Synthesis of [5-methyl-^{3}H]-Nitrendipine

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Summary

Synthesis of [5-methyl-^{3}H]-nitrendipine by means of a new method was described. Methyl-[^{3}H]-methanol was reacted with diketene forming methyl-[^{3}H]acetoacetate, followed by treatment with ammonia to give [methyl-^{3}H]-3-aminoacrotonate, which was reacted with methyl 2-[(3-nitrophenyl)-3-methylene]-3-oxobutanoate to synthesize [5-methyl-^{3}H]-nitrendipine. Its specific activity and radiochemical purify were 23 Ci/mmol and >98%, respectively.

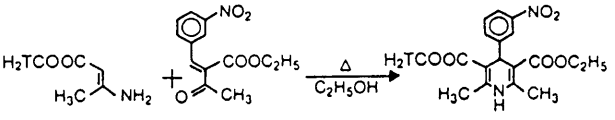
Key Words: Synthesis, [^{3}H]-3-aminocrotonate, [5-methyl-^{3}H]-nitrendipine

Introduction

Since nitrendipine,a calcium antagonist, was first synthesized by Meyer et al. in 1972[1], it has been found in a great many biologically active molecules and widely used in calcium channel[2,3]. To have this molecule enriched with tritium atoms for biological studies, the labelling position must be both chemically and metabolically stable. Only in labelling at the methyl group can both these conditions be fulfilled. D.J. Triggle reported the synthesis of [5-methyl-^{3}H]-nitrendipine by esterification of a dihydropyridine monocarboxylic acid. In that case, the carboxylic acid function is activated via the imidazolide, followed by methanolysis[4]. However, the imidazole reagent is unavailable here and we had to use a new method to synthesize [5-methyl-^{3}H]-nitrendipine. The starting material is [methyl-^{3}H]-methyl alcohol (specific activity 23Ci/mmol), which can be reacted with diketene to form methyl-^{3}H-acetoacetate in high yield and purification would not be required after reaction. Ammonia can then be introduced to that solution to prepare [methyl-^{3}H]-3-aminocrotonate. According to a cyclizing Michael[5] addition, [methyl-^{3}H]-aminocrotonate and methyl 2-[(3-nitrophenyl)methylene]-3-oxobutanoate were refluxed in ethanol for 20 hours to synthesize [5-methyl-^{3}H]-nitrendipine. Its specific activity and radiochemical purity were 23 Ci/mmol and 98%, respectively. Our scheme of synthesis was as follows:

(1) $CH_2TOH + H_2C{=}C(-O-C(=O)-CH_3)-H \longrightarrow CH_3COCH_2COOCH_2T$ (1)

(2) $CH_3COCH_2COOCH_2T + NH_3 \longrightarrow CH_3C(NH_2){=}CH_2COOCH_2T$ (2)

(3) 

(3) 3

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Experimental

Chemicals were available from manufacturers. Only [methyl]-^{3}H]-methyl alcohol required preparation . Ultraviolet spectra were determined on a UV-210 Spectrometer using ethanol as solvent. Radiochemical purity was measured in the thin layer radioscanner Model RTLS-A. Tritium was counted with a Packard Liquid Scintillation Counter Model FJ-353G.

(1) Synthesis of Methyl-[^{3}H]-acetoacetate 1

40 μL(9.70 mmol) of [methyl-^{3}H]-methyl alcohol, 20 μL of triethylamine and 85 μL(9.77 mmol) of diketene were placed in a 5mL reaction flask. The temperature of the reaction solution was then raised to 95°C and kept for 5 hours. The product was thus obtained without purification for the next reaction.

(2) Synthesis of [Methyl-^{3}H]-3-aminocrotonate 2

After methyl-[^{3}H]-acetoacetate was obtained, it was cooled to 0–5°C and 150 μL of anhydrous ethanol was added. While the reaction system was maintained at 5°C, ammonia(1mL/mit.) was introduced to that solution for 2 hours and the temperature of reaction system was kept below 5°C. Then the reaction flask was tightly stoppered and allowed to stand overnight in the refrigerator. The product, [methyl-^{3}H]-3-aminocrotonate, was obtained.

(3) Synthesis of [5-Methyl-^{3}H]-nitrendipine 3

A solution of 2mL of anhydrous ethanol and 25 mg of methyl 2-[(3-nitrophenyl) methylene]-3-oxobutanoate were added to the above reaction flask and refluxed for 20 hours. After cooling to room temperature, the product, [5-methyl-^{3}H]-nitrendipine, was purified on silica paper (solvent system was ethyl acetate : cyclohexane = 1:4 v/v) and eluted with 95% ethanol to afford the product for determining its tritium count in liquid scintillation counter and the chemical purity on the UV spectrometer using ethanol as a solvent. Finally, radiochemical purity was measured with a thin layer radioscanner.

Results and Discussion

(1) Methyl-^{3}H-acetoacetate In this reaction, if one wanted to prepare methyl acetoacetate by a routine process, diketene must be added dropwise to the solution of methanol so as to maintain the temperature of reaction system below 80°C, thus preventing a fast reaction that would liberate a huge amount of heat and cause an explosion. After the addition was completed the temperature of the reaction system was raised again to 95°C.

(2) [Methyl-^{3}H]3-aminocrotonate When methyl-^{3}H acetoacetate is reacted with ammonia, the temperature of reaction system should be kept below 5°C. If the temperature of the reaction system was higher than 5°C, the yield would be small. Also, if ethanol was not added to the reaction system, the solid product which formed on reaction would disappear again after it stood for a few minutes in air. Because the reaction was a reversible one, anhydrous ethanol (or methanol) must be added to remove the water, which was produced in the reaction.

(3) [5-Methyl-^{3}H]-nitrendipine Because the quantity of the product was very small and could not be recrystallized from ethanol, paper chromatography was used to purify the product. The UV spectrometer was adopted to analyze the quantity of the product, which was used to calculate the yield (>70% according to added quantity of methyl 2-[3-nitrophenyl) methylene]-3-oxobutanoate). The UV spectrum of standard nitrendipine and [5-methyl-^{3}H]-nitrendipine was the same (see Figures 1 and 2), which proved that [5-methyl-^{3}H]-nitrendipine was the product. If it is required to obtain high specific activity of [5-methyl-^{3}H]-nitrendipine, one must prepare high specific activity [methyl-^{3}H]-methyl alcohol to synthesize high specific activity [5-methyl-^{3}H]-nitrendipine by this method.

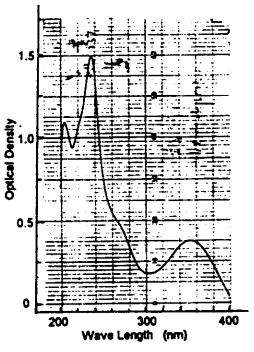

Figure 1 Spectrum of standard nitrendipine

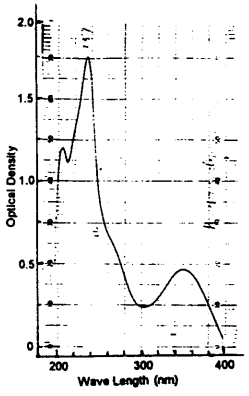

Figure 2 Spectrum of [5-methyl-^{3}H]-nitrendipine

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