Synthesis of Diethyl 4-(benzothiazol-[2-^{14}C]-2-yl) benzylphosphonate ([^{14}C]KB-944)

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SUMMARY

Diethyl 4-(benzothiazol-[2-^{14}C]-2-yl)benzylphosphonate ([^{14}C]KB-944), a new calcium antagonist, was prepared from labelled carbon dioxide.

The synthetic intermediate, [^{14}C]p-toluic acid, obtained by the Grignard reaction was condensed with 2-aminothiophenol, brominated with N-bromosuccinimide, and followed by the Arbuzov reaction.

[^{14}C]KB-944, having the specific activity 52.7 mCi/mmol, was obtained in 36% overall yield from [^{14}C]barium carbonate and its radiochemical purity was 99.3% in reverse isotope dilution analysis.

Key words: Calcium antagonist, Diethyl 4-(benzothiazol-2-yl) benzylphosphonate, Carbon-14, Arbuzov reaction

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INTRODUCTION

Diethyl 4-(benzothiazol-2-yl)benzylphosphonate (KB-944) is a newly synthesized calcium antagonist, and is expected to exert a long acting coronary vasodilatory effect[1] as well as hypotensive effect[2].

It is important to discuss the pharmacological effects and toxicities of the drugs correlating the pharmacokinetical parameters in them, and the utilization of radio-labelled compounds offers us useful data in understanding their pharmacokinetics.

It is generally accepted that the compound whose carbon atoms on the skelton in the chemical structure are replaced and labelled as ^{14}C does not easily expose the disconnection in their metabolism and isotope effect in comparison with ^{3}H labelled one. This paper deals with the synthesis of KB-944 labelled with ^{14}C at position 2 of the benzothiazole ring which is asterisked in Fig. 1.

RESULTS AND DISCUSSION

The synthetic pathway of [^{14}C]KB-944 (1) is shown in Fig. 1. [Carboxy-^{14}C]p-toluic acid (3) was synthesized by reacting [^{14}C]carbon dioxide obtained from [^{14}C]barium carbonate (2) with p-tolylmagnesium bromide. The reactions which directly introduce the carboxy group into a benzene ring with various

substituents have been reported relatively often. Thus, we referred to the method[3], which was used for the synthesis of mesitoic acid for the synthesis of 3.

When non-labelled barium carbonate was used, the yield of p-toluic acid based on barium carbonate increased from 70 to 75% by using from 2.5 to 4.0 molar equivalents of the Grignard reagent to barium carbonate. In the case of synthesis of the labelled compound, we used 4.0 molar equivalents of the Grignard reagent to 2, and 3 was obtained in 71% yield.

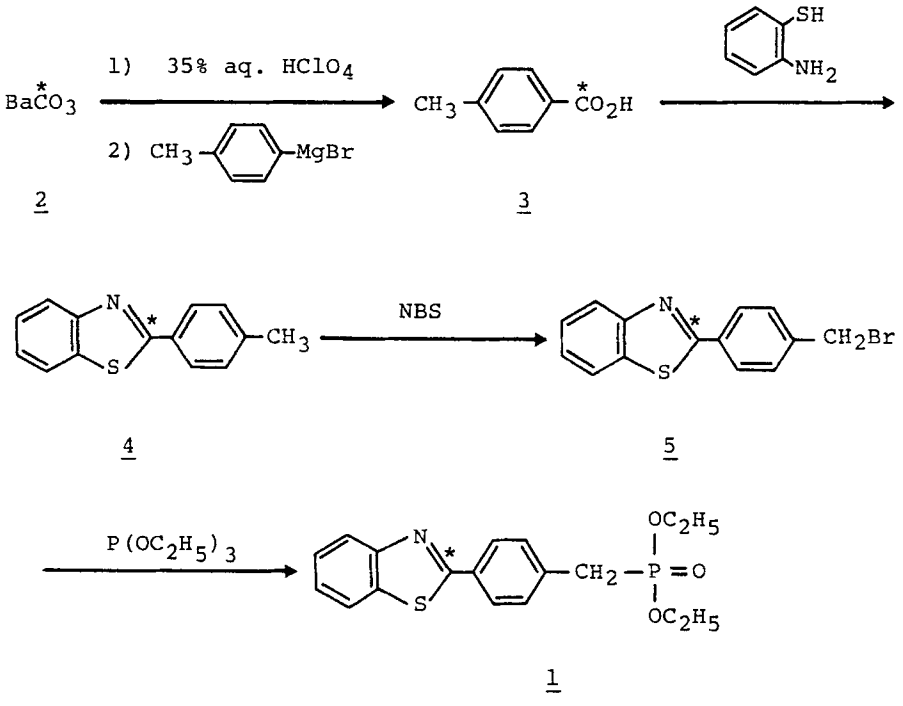

Fig. 1 Scheme for the synthesis of [^{14}C]KB-944

Non-labelled 2-(p-tolyl)benzothiazole (4) is able to be obtained from p-toluoyl chloride and o-aminothiophenol in 82% yield[4]. However, in this method [^{14}C]p-toluoyl chloride has to be synthesized from the acid 3. In our method, the acid 3 was direct condensed with o-aminothiophenol in the presence of polyphosphoric acid and 4 was obtained in 95% yield.

Benzothiazole 4 was brominated with N-bromosuccinimide (NBS)[5] to produce [^{14}C]2-(4-bromomethylphenyl)benzothiazole (5). In a preliminary experiment, non-labelled 5 was obtained from 4 in a yield of about 60%. In the case of synthesis using labelled compound 4, 1 was synthesized without isolation of 5, by the arbuzov reaction with triethyl phosphite in 54% yield based on 4 .

The overall yield from 2 to 1 was 36%. The radiochemical purity of 1 was 99.3% in reverse isotope dilution analysis, and the specific radioactivity was a 52.7 mCi/mmole.

EXPERIMENTAL

Materials and Methods

[^{14}C]Barium carbonate was obtained from New England Nuclear and the reagents and solvents of reagent grade from Wako Pure Chemical Industries, Ltd. were used without purification.

The radioactivity was determined by using a Packard Tri-Carb 3385 scintillation counter after adding scintillator Econofluor (NEN) or Dotite scintisol EX-H (Wako Pure Chemical Industries, Ltd.) to the samples.

Column chromatography was performed using a column which was filled with the mixture of silica gel for column chromatography (Wako gel C-200) and 10% of silica gel containing fluorescent indicator (Wako gel B-5F).

Thin layer chromatography (TLC) was made with plates from Merck (Kieselgel 60 F_{254}, 20 x 20 cm, 0.25 mm) and the spots were measured with a Packard Model 7201 Radiochromatogram scanner or detected by UV (250 - 400 nm) radiation.

[^{14}C]p-Toluic Acid (3)

To a solution of p-bromotoluene (496.4 mg, 2.90 mmol) in anhydrous ether (3.9 ml), magnesium turnings (70.5 mg, 2.90 mmol) and a small amount of iodide were added. The reaction mixture was refluxed for 5 min and additionally stirred for 1.5 h at room temperature to obtain ether solution of p-tolylmagnesium bromide.

[^{14}C]carbon dioxide was generated by adding 35% aqueous perchloric acid (ca. 1 ml) to [^{14}C]barium carbonate (2) (149.7 mg, 40 mCi) and transferred to p-tolyl magnesium bromide solution described above through the vacuum line stirring for 15 min at -5° to 0°C.

The reaction mixture was acidified with 2N sulfuric acid and extracted with ether. After back-extraction into 1N sodium hydroxide aqueous solution, the solution was again acidified with 2N sulfuric acid and extracted with ether.

The ether solution was washed with water, and dried over anhydrous magnesium sulfate.

Removal of the solvent under reduced pressure gave a crude solid, which was chromatographed on silica gel with chloroform to afford colorless crystals of 3 (73.6 mg, 28.5 mCi, 71%). The product showed only one spot on TLC and its Rf value was 0.71 (ethyl acetate).

[^{14}C]2-(p-Tolyl)benzothiazole (4)

A mixture of 3 (73.6 mg, 28.5 mCi), 2-aminothiophenol (275.2 mg, 2.20 mmol) and polyphosphoric acid (1.5 g) was stirred at 165°C for 1.5 h. After cooling, 0.02N sodium hydroxide aqueous solution was added to the reaction mixture, then the solution was extracted with ether. The ether solution was washed with 0.02N sodium hydroxide aqueous solution and water, and dried over anhydrous magnesium sulfate. Removal of the solvent gave a crude solid, which was chromatographed on silica gel with dichloromethane to afford colorless crystals of 4 (116.0 mg, 27.1 mCi, 95%).

The product showed only one spot on TLC and its Rf values were 0.69 (benzene-ethyl acetate = 20 : 1) and 0.68 (dichloromethane).

[^{14}C]KB-944 (1)

A suspension of 4 (116.0 mg, 27.1 mCi), N-bromosuccinimide (98.7 mg, 0.55 mmol) and benzoyl peroxide (2.9 mg) in carbon tetrachloride (2.7 ml) was stirred at 80°C for 4 h. The reaction mixture was filtered and the filtrate was evaporated to dryness under reduced pressure. Then the residue was mixed with

triethyl phosphite (0.85 ml), and stirred at 140°C under N_2 stream for 30 min.

After cooling to room temperature, n-hexane (8.5 ml) was added and the solution was kept at 4°C overnight to precipitate colorless crystals.

The crystals were washed with n-hexane and purified by column chromatography on silica gel with dichloromethane.

A solution of the crystals in ethyl acetate was washed with water, and dried over anhydrous magnesium sulfate.
Removal of the solvent under reduced pressure gave colorless crystals of 1 (100.3 mg, 14.6 mCi, 54%). The product showed only one spot on TLC and its Rf value was 0.31 (ethyl acetate).

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