2O$) 1.34 (b, 4H), 1.65 (b,4H), 2.90-3.40 (multiplets, 12H), 6.75 (dd,1H), 6.85 (s, 1H), 6.90 (d,1H), 7.37 (AA'BB' system, 4H) ppm; ^{2}H-nmr (δ, 55 MHz, H_2O) 7.40 (bs) ppm, no other resonances, m/z (FAB) 358 (unlabelled = 357), 234 (unlabelled = 233), 112 (unlabelled = 112), 106 (unlabelled = 105).

4-[2-[[6-[(2-Phenylethyl)amino]-[2,3,4,5-^{2}H]hexyl]amino]ethyl]-1,2-benzenediol dihydrochloride (8)

The unsaturated precursor 7 (40 mg) was dissolved in [O-^{2}H]methanol (4 cm^3) and was stirred with 5% rhodium on carbon (20 mg) under a deuterium

atmosphere until gas uptake was complete (3 hr). The suspension was filtered, reduced to dryness and the residue boiled with methanol (4 cm^3). Crystallisation from hot methanol yielded 8 (28 mg); λmax (EtOH) 282 nm; νmax (KBr) 3300, 2950, 2800, 2460, 1605, 1512, 1440, 770, 705 cm^{-1}; ^{1}H-nmr (δ,360 MHz, ^2H_2O) 1.35 (ca 1.4H), 1.65 (ca 3H), 2.90-3.40 (multiplets, 12H), 6.75 (dd, 1H), 6.85 (s, 1H), 6.90 (d, 1H), 7.40 (m, 5H) ppm; ^{2}H-nmr (δ,55 MHz, H_2O) 1.35 (bs), 1.63 (bs, ratio 5:1 respectively) ppm, no other resonances; m/z (FAB) 363, 362, 361, 360, 359 (max), 358, 357 (unlabelled = 357), 137 (unlabelled = 137), 105 (unlabelled = 105).

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