Syntheses of [6-^{14}C] and [5-carboxy, 6-$^{14}C_2$]Nitrendipine

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SUMMARY

[6-^{14}C]Nitrendipine synthesis started from barium[^{14}]carbonate, which was converted to [1-^{14}C]acetyl chloride. The acid chloride was condensed with Meldrum's acid (2,2-dimethyl-1,3-dioxane-4,6-dione). The resulting intermediate was treated with boiling methanol to give methyl [3-^{14}C]acetoacetate. The reaction with gaseous ammonia in toluene yielded the corresponding methyl 3-amino[3-^{14}C]crotonate which was condensed with ethyl 2-(3-nitro-benzylidene) acetoacetate to obtain [6-14C]nitrendipine.

[5-carboxy, 6-$^{14}C_2$]Nitrendipine was synthesized by an analogous procedure starting from methyl [1,3-$^{14}C_2$]acetoacetate.

Key words: calcium antagonist, carbon-14, Michael addition, Knövenagel condensation, Meldrum's acid

INTRODUCTION

Nitrendipine - (±) 3-ethyl 5-methyl-1,4-dihydro-2,6-dimethyl-4-(3-nitrophenyl)-pyridine-3,5-dicarboxylate - is a calcium antagonist showing antihypertensive activity (1). The ^{14}C-labelled compound was needed for the investigation of the pharmacokinetics and the metabolic fate in rat (2-4), dog and man (5). We decided to introduce the label into the dihydropyridine nucleus of nitrendipine due to its stability against metabolic attacks. The present communication describes the syntheses of nitrendipine labelled at position 6 as well as at the carboxyl group adjacent to position 5 and the 6-position of the dihydropyridine system.

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DISCUSSION

Dihydropyridines and pyridines, respectively, have been synthesized in a one-pot reaction by Hantzsch (6) starting from aldehydes, ß-ketoesters and ammonia. This approach was improved by Bossert, Meyer and Wehinger (7): The synthesis of nitrendipine started from the Knövenagel condensation of 3-nitrobenzaldehyde with ethyl acetoacetate and afforded a highly reactive α,ß-unsaturated ketone. This intermediate served as a substrate for the key reaction step of the synthesis - the cyclizing Michael addition with methyl 3-aminocrotonate (accessible from methyl acetoacetate by reaction with gaseous ammonia) which gave nitrendipine in good overall yields.

The ß-ketoester could easily be labelled with carbon-14. [1-^{14}C]Acetyl chloride was prepared starting from barium[^{14}C]carbonate according to the literature (8) and condensed with Meldrum s acid (2,2-dimethyl-1,3-dioxane-4,6-dione) followed by the reaction with boiling methanol (9). The labelled ß-ketoester was converted to the corresponding methyl 3-amino[3-^{14}C]crotonate in the presence of gaseous ammonia (cf. scheme 1). The reaction with ethyl 2-(3-nitrobenzylidene) acetoacetate yielded nitrendipine labelled at the 6-position with a specific activity of 1.55 MBq/mg (42 µCi/mg). The radiochemical purity exceeded 99.6 % (TLC).

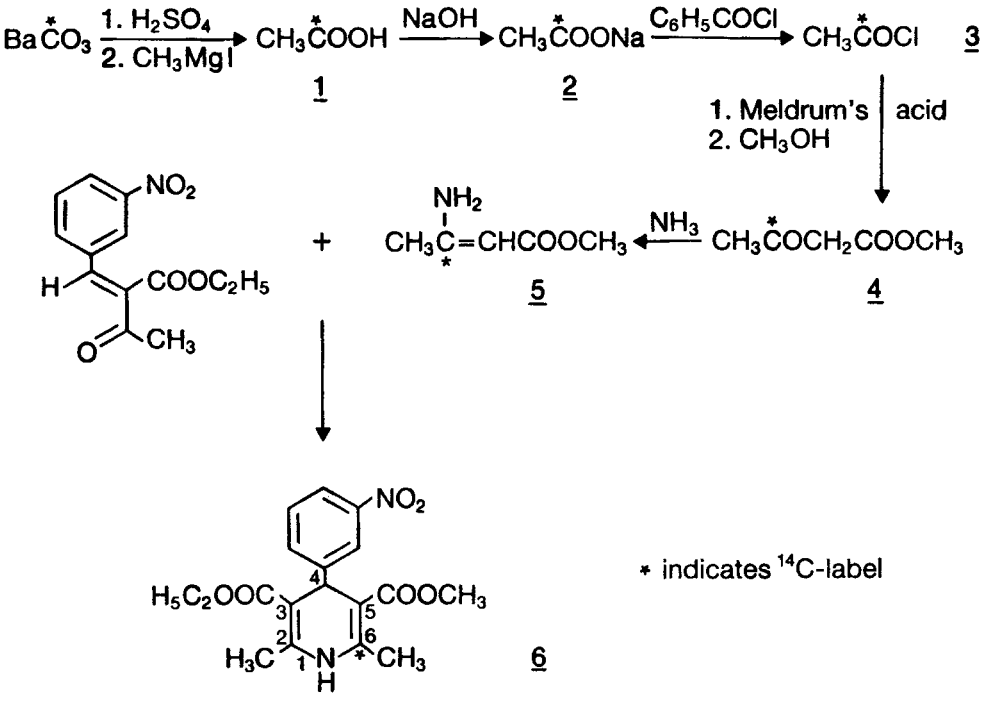
A second approach (cf. scheme 2) starting from methyl [1,3-$^{14}C_2$]acetoacetate afforded [5-carboxy,6-$^{14}C_2$]nitrendipine with a specific activity of 2 MBq/mg (54.3 µCi/mg) . The radiochemical purity was 98.3 and 98.6 % (HPLC and TLC, resp.).

EXPERIMENTAL PART

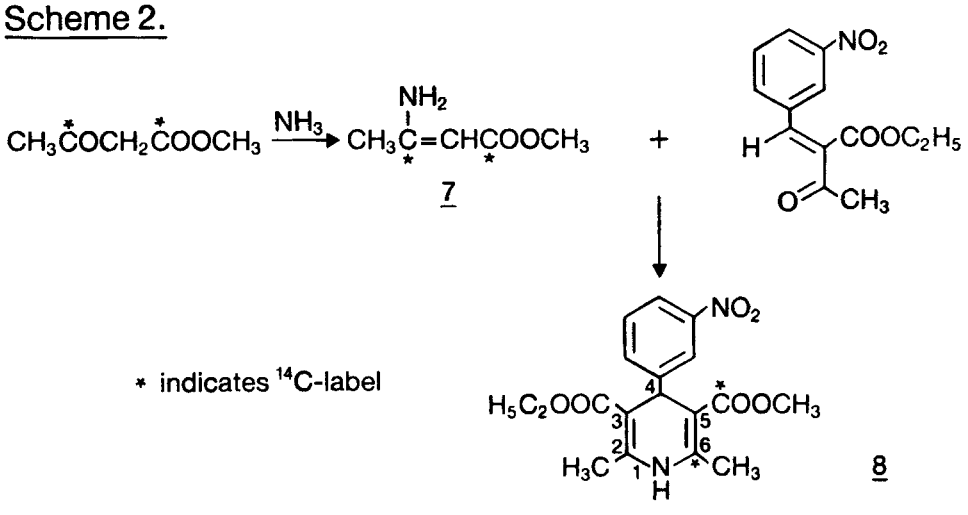
All reagents used were of analytical or HPLC grade and supplied by E.Merck (Darmstadt) unless otherwise stated.

Gas liquid chromatography was performed on the HP 5880 A or 5890 A gas chromatographs (Hewlett Packard, Waldbronn) with simultaneous detection by flame-ionization detector and by radioactivity monitor (containing platinum as catalyst operated at 740 °C and consisting of a 2 ml gas proportional counting tube LB 6231, FAG Measuring Channel FHT 7000 as amplifier/high voltage supply and Trilab$^{(R)}$ 3500 Multi-channel chromatography data system) using helium as carrier, hydrogen as auxiliary and argon/methane 9+1 as counting gas. The cross linked methyl silicone fused silica capillary column (25 m, i.d. 0.32 mm, manufactured by Hewlett

Scheme 1.



Scheme 2.



Packard) was operated either in a temperature programming mode or isothermically.

HPLC was performed on a Varian(R) 5060 chromatograph (Varian, Darmstadt) using the Vista(R) 401 chromatography data system and

the UV-100-detector (234 nm) as well as the on-line radioactivity monitor Ramona 4 (Raytest, Straubenhardt). For the HPLC-analyses Spherisorb(R) ODS-II was used as stationary phase (stainless steel column 250 x 4.6 mm, particle size 5 µm, purchased from Phase Separation, Runcorn, UK). The solvent system consisted of a 40+60-mixture of dist. water and methanol (by volume) at a flow rate of 1 ml/minute.

Purity checks by TLC were performed on precoated TLC-plates (silica gel 60, F 254, layer thickness 0.25 mm) using ethyl acetate/toluene 1+1 and ethyl acetate/chloroform 1+1 (by volume), respectively. Radioactive spots were detected by apposition autoradiography with Agfa Curix(R) RP 1 Cb X-ray film. Zones of silica gel corresponding to radioactive areas were scraped off, mixed with 10 ml Unisolve(R) I (Zinsser, Frankfurt) and 4 ml dist. water. The radioactivity was counted by liquid-scintillation technique. Alternatively the purity checks were established by the Linear Analyzer IM 3000 (Raytest, Straubenhardt).

Radioactivity of liquid samples was measured by the Philips liquid scintillation spectrometer PW 4700 using the external standard channel ratio method at 13 °C and Quickszint(R) 294 (Zinsser) as scintillation cocktail.

Ethyl 2-(3-nitrobenzylidene) acetoacetate

Ethyl 2-(3-nitrobenzylidene) acetoacetate was synthezised by Dr. H.Meyer, Bayer AG, Wuppertal.

Sodium[1-^{14}C]acetate (2)

Sodium[1-^{14}C]acetate (preparation cf. 8) was synthesized from barium[^{14}C]carbonate (768 mg, 3.89 mmol, 7.42 GBq from Hoechst AG, Frankfurt and 273 mg, 1.38 mmol, 3.02 GBq from Amersham Buchler GmbH & Co.KG, Braunschweig) in 86 % yield.

[1-^{14}C]Acetyl chloride (3)

371.5 mg (4.53 mmol) sodium[1-^{14}C]acetate was diluted with 858.5 mg (10.47 mmol) sodium acetate and reacted with 20 ml benzoyl chloride (cf. 8) to obtain [1-^{14}C]acetyl chloride in 98.6 % yield.

Methyl [3-^{14}C]acetoacetate (4)

2.55 ml (30 mmol) pyridine were added to a cooled (2 °C) and

stirred solution of 2.16 g (15 mmol) Meldrum's acid in 20 ml dichloromethane. 1.05 ml (14.8 mmol) [1-^{14}C]acetyl chloride in 2 ml dichloromethane was introduced dropwise within 10 minutes. Stirring at 2 °C was continued for 30 minutes and at room temperature for additional 40 minutes.

The reaction mixture was extracted once with 25 ml 1 N hydrochloric acid and twice with 10 ml dist. water. The organic phase was dried (sodium sulfate), filtered (glass wool) and evaporated under reduced pressure. The orange-red intermediate was dissolved in 20 ml methanol and refluxed for 60 minutes. The excess of reagent was removed under reduced pressure (30 °C/20 mbar) to obtain methyl [3-^{14}C]acetoacetate in 55.6 % yield based on sodium acetate.

Methyl 3-amino[3-^{14}C]crotonate (5)

Gaseous ammonia was introduced into a stirred solution of 966.9 mg (8.33 mmol) methyl [3-^{14}C]acetoacetate and 60 mg (0.3 mmol) p-toluene sulfonic acid in 40 ml boiling toluene within 4 hours. The cooled (room temperature) reaction mixture was extracted with 2.5 ml saturated sodium bicarbonate solution. The organic layer was dried (sodium sulfate), filtered (glass wool) and the excess of solvent was removed in vacuo (40 °C/20 mbar) to obtain 862.2 mg methyl 3-amino[3-^{14}C]crotonate (50 % based on sodium acetate) with 76.1 % purity (GLC).

3-Ethyl 5-methyl 1,4-dihydro-2,6-dimethyl-4-(3-nitrophenyl)-[6-^{14}C]pyridine-3,5-dicarboxylate (6)

Nitrendipine is sensitive to daylight, the next reaction step has to be carried out under exclusion of daylight. The light of a sodium vapor lamp may be used.

A solution of 862.2 mg (7.5 mmol) methyl 3-amino[3-^{14}C]crotonate and 1972.5 mg (7.5 mmol) ethyl 2-(3-nitrobenzylidene)-acetoacetate in 8 ml ethanol was refluxed for 16 hours. The reaction mixture was cooled to 0 °C. After 2 hours the pale yellow crystals of [6-^{14}C]nitrendipine (6) were filtered off, washed with cold ethanol and dried (1687.8 mg 6 = 31.3 % based on sodium acetate, radiochemical purity 97.5 %).

The crystals were washed again with cold ethanol and dried (1419 mg 6 = 26.3 % based on sodium acetate, radiochemical purity 99.6 % by TLC, specific activity 1.554 MBq/mg = 42 µCi/mg).

Methyl 3-amino[1,3-$^{14}C_2$]crotonate (7)

Methyl [1,3-$^{14}C_2$]acetoacetate (dissolved in 1.5 ml benzene) was supplied by ICI Chemicals and Polymers Group, Physics and Radioisotope Services, Billingham Cleveland, UK (3.515 GBq, spec. activity 1.221 GBq/mmol). 334 mg (2.88 mmol) methyl [1,3-$^{14}C_2$]acetoacetate was diluted with 235 mg (2.03 mmol) methyl acetoacetate and then treated with gaseous ammonia in 7.5 ml methanol over a period of 30 minutes. Stirring was continued for additional 30 minutes. The excess of solvent and reagent was removed in vacuo.

3-Ethyl 5-methyl 1,4-dihydro-2,6-dimethyl-4-(3-nitrophenyl)-[5-carboxy, 6-$^{14}C_2$]pyridine-3,5-dicarboxylate (8)

The resulting amino crotonate was treated with 1.289 g (4.9 mmol) ethyl 2-(3-nitrobenzylidene) acetoacetate dissolved in 5 ml ethanol. The reaction mixture was refluxed for 10 hours and then cooled to room temperature. The pale yellow crystals of [5-carboxy, 6-$^{14}C_2$]nitrendipine (7) were filtered off and washed several times with cold ethanol. Recrystallization from ethanol afforded 942 mg 7 (= 53.3 % based on methyl acetoacetate, radiochemical purity 98.3 % by HPLC and 98.6 % by TLC, respectively, specific activity approx. 2 MBq/mg = 54.3 µCi/mg).

REFERENCES

1. Stöpel K., Heise A. and Kazda S. - Arzneim.Forsch./Drug Res. 31, 2056 (1981)

2. Meyer H., Scherling D. and Karl W. - ibid. 33, 1528 (1983)

3. Meyer H., Bossert F., Wehinger E. and Scherling D. - in: Nitrendipine, edited by A.Scriabine, S.Vanov and K.Deck (Baltimore 1984: Urban & Schwarzenberg), p. 1

4. Rämsch K.-D., Graefe K.-H., Scherling D., Sommer J. and Ziegler R. - Am.J.Nephrol. 6: suppl. 1, 73 (1986)

5. Krol G.J., Lettieri J.T., Yeh S.C., Burkholder D.E. and Birkett J.P. - J.Cardiovasc.Pharmacol. 9 (suppl. 4), S 122 (1987)

6. Hantzsch A. - Liebigs Ann.Chem. 215, 1 (1892)

7. Bossert F., Meyer H. and Wehinger E. - Angew.Chem.Int.Ed.Engl. 20, 762 (1981)

8. Murray III A. and Williams D.L. - in: Organic Syntheses with Isotopes, Part I (Interscience Publishers, Inc., New York, 1958) pp. 34 and 376, respectively

9. Oikawa Y., Sugano K. and Yonemitsu O. - J.Org.Chem. 43, 2087 (1978)