SYNTHESIS OF ^{14}C- AND ^{2}H-LABELED (3S)-1-BENZYL-3-PYRROLIDINYL METHYL (4S)-2,6-DIMETHYL-4-(m-NITROPHENYL)-1,4-DIHYDROPYRIDINE-3,5-DICARBOXYLATE HYDROCHLORIDE (YM-09730-5), A POTENT CALCIUM ANTAGONIST

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SUMMARY

A potent and long acting calcium antagonist YM-09730-5, (3S)-1-benzyl-3-pyrrolidinyl methyl (4S)-2,6-dimethyl-4-(m-nitrophenyl)-1,4-dihydropyridine-3,5-dicarboxylate hydrochloride (7a·HCl), was labeled with carbon-14 and deuterium. The labeled materials were prepared by either the cyclizing Michael addition of 3-aminocrotonate (5c) to benzylidene acetoacetate (6) or the modified Hantzsch reaction using m-nitrobenzaldehyde, aminocrotonate(5a) and acetoacetate (3b). The ^{14}C-labeled material (7c·HCl) was synthesized from methyl [3-^{14}C]acetoacetate (4c) in a 32.0% radiochemical yield, at a specific activity of 60.9 mCi/mmol. The deuterium labeled material (7b·HCl) was synthesized from 1-benzyl-3-hydroxy-pyrrolidine-d_4 (2b) which was obtained by the reduction of succinimide (1) with $LiAlD_4$.

Keywords: Carbon-14, Deuterium, YM-09730-5, Dihydropyridine, Calcium antagonist

0362-4803/88/020161-10$05.00

Received June 1, 1987

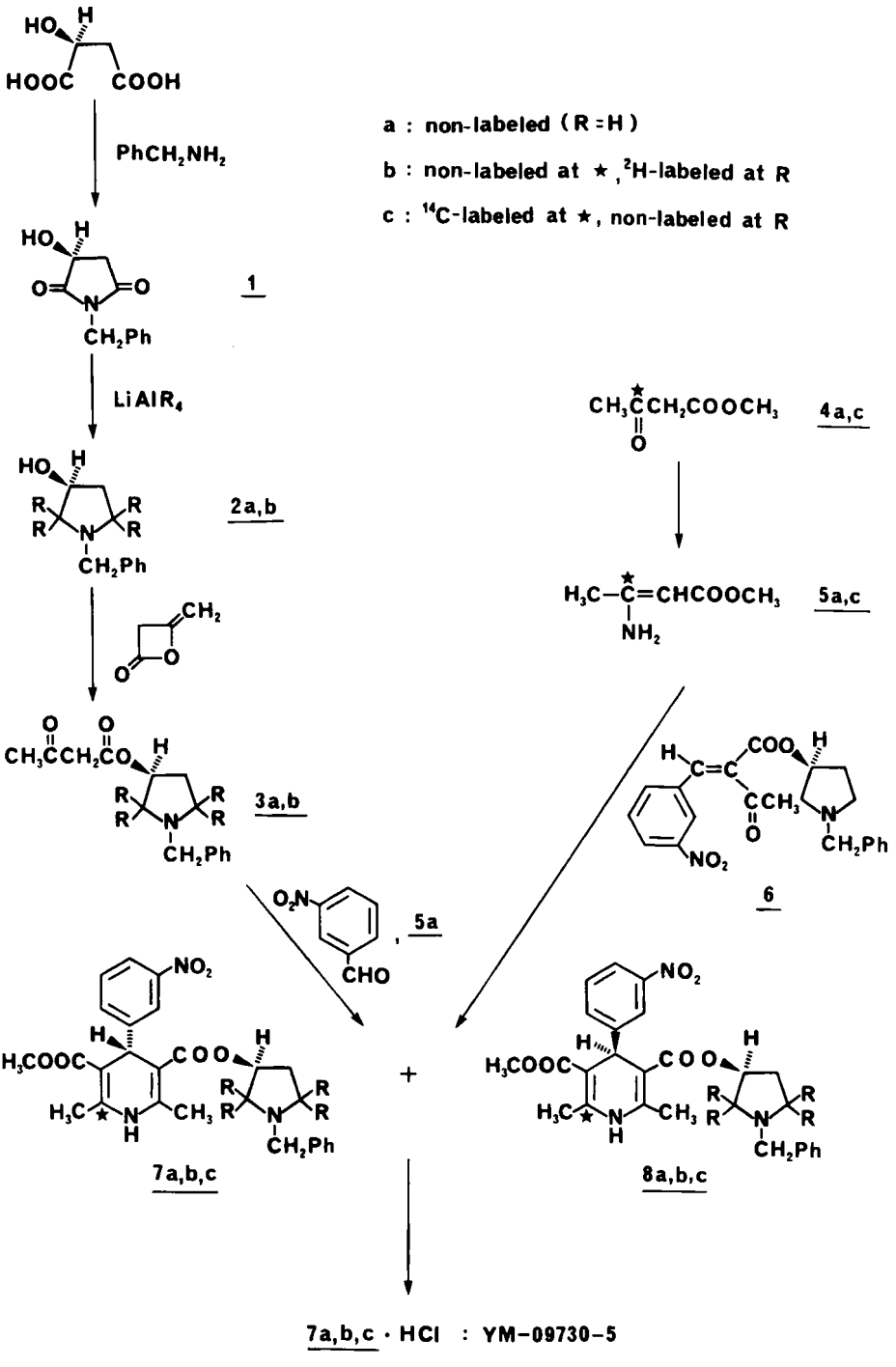
INTRODUCTION

A dihydropyridine compound with two chiral centers, (±)-1-benzyl-3-pyrrolidinyl methyl 2,6-dimethyl-4-(m-nitrophenyl)-1,4-dihydropyridine-3,5-dicarboxylate hydrochloride (YM-09730), was previously found to be very potent in producing marked hypotention of long duration.[1] Recently it was elucidated that the potent activity found in YM-09730 resided in the enantiomer 7a·HCl (YM-09730-5) with the absolute configurations of (S)-1,4-dihydropyridine-C4 and (S)-pyrrolidine-C3.[2,3] This report describes the synthesis of ^{2}H- and ^{14}C-labeled YM-09730-5 to enable studies on the metabolism and disposition of this promising agent.

RESULTS AND DISCUSSION

The preparation of [$^{2}H_{4}$]- and [^{14}C]-labeled YM-09730-5 (7b·HCl and 7c·HCl, respectively) was depicted in the following Scheme. The route is based essentially on the procedures developed for the non-labeled compound.[3] Introduction of 4 atoms of deuterium in the pyrrolidine ring was carried out by reduction of (S)-succinimide (1) with lithium aluminium deuteride. Mass and ^{1}H-NMR spectra of the product (2b) showed that the 2 and 5 positions of the pyrrolidine ring were completely deuterated. Enantiomeric purity of 2b was evaluated to be 84% ee by NMR analysis of its (R)-mandelate. Optically pure 2b was obtained by resolution with use of (R)-mandelic acid. Reaction of 2b with diketene gave acetoacetate (3b), which was subjected to modified Hantzsch reaction with m-nitrobenzaldehyde and methyl 3-aminocrotonate (5a) to yield a diastereomeric mixture of 7b and 8b. The desired 7b was separated from its isomer 8b by column chromatography.[4] The free base of 7b thus obtained, which was contaminated by a small amount of 8b and other impurities, was further purified by recrystallization of its (S)-malic acid salt. Yield of the malate was 25.2% based on 2b. Conversion of the malate into hydrochloride salt furnished [$^{2}H_{4}$]YM-09730-5 (7b·HCl). Isotopic purity of this material was greater than 99 atom % D. Yield of 7b·HCl from 2b was 22.4%.

Scheme


a : non-labeled (R = H)
b : non-labeled at ★, ^{2}H-labeled at R
c : ^{14}C-labeled at ★, non-labeled at R
PhCH2NH2
1
LiAlR4
2a,b
4a,c
5a,c
3a,b
6
5a
7a,b,c
8a,b,c
7a,b,c · HCl : YM-09730-5

Carbon-14 was introduced conventionally at 6 position of the dihydropyridine nucleus.[5,6] Reaction of methyl [3-^{14}C]acetoacetate (4c) with gaseous ammonia in methanol gave aminocrotonate (5c).[7] In order to maximize the yield based on the radiolabeled precursor, benzylidene acetoacetate (6) was chosen as the reaction partner in the final cyclization. Knoevenagel condensation of m-nitrobenzaldehyde with 3a yielded a E/Z mixture of 6, from which (E)-isomer was crystallized for the purpose of purification. After reaction of 5c and 6, the diastereomeric mixture (7c and 8c) was worked up by the same manner described above to provide [^{14}C]YM-09730-5 (7c·HCl) with a specific activity of 60.9 mCi/mmol and a radiochemical purity of greater than 99%. Overall radiochemical yield was 32.0%.

EXPERIMENTAL

The purity and identity of deuterium labeled compounds were confirmed by routine spectra and analytical techniques. ^{1}H- and ^{13}C-NMR spectra were recorded on a JEOL FX-100 NMR spectrometer. Chemical shifts are in parts per million (δ) relative to tetramethylsilane. Coupling constants (J values) are in Hertz (Hz). MS spectra were recorded on a Hitachi M-80 mass spectrometer. TLC analyses were conducted on Merck silica gel 60F$_{254}$ plates. HPLC analyses were performed on a Waters 6000A chromatograph; column: Nucleosil 5C$_{18}$, 4.6 mm X 300 mm (Marchery Nagel); mobile phase: 0.05M KH_2PO_4 (adjusted to pH 3 with H_3PO_4)/CH_3CN containing 3 mmol tetra-n-pentylammonium bromide (1:4 v/v)[8]; flow rate : 0.9 ml/min; UV wave length : 254 nm. The diastereomeric ratio of 7 to 8 was determined by HPLC analysis.[3] The optical purity of 2b was evaluated by ^{1}H-NMR analysis of its (R)-mandelate according to the reported method.[3] Specific rotations were determined with a Perkin-Elmer 241 polarimeter. Melting points and boiling points are uncorrected.

The identity of the ^{14}C-labeled compounds was determined by comparison of their chromatographic properties with those of standard non-labeled

materials. The radioactivity was measured with a Packard Model 4640 Liquid Scintillation Spectrometer. The radiochemical purity was determined by TLC with a Berthold Radio-TLC Scanner LB 2723.

(S)-1-Benzyl-3-hydroxy succinimide (1a)

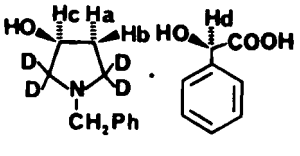
This material prepared from (S)-malic acid was purified according to the reported method[3]: mp 105-107°C (lit.[3] mp 99-101°C); $[\alpha]^{20}_{D}$ -54.8° (c 1, MeOH) (lit.[3] $[\alpha]^{20}_{D}$ -51.1° (c 1, MeOH)).

(S)-1-Benzyl-3-hydroxy[2,2,5,5-d_4]pyrrolidine (2b)

A solution of 1a (12.3 g, 60 mmol) in dry THF (130 ml) was added to a suspension of $LiALD_4$ (6.45 g, 154 mmol, minimum isotopic purity 98 atom % D, Merck) in dry THF (230 ml). The mixture was heated under reflux for 2.5 hr with stirring. After cooling, the reaction mixture was quenched successively with dropwise addition of H_2O (5.1 ml), 4 N NaOH (5.1 ml), and H_2O (15.4 ml). The solid was removed by filtration, and the filtrate was concentrated. The residual oil was distilled to afford 9.9 g (91.2% based on 1a) of crude 2b, bp 107-110°C (1.1 mmHg). The optical purity of 2b was 84% ee. Resolution of the crude alcohol (9.8 g, 54.1 mmol) with use of (R)-mandelic acid by the same procedure for non-labeled 2a described before[3] gave 6.0 g (61.3%) of optically pure 2b.

2b (R)-mandelate: mp 104-105°C, $[\alpha]^{20}_{D}$ -44.9° (c 1, MeOH). Anal. calcd. for $C_{19}H_{19}D_4NO_4$: C, 68.44; H, 5.74; D, 2.42; N, 4.20. Found: C, 68.49; H, 5.76; D, 2.36; N, 4.17.

^{1}H-NMR ($CDCl_3$): δ 1.95 (1H, dd, J_{HaHb}=14, J_{HbHc}=4, Hb), 2.17 (1H, dd, J_{HaHb}=14, J_{HaHc}=6, Ha), 4.03 (2H, ABq, J=12, $\underline{CH_2}Ph$), 4.38 (1H, dd, J_{HaHc}=6, J_{HbHc}=4, Hc), 4.99 (1H, s, Hd), 7.20-7.68 (10H, m, phenyl-H).



No signal of the methylene protons in the benzyl group correspoding to (R)-isomer (δ 4.01, ABq, J=14)[3] was detected.

Optically pure 2b: bp 111-113°C (1.7 mmHg), $[\alpha]^{20}_{D}$ -4.1° (c 1, MeOH). MS: m/z 181(M^+), 137, 104, 91.

^{1}H-NMR (CD_3OD): δ 1.72 (1H, dd, J_{HaHb}=15, J_{HbHc}=4, Hb), 2.24 (1H, dd, J_{HaHb}=15, J_{HaHc}=8, Ha), 3.68 (2H, s, $\underline{CH_2}$Ph), 4.35 (1H, dd, J_{HaHc}=8, J_{HbHc}=4, Hc), 7.36 (5H, m, phenyl-H). MS and NMR spectra showed complete deuteration at both the 2 and 5 positions of the pyrrolidine ring.

(3S)-1-Benzyl-3-[2,2,5,5-d_4]pyrrolidinyl methyl (4S)-2,6-dimethyl-4-(m-nitrophenyl)-1,4-dihydropyridine-3,5-dicarboxylate hydrochloride (7b·HCl)

Freshly distilled diketene (1.28 g, 15.2 mmol) was added dropwise to a solution of 2b (2.76 g, 15.2 mg) in benzene (12 ml) at 50-60°C, and the mixture was heated at 70-80°C for 3 hr. After evaporation of the solvent, (3S)-1-benzyl-3-[2,2,5,5-d_4]pyrrolidinyl 3-oxobutyrate (3b) was obtained quantitatively as an oil. A solution of 3b (4.04 g, 15.2 mmol), m-nitrobenzaldehyde (2.30 g, 15.2 mmol), and methyl 3-aminocrotonate (5a, 1.75 g, 15.2 mmol) in isopropanol (10 ml) was heated under reflux for 8hr. After evaporation of the solvent, (3S)-1-benzyl-3-[2,2,5,5,-d_4]pyrrolidinyl methyl (4R/S)-2,6-dimethyl-4-(m-nitrophenyl)-1,4-dihydropyridine-3,5-dicarboxylate (7b and 8b) was afforded as a caramel. The diastereomeric ratio of the product was about 1:1. The product was subjected to column chromatography on silica gel (2 kg, Wakogel C-200). The column was eluted with n-hexane/ethyl acetate (1:1 v/v). The fractions were checked by HPLC and those containing only the diastereomer 7b were collected and concentrated to obtain 2.59 g (34.4% based on 2b) of free base 7b as a caramel. To a solution of the caramel (2.59 g, 5.23 mmol) in acetone (6 ml) was added a solution of (S)-malic acid (700 mg, 5.22 mmol) in acetone (7 ml), and the resulting solution was stirred at 5°C overnight to crystallize 7b (S)-malate. The crystals were collected by filtration and recrystallized from MeOH (42 ml) to provide 2.41 g (25.2% based on 2b) of the optically pure malate: mp 195-196°C (decomp.), $[\alpha]^{20}_D$ +82.2 (c 0.5, MeOH). Anal. Calcd. for $C_{31}H_{31}D_4N_3O_{11}$: C, 59.13; H, 4.96; D, 1.28; N, 6.67. Found: C, 58.87; H, 4.99; D, 1.25; N, 6.56.

^{1}H-NMR (CD_3OD): δ 1.94 (1H, dd, J_{HaHb}=15, J_{HbHc}=4, Hb), 2.29 (1H, dd, J_{HaHb}=15, J_{HbHc}=7, Ha), 2.32 (6H, s, 2,6-CH_3), 2.52 (1H, dd, J_{HeHf}=16, J_{HeHg}=8, He), 2.80 (1H, dd, J_{HeHf}=16, J_{HfHg}=6, Hf), 3.63 (3H, s, OCH_3), 4.18 (2H, s, $\underline{CH_2}$Ph), 4.33(1H, dd, J_{HeHg}=8, J_{HfHg}=6, Hg), 5.05 (1H, s, 4-H), 5.22 (1H, dd, J_{HaHc}=7, J_{HbHc}=4, Hc), 7.32-8.16 (9H, m, phenyl-H).

The malate (2.2 g, 3.49 mmol) in $CHCl_3$ (10 ml) was treated successively with saturated aq. $NaHCO_3$ (10 mlx2), H_2O (10 ml), and 1 N HCl (10 mlx2). The organic layer was separated followed by concentration, and the residue was crystallized from MeOH (9 ml) to obtain 1.65 g (88.8% based on the malate) of 7b·HCl: mp 226-228°C (decomp.), $[\alpha]^{20}_{D}$ +115.2°(c 1, MeOH). Anal. Calcd. for $C_{27}H_{26}D_4N_3O_6Cl$: C, 60.95, H, 4.93; D, 1.51; N, 7.90; Cl, 6.66. Found: C, 60.78; H, 4.94; D, 1.48; N, 7.87; Cl, 6.76. MS: m/z 495 (M^+), 478, 464, 373, 315, 162. The isotopic purity was estimated by the comparison of MS spectra of 7b HCl with those of 7a·HCl to be greater than 99%.

^{1}H-NMR (CD_3OD): δ 2.10 (1H, dd, J_{HaHb}=15, J_{HbHc}=4, Hb), 2.2-2.5 (1H, dd, J_{HaHb}=15, J_{HaHc}=7, Ha), 2.32 (3H, s, 6-CH_3), 2.34 (3H, s, 2-CH_3), 3.66 (3H, s, OCH_3), 4.43 (2H, ABq, J=13, $\underline{CH_2}$Ph), 5.08 (1H, s, 4-H), 5.30 (1H, dd, J_{HaHc}=7, J_{HbHc}=4, Hc), 7.3-8.16 (9H, m, phenyl-H).

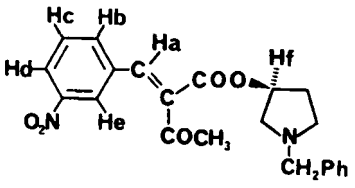
Methyl 3-amino[3-^{14}C]crotonate (5c)

To a glass ampoule containing methyl [3-^{14}C]acetoacetate (4c; 40 mCi, 60.9 mCi/mmol, 0.657 mmol; Amersham International plc, England) were added under ice-cooling 1.7 ml of MeOH and 0.55 ml of a saturated solution of NH_3 in MeOH (about 19 w/v% at 0°C). The mixture was allowed to stand at room temperature for 20 hr. The solvent and an excess of NH_3 were evaporated to obtain crystals of 5c (75.8 mg, 98.6%). Radiochemical purity: 94.5% by TLC (n-hexane/ether, 2:1 v/v, Rf 0.25). The product was used in the next step without further purification.

(3S)-1-Benzyl-3-pyrrolidinyl (E)-2-(m-nitrobenzylidene)acetoacetate (6)

To a benzene solution of (S)-1-benzyl-3-pyrrolidinyl 3-oxobutyrate (3a), which was prepared by the reaction of (S)-1-benzyl-3-hydroxy-pyrrolidine (2a,[3,9] 5.22 g, 29.5 mmol) and diketene (2.48 g, 29.5 mmol), were added m-nitro-benzaldehyde (4.46 g, 29.5 mmol), piperidine (0.1 ml), and AcOH (0.3 ml). The mixture was heated under reflux for 3 hr using Dean-Stark trap. After cooling, the reaction mixture was applied to silica gel column chromatography (Wakogel C-200, 470 g). The column was eluted with benzene/ethyl acetate (3:1 v/v) to obtain 9.63 g (82.9%) of 6 as an oil. The product was a E/Z-mixture and contaminated by a small amount of impurities. By addition of ether (19 ml) followed by standing at 0°C for 2 hr, pure (E)-isomer was crystallized. Yield: 7.67 g (66.6%): mp 84-85°C, $[\alpha]^{20}_{D}$ -12.5° (c 1, MeOH). Anal. Calcd. for $C_{22}H_{22}N_2O_5$: C, 66.99; H, 5.62; N, 7.10. Found: C, 66.97; H, 5.62; N, 7.06.

^{1}H-NMR ($CDCl_3$): δ 1.6-3.2 (6H, m, $CH_2NCH_2CH_2$), 2.44 (3H, s, CH_3CO), 3.60 (2H, ABq, J=12, $\underline{CH_2}Ph$), 5.44 (1H, m, Hf), 7.28(5H, s, $CH_2\underline{Ph}$), 7.52 (1H, t, $J_{HbHc}=J_{HcHd}=8$, Hc), 7.60 (1H, s, Ha), 7.76 (1H, m, Hb), 8.24 (1H, m, Hd), 8.32 (1H, m, He). Any signal of 2.38 (CH_3CO), 3.65($\underline{CH_2}Ph$), and 7.34 ($CH_2\underline{Ph}$) which corresponded to (Z)-isomer was not detected.



^{13}C-NMR ($CDCl_3$): δ 31.4 ($\underline{CH_3}CO$), 166.6 ($\underline{COO}$), 193.8 ($CH_3\underline{CO}$). Any signal of 31.8, 163.6, and 201.4 which corresponded to (Z)-isomer was not detected.

(3S)-1-Benzyl-3-pyrrolidinyl methyl (4S)-2,6-dimethyl-4-(m-nitrophenyl)-1,4-dihydro[6-^{14}C]pyridine-3,5-dicarboxylate hydrochloride (7c·HCl)

A mixture of 5c (75.8 mg, 0.648 mmol), 6 (281 mg, 0.713 mmol) and isopropanol (2 ml) was heated under gentle reflux for 14 hr. After evaporation of the solvent, the diastereomeric product (7c and 8c; 1:1 by HPLC) was applied to a Lobar® column (LiChroprep® Si 60, Size C, Merck). The column was eluted with n-hexane/ethyl acetate (1:1 v/v) at a flow rate

of 20 ml/min. The fraction eluted from 160 to 200 min was collected and concentrated. The residual caramel (156.1 mg, 0.316 mmol, 7c:8c=97.6:2.4) was treated with (S)-malic acid (43 mg, 0.32 mmol) in acetone (1 ml) to obtain crystals of 7c (S)-malate (182.6 mg), which was recrystallized from MeOH (2.7 ml) to provide the optically pure product. Yield: 155.0 mg (38.1% based on 5c). The malate was converted into hydrochloride salt by the same manner described above to yield 7c·HCl. Yield: 111.4 mg, (32.0% from 5c); 12.8 mCi. Specific activity: 115 μCi/mg, 60.9 mCi/mmol; Radiochemical purity: greater than 99% by TLC analysis ($CHCl_3$/MeOH (10:1 v/v), Rf 0.53); Chemical purity: 99.8% by HPLC analysis.

ACKNOWLEDGMENT

We are indebted to Messrs. H. Kaniwa and T. Tokunaga for the NMR analysis, to Messrs. M. Shimizu and H. Matsumoto for obtaining MS spectra, and to Miss T. Yahagi and Mrs. E. Suzuki for the elemental analysis.

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7) Methanol was an effective solvent for both the enamine formation and the suppression of 2-aminocrotonamide as a by-product.

8) The mobile phase shown in the preceding paper (Ref.3) is erroneous. It should be corrected as described in this section.

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