SYNTHESIS OF ^{3}H-LORCAINIDE MONOHYDROCHLORIDE (R 15 889)

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SUMMARY

Lorcainide monohydrochloride, N-(4-chlorophenyl)-N-/1-(1-methylethyl)-4-piperidinyl/benzeneacetamide monohydrochloride, is a new orally active antiarrhythmic drug.

Incorporation of tritium was achieved by reduction of 4-chloro-N-/1-(1-methylethyl)-4-piperidinylidene/benzenamine with tritiated sodium borohydride to N-(4-chlorophenyl-1-(1-methylethyl)-4-piperidinamine. This compound was converted in situ into the amide derivative.

The radioactive yield of the last two synthesis steps was 82.7 % spread over two fractions with specific activities of 1.1 Ci/mmol and 0.046 Ci/mmol. The labelled compounds were radiochemically pure according to thin-layer chromatography in three solvent systems, and high-performance liquid chromatography. The radiochemical stability of lorcainide monohydrochloride investigated in both acidic and alkaline media for 1 hour at 60°C was found to be excellent.

Key words: ^{3}H-lorcainide monohydrochloride, sodium borohydride-^{3}H.

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INTRODUCTION

Lorcainide monohydrochloride (Janssen Pharmaceutica, R 15 889) is a new antiarrhythmic drug (1); its chemical structure is shown in Figure 1.

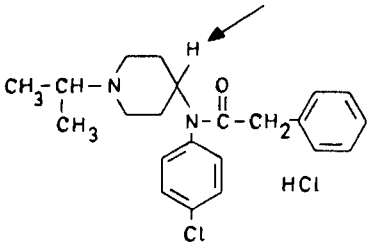

Fig. 1. Chemical structure of lorcainide monohydrochloride, N-(4-chlorophenyl)-N-[1-(1-methylethyl)-4-piperidinyl]benzeneacetamide monohydrochloride. The position of the tritium-label is denoted by an arrow.

This compound was selected because of its potent and long-lasting action, its good oral absorption and bioavailability on chronic administration and its relatively low toxicity (1-3).

Biotransformation and pharmacokinetic studies are facilitated by the use of radio-labelled drugs. For lorcainide, a drug which can be metabolized by various routes (e.g. N-dealkylation, amide hydrolysis and aromatic hydroxylation), it was obvious that the label must be placed in a position which was uninvolved in, or only minimally subject to, metabolic attack. We therefore selected the 4-position of the piperidine ring. The introduction of a tritium label at this position seemed to be the most convenient procedure since the desired product could be obtained by a two-step reaction, utilizing tritiated sodium borohydride,

which is relatively cheap, easily treatable and available at high specific activities.

The reaction scheme for the synthesis of lorcainide-^{3}H is shown in Figure 2. Using tritiated sodium borohydride, 4-chloro-N-/1-(1-methylethyl)-4-piperidinylidene/benzenamine (II) was reduced to N-(4-chlorophenyl)-1-(1-methylethyl)-4-piperidinamine (III), according to the method of Horii et al. (4). Lorcainide-^{3}H was obtained after acetylation of (III) with benzeneacetyl chloride, using the slightly adapted method of Delaby et al. (5).

^{3}H-lorcainide could be isolated as its monohydrochloride salt, which has been used in various pharmacokinetic and biotransformation studies, to be published elsewhere (6, 7 and 8).

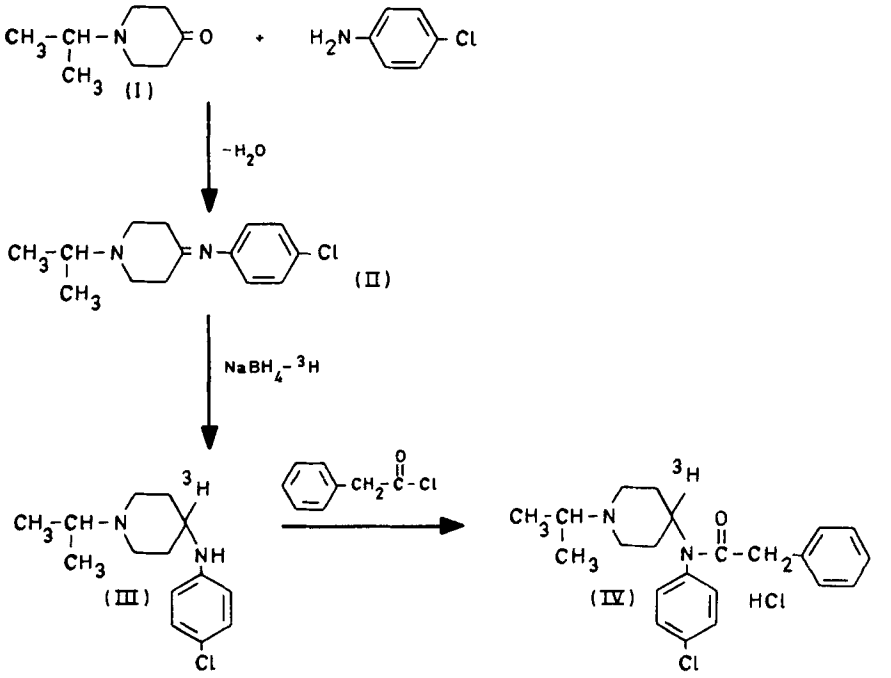

Fig. 2. Reaction scheme for the synthesis of ^{3}H-lorcainide monohydrochloride (IV).

EXPERIMENTAL

ANALYTICAL PROCEDURE

Radioactivity measurements

The specific activity of labelled lorcainide monohydrochloride was measured by liquid scintillation spectrometry (Packard Tri-Carb 3380, equipped with an automatic activity analyzer 544). The radioactivity of the samples was counted in 10 ml of a scintillator solution, containing 5 g of PPO and 0.1 g of dimethyl-POPOP in a 1-litre mixture of toluene: 2-propanol (8:2, v/v).

Determination of the radiochemical purity

A. Thin-layer chromatography (TLC)

The labelled compound, dissolved in methanol (1 mg/ml) was chromatographed on glass plates (20 x 20 cm) precoated with 0.2 mm of silica gel 60F254 (Merck AG, Darmstadt, Germany) using three solvent systems: - chloroform:methanol (90:10, v/v); - chloroform:methanol: ammonia (85:15:1, v/v); - acetate buffer pH 4.8:chloroform:methanol: ethyl acetate (5:23:18:54, v/v). The radioactivity on the plates was scanned with a Berthold radiochromatogram scanner (LB 2723) and spots were visualized by viewing under UV-light at 254 nm.

B. High performance liquid chromatography (HPLC)

The apparatus consisted of two Waters Associates model 6000 A pumps with a Waters model 660 solvent programmer for gradient elution. Stainless steel columns (4.6 mm I.D. x 30 cm) were packed with

Lichrosorb RP-8 (5 μm) bonded phase. The samples (about 0.45 μCi of each fraction) were injected using a Waters model U6K universal injector and eluted with a linear gradient running from water-0.2 % N-(1-methylethyl)-2-propanamine to acetonitrile-0.2 % N-(1-methylethyl)-2-propanamine over a 30-minute period (flow rate: 1 ml/min). On-line radioactivity detection of the HPLC-eluates was carried out with a Berthold Radioactivity Monitor LB 5025 HP system, using a flow-through cell of 200 μl. The eluate was mixed with Pico-fluor TM 30 (Packard) (used as a scintillation cocktail) in an LKB Ultrograd mixing unit. The normalized areas of the radioactivity peaks were computed by a SP 4000 system (Spectra-Physics).

Determination of the stability of ^{3}H-lorcainide in aqueous solutions

In test tubes, 0.050-ml aliquots of the methanol solution of labelled lorcainide monohydrochloride (1 mg/ml) were mixed with equal volumes of aqueous solutions of either hydrochloric acid (0.01, 0.1 and 1 N) or sodium hydroxide (0.01, 0.1 and 1 N) respectively. The six mixtures were heated at 60°C for 60 minutes, diluted with methanol, spotted on a silica gel plate and eluted with chloroform: methanol (90:10) as a moving liquid.

REAGENTS

$NaBH_4$-^{3}H was purchased from I.R.E. (Fleurus, Belgium), 4-chlorobenzenamine and benzeneacetyl chloride were obtained from Aldrich (Beerse, Belgium). All other chemicals were used as purchased and were reagent grade where available.

SYNTHESIS

4-chloro-N-(1-methylethyl)-4-piperidinylidene/benzenamine (II)

98.7 g (0.7 mol) of 1-(1-methylethyl)-4-piperidinone (I) and 90.75 g (0.75 mol) of 4-chlorobenzenamine in 350 ml of toluene were refluxed with a few drops of glacial acetic acid until 12.7 ml (0.7 mol) of reaction water was collected in a water separator. The toluene was distilled off and the residue was fractionated yielding 140 g (80 %) of (II). Bp 13 mm/145-147°C.

N-(4-chlorophenyl)-1-(1-methylethyl)-4-piperidinamine-4-^{3}H (III)

In a one-necked flask of 10 ml, 2.48 mg (0.066 mmol) of tritiated sodium borohydride (corresponding to 210 mCi; specific activity 3.3 Ci/mmol) dissolved in 3.5 ml of methanol was mixed with 50 mg (0.2 mmol) of (II).

After stirring the solution at room temperature for 6 hours, the reduction of (II) was completed by addition of 4.96 mg (0.13 mmol) of non-radioactive sodium borohydride. The reaction mixture was stirred for an additional 15 h. The solvent was evaporated at 45°C under a dust-free stream of nitrogen. The solid residue was dissolved in 2 ml of 0.1 N hydrochloric acid and after alkalinization with 25 % ammonia, extracted repeatedly with small volumes of chloroform. The combined organic layers were filtered over a plug of cotton wool and evaporated under a gentle stream of nitrogen. As indicated by TLC, the residue contained only (III) and it was used in the next reaction step.

N-(4-chlorophenyl)-N-[1-(1-methylethyl)-4-piperidinyl-4t]benzeneacetamide monohydrochloride (IV)

A solution of 52.6 mg [0.34 mmol] of benzeneacetyl chloride in 1 ml of 4-methyl-2-pentanone was added dropwise to a solution of (III) in 2 ml of 4-methyl-2-pentanone under stirring at room temperature. The mixture was refluxed for 2 hours. After cooling to room temperature, the precipitate was collected by filtration and washed with 4-methyl-2-pentanone yielding 57 mg ^{3}H-lorcainide monohydrochloride corresponding to 153.9 mCi (radioactive yield 73.3 %).

The mother liquor was fortified with additional non-radioactive lorcainide monohydrochloride [203 mg; 0.5 mmol]. The solvent was evaporated and crystallization from 2-propanol yielded 173 mg corresponding to 19.7 mCi (radioactive yield 9.4 %).

The overall yield of ^{3}H-lorcainide monohydrochloride (IV) was 173.6 mCi or 82.7 % starting from sodium borohydride-^{3}H.

The specific activity was found to be 2.7 mCi/mg or 1.1 Ci/mmol for the "first fraction" and 113.7 μCi/mg or 46.3 mCi/mmol for the "second fraction".

Both fractions were radiochemically pure as tested by thin-layer chromatography (TLC) and high-performance liquid chromatography (HPLC).

REFERENCES

1. Carmeliet E., Janssen P.A.J., Marsboom R., Van Nueten J.M. and Xhonneux R. - Arch. Int. Pharmacodyn. 231: 104 (1978)

2. Kesteloot H. and Stroobandt R. - Arch. Int. Pharmacodyn. 230: 225 (1977)

3. Klotz U., Müller-Seydlitz P. and Heimburg P. - Clin. Pharmacokin. 3: 407 (1978)

4. Horii Z., Sakai T. and Inoi T. - J. Pharm. Soc. Jap. 75: 1161-2 (1955)

5. Delaby R., Reynaud P. and Lilly F. - Bull. Soc. Chim. France, 2067 (1961)

6. Meuldermans W., Hurkmans R., Swijsen E., Hendrickx J., Lauwers W. and Heykants J. - in preparation (1981)

7. Michiels M., Woestenborghs R., Xhonneux R., Marsboom R. and Heykants J. - in preparation (1981)

8. Heykants J., Michiels M., Meuldermans W., Woestenborghs R. and Xhonneux R. - in preparation (1981)