Syntheses of [4-^{14}C] and [6-^{14}C]Nisoldipine.

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SUMMARY

The synthesis of [4-^{14}C]nisoldipine started from dimethyl [^{14}C]formamide which was allowed to react with 2-nitrophenyl-lithium yielding 2-nitro[7-^{14}C]benzaldehyde. Knövenagel condensation with isobutyl acetoacetate yielded isobutyl 2-(2-nitro[7-^{14}C]benzylidene) acetoacetate. Key reaction step was the cyclizing Michael addition with methyl 3-aminocrotonate to obtain 3-isobutyl 5-methyl 1,4-dihydro-2,6-dimethyl-4-(2-nitrophenyl)-[4-^{14}C]pyridine-3,5-dicarboxylate (= [4-^{14}C]nisoldipine).

Starting material of the synthesis of [6-^{14}C]nisoldipine was barium[^{14}C]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. Reaction of the ß-ketoester with gaseous ammonia in toluene afforded the corresponding aminocrotonate, which was condensed with isobutyl 2-(2-nitrobenzylidene)-acetoacetate to yield [6-^{14}C]nisoldipine.

Key Words: calcium antagonist, carbon-14, cyclizing Michael addition, Knövenagel condensation, Meldrum´s acid, ß-ketoester

INTRODUCTION

Nisoldipine [(±)3-isobutyl 5-methyl 1,4-dihydro-2,6-dimethyl-4-(2-nitrophenyl)-pyridine-3,5-dicarboxylate] is a 1,4-dihydropyridine calcium antagonist with non-identical ester functions. It has been developed as an antihypertensive and antianginal drug. The ^{14}C-labelled compound was needed for the investigation of pharmacokinetics and metabolic fate in rat, dog and monkey (1) as

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well as in man (2). The requirement for a moderate specific activity (e.g. 1.85 MBq/mg = 50 /uCi/mg) and a radioactive label stable during long-term biological experiments suggested the use of carbon-14 as the labelling isotope.

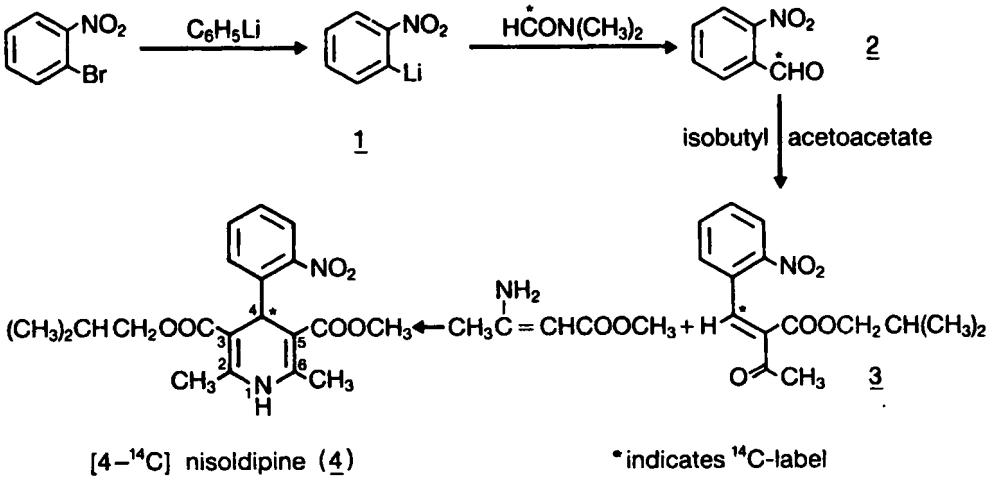
DISCUSSION

Major contributions to the synthesis of various heterocyclic compounds, e.g. pyridines, were made by A. Hantzsch (3). Starting from aldehydes, ß-ketoesters and ammonia, 1,4-dihydropyridines are obtained as stable intermediates which can be oxidized to the corresponding pyridines. This classical Hantzsch approach did not permit the synthesis of dihydropyridines with non-identical ester functions due to the formation of many reaction products which are difficult to separate.

Therefore, the synthesis of nisoldipine starts from the Knövenagel condensation of 2-nitrobenzaldehyde and isobutyl acetoacetate yielding a highly reactive a,ß-unsaturated ketone. This intermediate serves as substrate for the cyclizing Michael addition of methyl 3-aminocrotonate (accessible from methyl acetoacetate and ammonia) yielding nisoldipine in good overall yields (4).

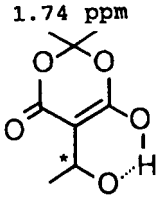
The aromatic aldehyde and the ß-ketoester, respectively, can be easily labelled with carbon 14. According to Johnson and Covington (5) 2-nitro[7-^{14}C]benzaldehyde (2) was prepared in 22 % yield by lithiation of 1-bromo-2-nitrobenzene followed by formylation using radioactive N,N-dimethylformamide (cf. scheme 1). The poor yield probably resulted from the presence of water forming unlabelled nitrobenzene. The following reaction steps - the Knövenagel

Scheme 1.



condensation as well as the Michael addition - could be performed in good yields obtaining nisoldipine (4) labelled with carbon 14 at the 4-position of the dihydropyridine nucleus.

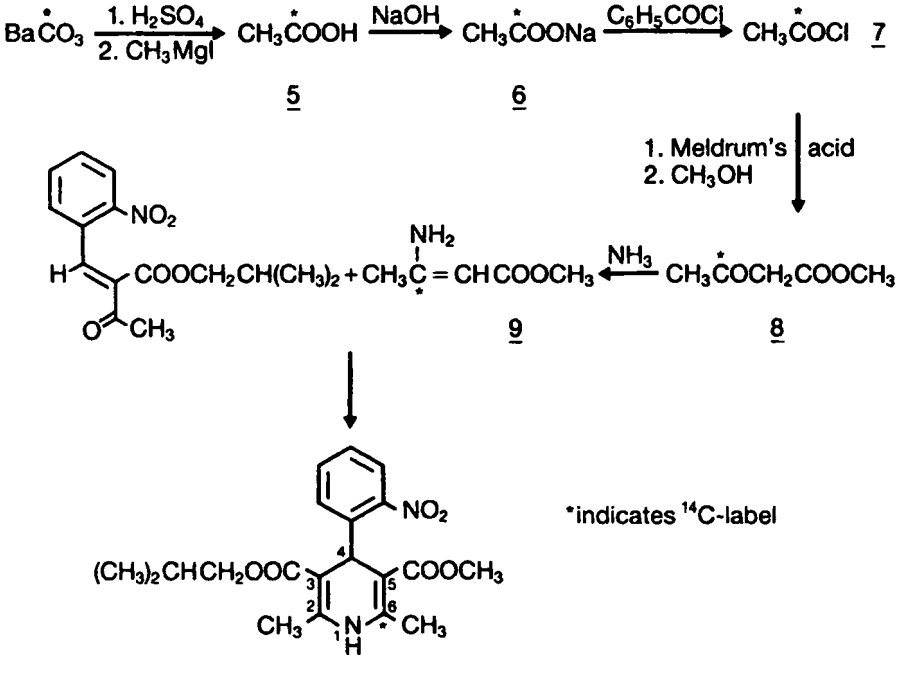
An alternative synthetic approach started from methyl [3-^{14}C]acetoacetate which was formed by condensation of [1-^{14}C]acetyl chloride and Meldrum's acid (= 2,2-dimethyl-1,3-dioxane-4,6-dione) and subsequent reaction with boiling methanol (6). The orange-red intermediate - acetyl Meldrum's acid - has been isolated in a previous synthesis. The ^{1}H-NMR-spectrum clearly demonstrated the enol structure of this intermediate showing a broad signal at 15 ppm in deuterochloroform (tetramethylsilane



* indicates ^{14}C-label

as internal standard). The enol structure prevents a second acetylation. The following reaction steps (cf. scheme 2) via methyl

Scheme 2.



[6-^{14}C] nisoldipine (10)

3-amino[3-^{14}C]crotonate obtained nisoldipine (10) labelled with carbon 14 at the 6-position of the dihydropyridine system in good overall yields.

The acetylation of Meldrum´s acid can generally be used for the preparation of ß-ketoesters labelled at the 3-position. In the case of higher boiling alcohols (> 100 °C) the reaction is performed in toluene as solvent using 1.5 to 3 equivalents of the desired alcohol.

EXPERIMENTAL PART

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

The ^{1}H-NMR-spectra were recorded at 60 MHz on a Jeol C-60 H using deuterochloroform as solvent and tetramethylsilane as internal standard.

HPLC was performed on a single-pump gradient HPLC system (Pharmacia LKB GmbH, Freiburg - consisting of the 2150 solvent delivery system, the 2152 LC controller and the 2151 variable wavelength detector operated at 237 nm) with on-line radioactivity monitoring using the Ramona D (Raytest, Straubenhardt). Analyses were performed on a RP 18 column (stainless steel column 125 x 4 mm, Shandon Hypersil ODS, particle size 5 µm, purchased from Bischoff Analysentechnik, Leonberg) using methanol/tetrahydrofuran/dist. water 55+5+40 (by volume) as solvent system.

Gas liquid chromatography was performed on the HP 5880 A or 5890 A gaschromatographs (Hewlett Packard, Waldbronn) with simultaneous detection by flame-ionization detector and 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 3500 Multi-channel chromatography data system as recorder and integrator) 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, supplied by Hewlett Packard) was operated either in a temperature programming mode or isothermically.

Purity checks by TLC were performed on precoated TLC-plates (silica gel 60, F 254, layer thickness 0.25 mm) using toluene/acetone 8+2 and ethyl acetate/diisopropyl ether 1+1 (by volume),

respectively. Radioactive spots were detected by apposition autoradiography using Agfa Curix RP 1 Cb X-ray film. Zones of silica gel corresponding to radioactive areas were scraped off, mixed with 10 ml Unisolve 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 294 (Zinsser) as scintillation cocktail.

Isobutyl 2-(2-nitrobenzylidene) acetoacetate

A solution of 2.5 g (30 mmol) piperidine and 1.8 g (30 mmol) acetic acid in 25 ml 2-propanol was added to a solution of 75.7 g (500 mmol) 2-nitrobenzaldehyde and 79.1 g (500 mmol) isobutyl acetoacetate in 300 ml 2-propanol. The reaction mixture was stirred at room temperature for 5 hours, poured into dist. water and extracted with dichloromethane. The combined organic extracts were dried (sodium sulfate) and evaporated to dryness. The oily residue was recrystallized from ethanol to obtain 72.2 g (49.6 %) isobutyl 2-(2-nitrobenzylidene) acetoacetate showing a m.p. of 68-69 °C.

2-Nitro[7-^{14}C]benzaldehyde (2)

The reaction sequence was carried out under an atmosphere of dry nitrogen. A solution of 430 mg (2.13 mmol) 1-bromo-2-nitrobenzene in 12 ml dry tetrahydrofuran was added dropwise to 1.3 ml of a stirred solution (2 molar) of phenyllithium in benzene/diethyl ether at -100 °C (diethyl ether/liquid nitrogen) within 20 minutes. The reaction mixture was stirred at -100 °C for 1 hour. The reaction was continued by addition of 129.9 mg (1.78 mmol, 3.96 GBq) dimethyl[^{14}C]formamide (purchased from ICI Chemicals and Polymers Group, Physics and Radioisotope Services, Billingham Cleveland, UK) dissolved in 2 ml tetrahydrofuran and stirring at -100 °C for additional 45 minutes. Then the reaction mixture was set to -80 °C, 10 ml dist. water were added dropwise and the cooling bath was removed simultaneously.

The reaction mixture was transferred into a liquid extractor and extracted with 30 ml diethyl ether. The ether was distilled off at

-10 °C in vacuo. The residue was purified on a Bond Elut silica gel cartridge (ict Handelsgesellschaft mbH, Frankfurt) using hexane/ethyl acetate 15+7 (by volume) as solvent system. The total eluate was applied to a Lobar column type B (silica gel) and eluted with the same solvent system (flow rate 2 ml/minute). The fractions (6 ml volume each) containing the 2-nitro[7-14C]benzaldehyde (2) were combined and lyophilized to obtain 59.8 mg (22 %) of the desired aldehyde 2. The radioactive material was diluted with 29.2 mg unlabelled aldehyde.

Isobutyl 2-(2-nitro[7-^{14}C]benzylidene) acetoacetate (3)

110.6 mg (0.7 mmol) isobutyl acetoacetate, 3.02 mg (0.036 mmol) piperidine and 2.16 mg (0.036 mmol) acetic acid were added slowly to a solution of 89 mg (0.59 mmol) 2-nitro[7-^{14}C]benzaldehyde in 2 ml 2-propanol. The reaction mixture was stirred at room temperature for 5 hours and then lyophilized to obtain 171 mg crude 3 (21.1 % based on dimethyl formamide by radio-GLC/GLC).

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

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

A solution of 171 mg (0.57 mmol) isobutyl 2-(2-nitro[7-^{14}C]-benzylidene) acetoacetate and 77 mg (0.67 mmol) methyl 3-aminocrotonate in 2 ml ethanol was refluxed for 20 hours. The volume of the reaction mixture was reduced to a volume of about 0.5 ml, diluted with 5 ml hexane/acetone 6+4 (by volume) and applied to a Lobar column type B (silica gel) using the same solvent system at a flow rate of 2 ml/minute. Fractions 24-27 (6 ml volume each) containing [4-^{14}C]nisoldipine (4) were combined and lyophilized to obtain 127 mg 4 (12.2 % based on dimethyl formamide). The specific activity was determined to be 3.07 MBq/mg (83.1 µCi/mg) and the radiochemical purity exceeded 99.1 % by TLC (97.7 % by HPLC at 237 nm and 98.9 % by GLC).

Sodium[1-^{14}C]acetate (6)

Sodium[1-^{14}C]acetate (preparation cf. 7) was synthesized from barium[^{14}C]carbonate (1140 mg, 5.78 mmol, approx. 11 GBq from Hoechst AG, Frankfurt) in 93.8 % yield.

[1-^{14}C]Acetyl chloride (7)

444.4 mg (5.42 mmol) sodium[1-^{14}C]acetate was diluted with 785.6 mg (9.58 mmol) sodium acetate and reacted with 20 ml benzoyl chloride (cf. 7) to obtain [1-^{14}C]acetyl chloride in 93.9 % yield.

Methyl [3-^{14}C]acetoacetate (8)

2.5 ml (28.5 mmol) pyridine were added to a cooled (2 °C) and stirred solution of 2.052 g (14.25 mmol) Meldrum's acid in 15 ml dichloromethane. 1 ml (14.1 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 residue was dissolved in 30 ml methanol and refluxed for 60 minutes. Excess of reagent was removed under reduced pressure (40 °C/20 mbar) to obtain methyl [3-^{14}C]acetoacetate in 70.9 % yield based on sodium acetate.

Methyl 3-amino[3-^{14}C]crotonate (9)

Gaseous ammonia was introduced into a stirred solution of 1.234 g (10.64 mmol) methyl [3-^{14}C]acetoacetate and 60 mg (0.3 mmol) p-toluene sulfonic acid in 30 ml boiling toluene within 4 hours. The cooled (room temperature) reaction mixture was extracted with 10 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 820 mg methyl 3-amino[3-^{14}C]crotonate (47.5 % based on sodium acetate) with a purity of 81.8 % (by GLC).

3-Isobutyl 5-methyl 1,4-dihydro-2,6-dimethyl-4-(2-nitrophenyl)-[6-^{14}C]pyridine-3,5-dicarboxylate (10)

A solution of 820 mg (7.13 mmol) methyl 3-amino[3-^{14}C]crotonate and 2075 mg (7.13 mmol) isobutyl 2-(2-nitrobenzylidene) acetoacetate in 13 ml ethanol was refluxed for 20 hours (reaction under exclusion of daylight). Excess of solvent was removed under reduced pressure. The oily residue was dissolved in 2.5 ml acetone, and 4 ml hexane were added slowly. After 20 hours at 4 °C the pale yellow crystals of [6-^{14}C]nisoldipine were filtered off,

washed with hexane and dried (1711 mg 10 = 29.4 % based on sodium acetate, radiochemical purity 95.2 % by TLC).

After recrystallization from acetone/hexane 1140 mg 10 (19.6 % based on sodium acetate) were isolated showing a radiochemical purity of more than 98.5 % and a specific activity of 1.665 MBq/mg (45 µCi/mg). A second synthesis starting from methyl [3-^{14}C]acetoacetate afforded [6-^{14}C]nisoldipine (400 mg) with a specific activity of 1.96 MBq/mg (53 µCi/mg) and a radiochemical purity of 99.1 % (by HPLC/TLC).

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