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Synthesis of [Allyl-2,3-^{3}H]-MN-9201

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Summary

Key step of the synthesis of calcium antagonist MN-9201 was the cyclizing addition of the allyl-3-amino-crotonate onto methyl 2-[(3-nitrophenyl) methylene]-3-oxobutanoate. It was then used to synthesize [Allyl-2,3-^{3}H]-MN-9201 by the catalytic addition of tritium gas. Its radiochemical purity and specific activity were 98% and 2.1TBq/mmol, respectively.

Key words: synthesis, Tritium addition, MN-9201, [Allyl-2,3-^{3}H]-MN-9201.

Introduction

Since nifedipine, a calcium antagonist, was first synthesized by Bossert et al[1] in 1968, a series of calcium antagonist, such as nimodipine, nicadipine, nitrendipine and nisodipine had been prepared and widely investigated [2,3] in calcium channel studies. Now MN-9201, another calcium antagonist had been synthesized by us using the Michael et al reaction[4,5]. Here we describe the synthesis of MN-9201 and report the catalytic addition of tritium gas into the double bonds of MN-9201. Diketene is an active reagent, which reacted with alcohol to form acetoacetate esters. This principle of reaction has been used to prepare allyl acetoacetate. At low temperature, the oxygen of acetyl group was easily replaced by ammonia to form amino-crotonate. The compound was used to synthesize allyl-3-amino-crotonate. It then reacted with methyl 2-[(3-nitrophenyl) methylene]-3-oxobutanoate to form MN-9201. This compound was required in a tritiated form, and the labelling position must be both chemically and metabolically stable. Labelling in the allyl group fulfill these conditions. We had therefore investigated the possibilities of tritium labelling MN-9201 in the allyl group using the catalytic addition of tritium gas. Usually, the hydrogenating procedure was that the unsaturated compound was dissolved in a suitable solvent, and then reacted with tritium gas in the presence of a catalyst[6]. Most of the well known catalyst, for example, platinum or palladium supported on charcoal, calcium carbonate and barium sulfate, can be used in this reaction. When maximum specific activity was required, hydrogenation should be best carried out in no-polar solvent, that is solvent which do not contain any labile hydrogen position. Ethyl acetate, of course fitted this requirements as a solvent. The scheme of synthesis is as follows:

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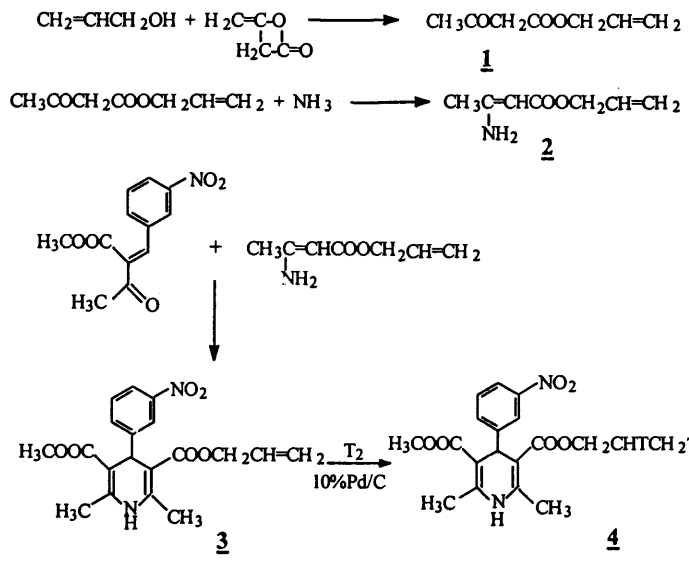

Experimental

All chemicals used were available from manufacturers. Melting point was determined on the Microscopic melting point apparatus. Radiochemical purity was recorded using thin layer radioscanner Model, RTLS-A. Tritium was counted with a Packard liquid scintillation counter, Model FJ-353G. Element analysis was measured on the Elementary Analyzer, Model 1106.

(1) Synthesis of allyl acetoacetate 1

To a 150 ml, three-necked flask was added 10 ml (0.147 mole) of allyl alcohol. When it was heated to 70°C, 13 ml (0.169 mole) of diketene was added dropwise (20 drop/min.) to that solution. After the addition was completed, it was reacted for 5 hours at 80°C. This reaction solution was then distilled at reduced pressure to receive 70°C cut product (17 ml, yield was more than 80%), that was our product, allyl acetoacetate. It was then submitted for elementary analysis.

Anal. Calcd. $C_7H_{10}O_3$: C, 59.10; H, 7.03; Found, C, 59.05; H, 7.10

(2) Synthesis of allyl-3-amino-crotonate 2

To a 100 ml, round-bottomed flask was added 10 ml of allyl acetoacetate and 10 ml of anhydrous ethanol. When it cooled to 0-5°C, ammonia was introduced (3 ml/min.) to the reaction system for three hours. At this time a lot of white crystalline was precipitated in the solution, it was then kept in refrigerator for overnight. The product, allyl-3-amino-crotonate was separated by filtration and washed with distilled water to get 9.4 g.(yield was over 90%) of white crystalline product. It was then submitted for elemental analysis.

Anal. Calcd. $C_7H_{11}NO_2$: C, 59.57; H, 7.80; N, 9.92. Found, C, 59.60; H, 7.75; N, 9.91.

(3) Synthesis of MN-9201 3

In a 250 ml, three-necked, round-bottomed flask was added 50 ml of anhydrous ethanol and 3 g.(12 mmole) of methyl 2-[(3-nitrophenyl) methylene]-3-oxobutanoate as well as 2 g.(0.03 mmol) of allyl-3-amino-crotonate. It was then refluxed for 24 hours. After most of ethanol was removed by evaporation, the solution of product was cooled to room temperature and then stood in refrigerator for overnight to crystallize the product. It was then to recrystallize in ethanol, getting 3 g. of pale yellow crystalline product (yield was >60%) of MN-9201. The result of elemental analysis was as follows:
Anal. Calcd. $C_{19}H_{20}N_2O_6$: C, 61.29; H, 5.41; N, 7.52. Found, C, 61.46; H, 5.33; N, 7.45

(4) Synthesis of [Allyl-2,3-^{3}H]-MN-9201 4

5 mg of MN-9201 and 1.5 ml of ethyl acetate were added to a 15 ml of reaction flask which was fitted with electromagnetic stick. 10 mg of 10% Pd/C catalyst was added to that flask, which was then cooled with liquid nitrogen and evacuated to 0.13 Pa. At this time 66Kpa of tritium was introduced into the flask and then reacted for three hours in the presence of catalyst. After the unreacted tritium was recovered to tritium storage tank, the catalyst was removed by filtration. The product, [allyl-2,3-^{3}H]-MN-9201 was purified by silica paper (solvent system was ethyl acetate : cyclohexane = 1: 4 v/v). Then 95% ethanol was used to elute the product (yield >90%). It was then to determine its chemical quantity(4.6mg) by UV spectrometer using ethanol as a solvent and total radioactivity(0.7Ci) with liquid scintillation counter. The calculated specific activity was 2.1 TBq/mmol.

(5) Determination of radiochemical purity of [allyl-2,3-^{3}H]-MN-9201

When the product was obtained, 2 μ l (0.5 μ Ci) of [allyl-2,3-^{3}H]-MN-9201 was spotted on sheet of Whatman No. 1 paper (2 x 25 cm), where standard MN-9201 was spotted before. It was then chromatographed in solvent system (n-butanol: water: acetic acid = 36:30:10 v/v) for three hours, during which time the front migrated about 19 cm. The sheet of Whatman paper was used to determine its radiochemical purity with thin layer radioscanner (see Figure 1). Thin layer Radioscanner gives the background counter, peak counter and total counter were 256, 2609, and 2650 cpm respectively. Calculation of radiochemical purity showed that it was more than 98%.

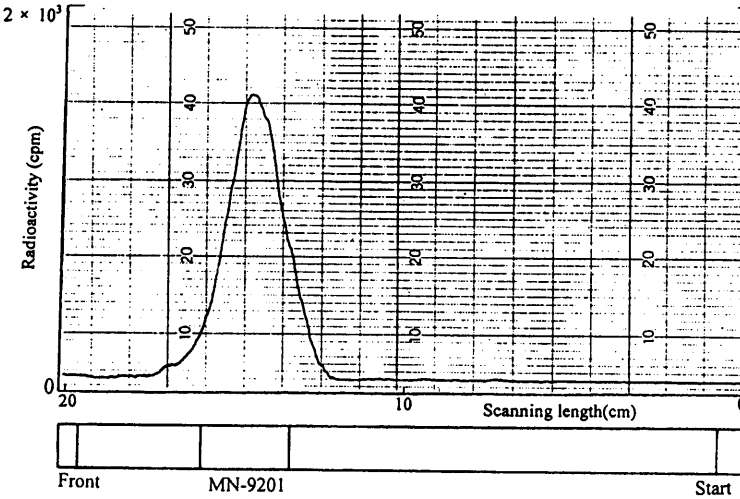

Figure 1 Spectrum of radiochemical purity of [allyl-2,3-^{3}H]-MN-9201

Results and Discussion

Synthesis of allyl acetoacetate When allyl alcohol reacted with diketene at room temperature, it did not react. This required to raise temperature to 70°C. If the temperature was higher than 90°C, the rate of reaction was too fast to control so as to reduce the yield of product. Also if diketene was added fast, the rate of reaction was so fast that a lot of impurity was produced. The rate of addition was controlled at 20 drop/min.

Synthesis of allyl-3-amino-crotonate At this reaction step, anhydrous ethanol must be added to the reaction system to remove water, which was produced in the reaction. Otherwise no product was produced. The reaction temperature should be controlled below 5°C. If the reaction temperature was higher than 5°C, the reaction did not take place. In order to raise yield of the product, ammonia was introduced to the reaction system should be more than 3 hours and then put it in refrigerator for overnight. The product was soluble in ethanol a little and not soluble in water. So water was used to wash the product to remove the impurity.

Synthesis of MN-9201 Reaction partners of the final cyclizing Michael addition in the synthesis of MN-9201 were allyl-3- amino-crotonate and methyl 2-[(3-nitrophenyl)methylene]-3-oxobutaboate. Both were readily accessible starting from the corresponding acetoacetate and by enamine formation or Knoevenagel condensation, respectively. The correct chemical name of MN-9201 is 3-allyl 5-methyl 1,4-dihydro-2,6-dimethyl-4-(3-nitropheny)-3,5-pyridine dicarboxylate. The compound has a molecular $C_{19}H_{20}N_2O_6$ and a molecular weight of 372.36. It forms pale yellow crystal of mp 131-132°C.

Synthesis of [Allyl-2,3-3H]-MN 9201 In the tritium addition reaction, the result was indicative of the selective of C=C double bond in MN-9201 **3**, since the theoretical incorporation of tritium was achieved in either case. If the maximum specific activity is required, hydrogenation should be carried out in no-polar solvent. To achieve maximum specific activities it was therefore necessary to block any labile hydrogen position by the preparation of suitable solvent, such as the methyl ester, methyl ether or acetate, thus preventing the alternative competing exchange reaction. So we tested the two solvents, ethyl acetate and acetone. The results indicated that the ethyl acetate was better than the acetone (see Table 1) and should be candidate of solvent for tritiation .

Table 1 Effect of solvent on the balanced time of tritiation

Solvent	MN-9201(mg)	6% Pd/C (mg)	Balanced time (min.)
ethyl acetate	30	30	140
acetone	30	30	250

Also reaction time was an important factor to guarantee the tritium addition was completed or not (see Table 2) on the tritiation condition. If the reaction time was in 120 minutes, the tritiation onto the C=C double bond was not completed. Therefore over 150 minutes of reaction time should be adopted. In table 1 and table 2, we use hydrogen instead of tritium for reaction to get all data, because of the properties of hydrogen similar as tritium.

Table 2 Effect of reaction time on hydrogen absorbed

Reaction time (min.)	Ethyl acetate (ml)	MN-9201 (mg)	6% Pd/C (mg)	Amount of hydrogen absorbed (kPa)
20	1.5	30	30	14
40	1.5	30	30	21
60	1.5	30	30	26
80	1.5	30	30	30
100	1.5	30	30	32
120	1.5	30	30	33
140	1.5	30	30	34
150	1.5	30	30	34

The product dissolved in ethanol was used to confirm its structure by UV spectrometer. The spectra of the product and standard MN9201 were showed in Fig.2 and Fig.3. The similar spectra indicated that our product is [allyl-2,3-^{3}H]-MN-9201. (On Figure 2, a little peak appeared at about 210 nm wave length, that is a solvent peak. Because the product eluted from silica paper, it contained trace solvent to cause a little peak.)

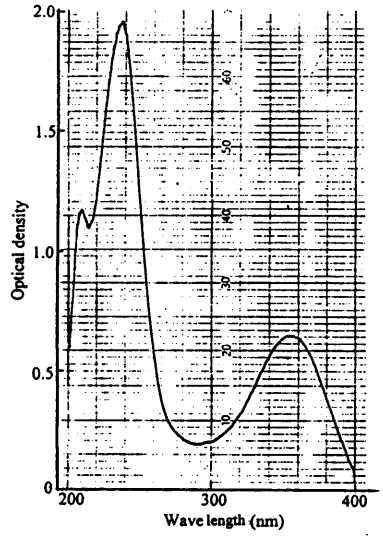

Figure 2 spectrum of [allyl-2,3-^{3}H]-MN-9201

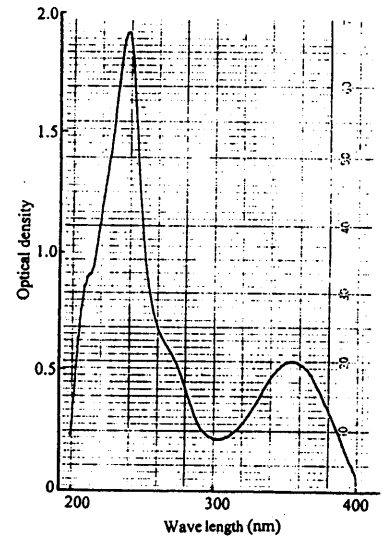

Figure 3 spectrum of standard MN-9201

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