SYNTHESIS OF ^{3}H- AND ^{14}C-LABELLED ASTEMIZOLE (R 43 512)

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SUMMARY

Astemizole, a new antihistamine drug, was specifically labelled with tritium and ^{14}C at three distinct positions. Tritium was introduced either in the 4-methoxyphenyl (II) or in the 4-fluorophenylmethyl (IV) moiety by catalytic dehalogenation of the corresponding halogenated analogues with tritium gas, yielding ^{3}H-astemizole with specific activities of 28.7 and 5.0 Ci/mmol respectively. Starting from [^{14}C]urea, [^{14}C]astemizole was synthesized with the label being placed in the 2-position of the benzimidazole moiety. The radioactive yield of the three-step synthesis was 22.4 %, spread over three fractions with specific activities of 4.5 mCi/mmol, 1.7 mCi/mmol and 0.4 mCi/mmol. The labelled compounds were radiochemically pure according to thin-layer (TLC) and high-performance liquid chromatography (HPLC).

Key Words: [^{3}H]astemizole, [^{14}C]astemizole, antihistamine drug, catalytic dehalogenation.

INTRODUCTION

Astemizole (Janssen Pharmaceutica, R 43 512) is a new antihistamine drug (1); its chemical structure is shown in Figure 1.

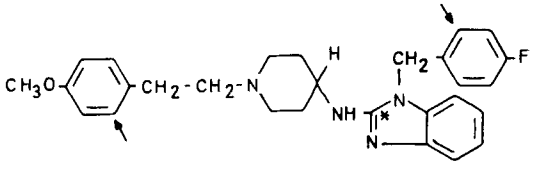

Fig. 1. Chemical structure of astemizole, 1-[(4-fluorophenyl)methyl] -N-[1-[2-(4-methoxyphenyl)ethyl]-4-piperidinyl]-1H-benzimidazol-2-amine. The positions of the tritium-label are denoted by an arrow, and that of the carbon-14-label is indicated by an asterisk.

This compound was selected because of its potent and long lasting action, and its lack of sedative and anticholinergic effects.

In order to fully investigate the metabolism of astemizole, three different positions in the molecule were selected for labelling either

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with tritium or with ^{14}C. Out of several possibilities, the ortho-positions of both phenyl groups were chosen for labelling with tritium by catalytic dehalogenation of the corresponding halogenated analogues of astemizole (Figure 2). The suitable precursors (I) and (III) could easily be prepared following the usual route for the preparation of astemizole (2).

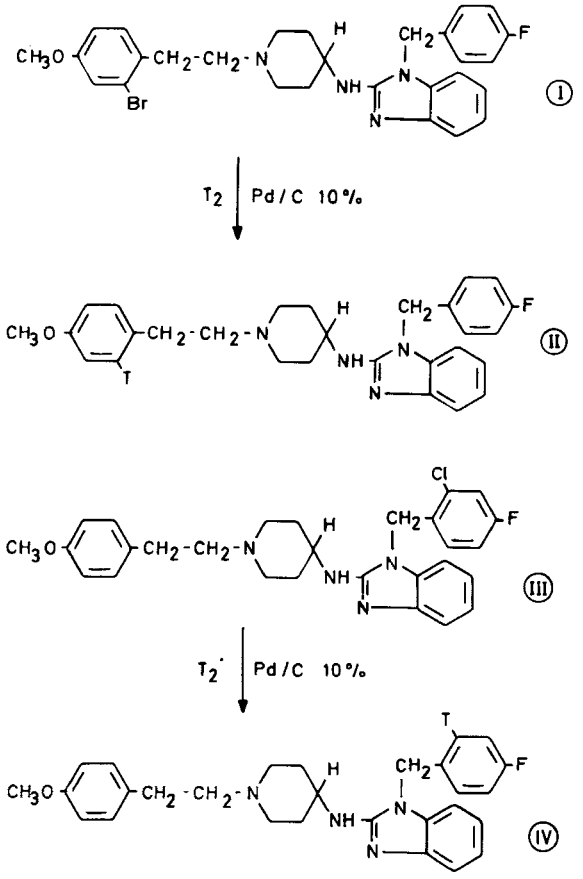

Fig. 2. Reaction scheme for the synthesis of [^{3}H]astemizole.

Considering the possible metabolic pathways of astemizole, ^{14}C was incorporated at the 2-position of the benzimidazole moiety. The usual route for the preparation of astemizole could not be adapted to the miniscale synthesis due to complicated isolation and purification procedures. Moreover using [^{14}C]urea, [^{14}C]astemizole could be obtained in a more efficient and economical three-step synthesis (Figure 3).

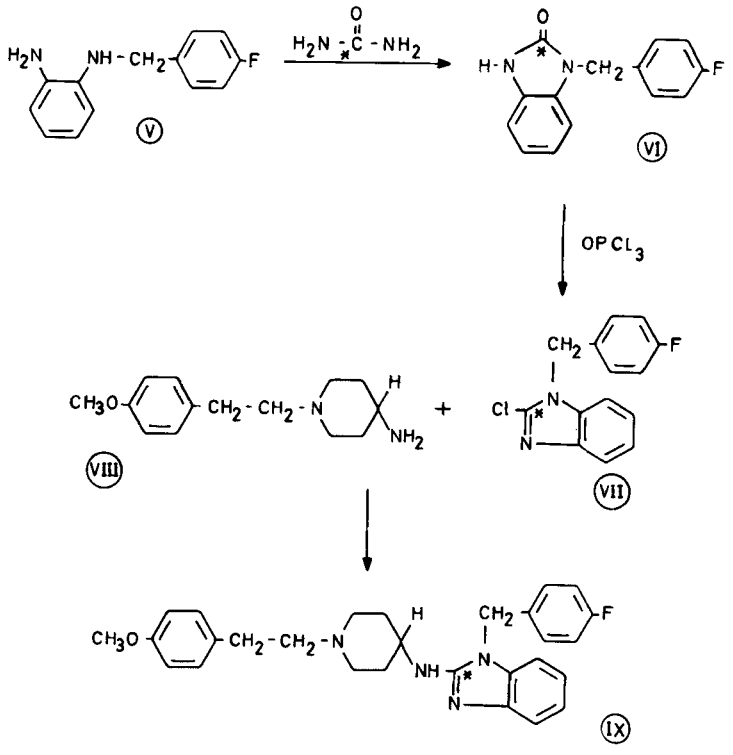

Fig. 3. Reaction scheme for the synthesis of [^{14}C]astemizole.

Thus cyclization of N^{1}-[(4-fluorophenyl)methyl]-1,2-benzenediamine (V) with [^{14}C]urea, performed according to the method of Clark et al (3), resulted in ^{14}C-labelled 1-[(4-fluorophenyl)methyl]-1,3-dihydro-2H-benzimidazol-2-one (VI), which was chlorinated with phosphorus oxychloride giving (VII), 2-chloro-1-[(4-fluorophenyl)methyl]-1H-benzimidazole, using the slightly adapted method of Rieci (4). Reaction of (VII) with 1-[2-(4-methoxyphenyl)ethyl]-4-piperidinamine resulted after chromatographic purification in [^{14}C]astemizole. Whereas ^{14}C-labelled astemizole was mainly used to study the excretion and biotransformation in animals and man, ^{3}H-labelled astemizole was also applied in receptor binding studies (5) and in the radioimmunologic determination of astemizole and metabolites in biological samples.

EXPERIMENTAL

ANALYTICAL PROCEDURES

Radioactivity measurements

The specific activity of the labelled compounds was measured by liquid scintillation spectrometry (Packard Tri-Carb 3380, equipped with a Wang 2200 PCS II, 4/2). The radioactivity of the samples was counted in 10 ml of Plasmasol (Packard 185) as a scintillation cocktail.

Determination of the radiochemical purity

A. Thin-layer chromatography (TLC)

An appropriate amount of the labelled compound was chromatographed on glass plates (20 x 10 cm) precoated with 0.2 mm of silica gel 60F 254 (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 U.V.-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 ID 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 100 % of 0.1 M ammonium acetate to 100 % of 1 M ammonium acetate:methanol:acetonitrile (10:45:45; v/v) 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 a scintillation cocktail (Pico-fluor ™30, Packard) in an LKB Ultrograd mixing unit. The normalized areas of the radioactivity peaks were computed by a SP 4000 system (Spectra-Physics).

SYNTHESIS

1. [^{3}H]Astemizole, labelled in the 4-methoxyphenyl moiety (II)

To a solution of 100 mg (0.186 mmole) of (I) in tetrahydrofuran (5 ml) were successively added 100 mg of 10 % Pd/C (catalyst) and 0.2 ml triethylamine (HBr acceptor). To avoid possible debenzylation, 0.015 ml of a 4 % thiophene solution in tetrahydrofuran was also added to the mixture. Tritiation [I.R.E., Fleurus, Belgium] of compound (I) was performed at room temperature for 15 hours, using approximately 30 Ci of tritium gas. The excess of tritium was adsorbed on active charcoal (Merck No 2184). The catalyst was removed by Millipore filtration and the filtrate was lyophilized twice with methanol to remove labile tritium. The residue was purified by preparative thin-layer chromatography on a silica gel plate (60 F 254, Merck 20 x 20 x 0.2 cm) using chloroform: ethyl acetate: methanol (4:4:2, v/v) as developing solvent. The radioactive zone corresponding to authentic astemizole was scraped off and eluted with ethanol yielding 2.17 Ci pure [^{3}H]astemizole (II) with a specific activity of 28.7 Ci/mmol (radiochemical yield 40 %).

2. [^{3}H]Astemizole, labelled in the (4-fluorophenyl)methyl moiety (IV)

To a solution of 100 mg (0.203 mmole) of (III) in tetrahydrofuran (5 ml) were added 100 mg of 10 % Pd/C (catalyst) and 0.2 g of calcium oxide (HCl acceptor). Tritiation [I.R.E., Fleurus, Belgium] of compound (III) was performed at room temperature for 15 hours, using approximately 30 Ci of tritium gas. After removal of the excess and labile tritium and the catalyst as described above, the residue was dissolved in 0.5 ml of chloroform. This solution was purified on a Lichrosorb RP-18 (10 µm) bonded phase (Chrompack 25 cm x 0.9 cm) column. The sample was eluted with a mixture of acetonitrile: water: diisopropylamine (60:40:0.1; v/v) at a flow rate of 8 ml/min. The elution of the products was visualized by UV-absorption at 280 nm (Perkin Elmer LC-75 variable U.V detector) and yielded 430 mCi pure [^{3}H]astemizole with a specific activity of 5.0 Ci/mmol (radiochemical yield 7 %).

3. [^{14}C]Astemizole(IX)

1-[(4-fluorophenyl)methyl]-1,3-dihydro-2H-[2-^{14}C]benzimidazole-2-one (VI)

In a two-necked flask of 10 ml, 60.2 mg (1 mmole) of [^{14}C]urea (spec. act. 5.14 mCi/mmol) [AMERSHAM INTERNATIONAL plc, Amersham (U.K.)] and 212 mg (1 mmole) of (V) were suspended in 1.5 ml of xylene and heated in an oil-bath at 140° for 24 hours. The solvent was evaporated and the residue was used in the next reaction step without further purification.

2-chloro-1-[(4-fluorophenyl)methyl]-1H-[2-^{14}C]benzimidazole (VII)

The flask was equipped with a gas-inlet and with a condenser connected to a drying tube. 5 ml of phosphorus oxychloride was added to the residue (VI) and the mixture was heated in an oil-bath at 140°C for 8 hours, hydrogen chloride being passed during the last 7 hours. The reaction mixture was concentrated and the residue was treated with 5 ml

of ice-water and 1.5 ml of ammonium hydroxide (25 %). The resulting alkaline mixture was extracted repeatedly with chloroform (1 x 10 ml, 3 x 5 ml). The combined organic layers were dried over molecular sieves 4 Å (Merck art 5708) and evaporated to dryness. According to TLC (chloroform:hexane:methanol; 55:35:10;v/v), the residue contained mainly (VII), and was used in the next reaction step without further purification.

1-[(4-fluorophenyl)methyl]-N-[1-[2-(4-methoxyphenyl)ethyl]-4-piperidinyl]-1H-[2-^{14}C]benzimidazol-2-amine (IX)

The residue containing (VII) was mixed with 703 mg (3 mmole) of (VIII) and heated in an oil-bath at 130°C for 41 hours. After cooling to room temperature, 10 ml of water was added and the mixture was extracted repeatedly with chloroform (1 x 10 ml, 3 x 5 ml). The combined extracts were dried over molecular sieves 4 Å (Merck, art 5708) and evaporated *in vacuo*. The residue was purified by silica gel column chromatography, followed by preparative thin-layer chromatography on a silica gel plate, (Merck 60F 254, 20 x 20 x 0.2 cm). In both cases, chloroform:methanol (90:10; v/v) was used as the eluting solvent. The radioactive zone corresponding to authentic astemizole was scraped off and eluted with methanol. The solvent was evaporated and crystallization from 2-propanol yielded a first fraction of [^{14}C]astemizole (546 µCi; radioactive yield 10.9 %). Fortification of the mother liquor with two additional 100-mg amounts of unlabelled astemizole resulted in second and third fractions, containing respectively 467 µCi (9.3 %) and 107 µCi (2.1 %) of [^{14}C]astemizole. The overall yield of [^{14}C]astemizole (IX) was 1.12 mCi or 22.4 % starting from [^{14}C]urea. The specific activities of the three fractions were 4.55, 1.71 and 0.43 mCi/mmol respectively and were shown to be radiochemically pure by thin-layer chromatography and by high-performance liquid chromatography.

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