SYNTHESIS OF ^{3}H-LABELLED ENALAPRIL MALEATE

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SUMMARY

The synthesis of enalapril maleate (MK 421) labelled with tritium at its proline ring is described. Thus, the N'-hydroxysuccinimidyl ester of a N-carboxyalkyl alanine derivative was reacted with L-[4,5-^{3}H] proline and the crude reaction mixture treated with maleic acid to give the maleate salt of enalapril in 27% yield.

Key words : ^{3}H-enalapril maleate , antihypertensive agent, tritium.

INTRODUCTION

1-(N-(1(S)-(Ethoxycarbonyl)-3-phenylpropyl)-L-alanyl)-L-proline maleate (enalapril maleate, MK 421) is a potent angiotensin converting enzyme inhibitor[1]. It is a very biologically active compound which is administered at very low doses for the treatment of hypertension. For this reason, measurement of plasma levels by spectrophotometric or chromatographic methods is not feasible. To carry out some pharmacokinetic studies we developed a short synthesis of this compound[2] in which the labelled proline unit was introduced in the last step, thus maximizing yields and avoiding undesired handling of radioactive synthetic intermediates.

RESULTS AND DISCUSSION

The synthesis consisted in four steps from commercially available compounds (Scheme I). Ethyl 2-oxo-4-phenylbutanoate (1) was prepared according to a published procedure[3] in 68% yield by treatment of the Grignard derivative of 2-phenylethylbromide with an excess of diethyloxalate in THF. This α-oxoester was then reductively alkylated with L-alanine ($NaBH_3CN$, AcOH-EtOH, 18h, 20°C) to give compound **2** as a mixture of diastereomers at the new sp^3 carbon (SS:RS = 60:40) in 57% yield.

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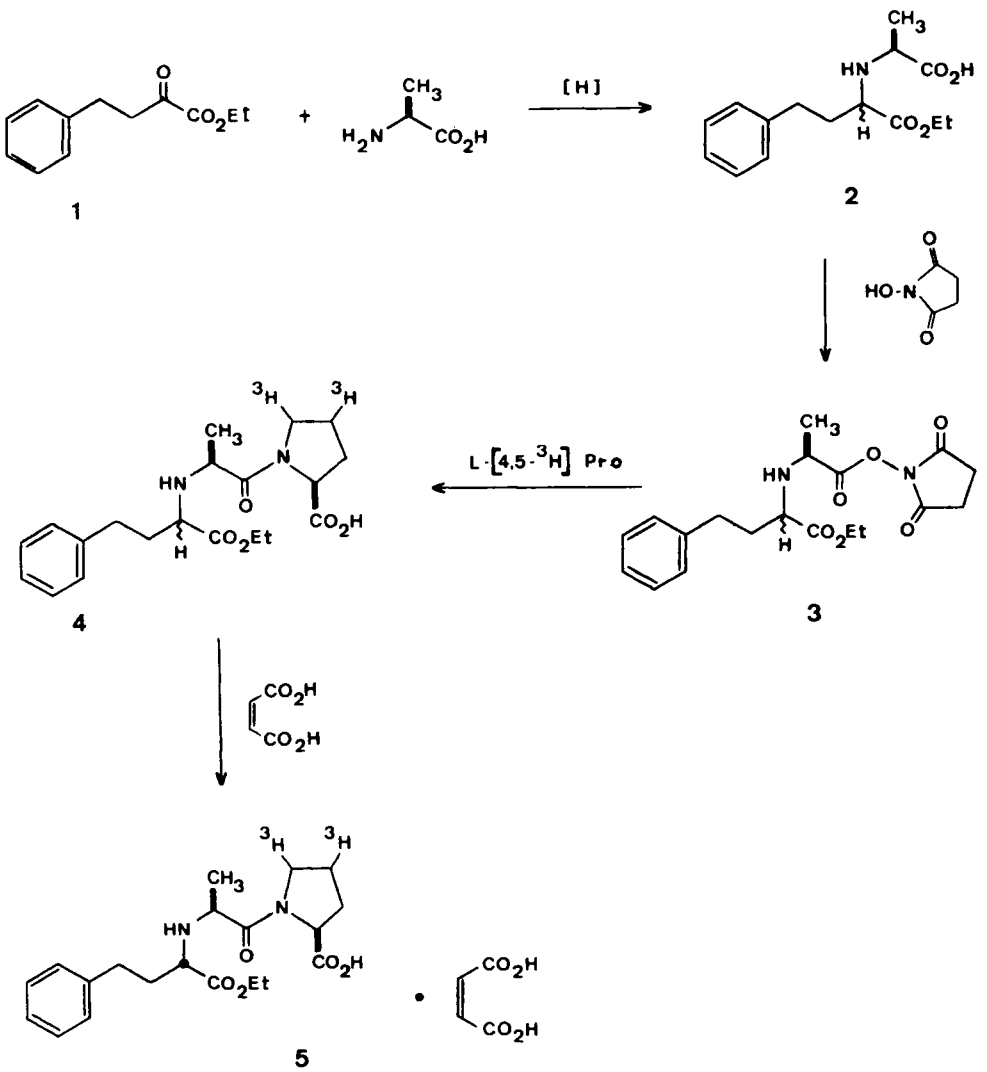
Scheme I



Due to the inductive effect of the two carbonyl substituents at the α and α' positions of the nitrogen atom of 2, this atom did not need to be protected for the peptide coupling step. Thus, 2 was converted into its N-hydroxysuccinimidyl ester, 3, (HONSU, DCC, 1,2-DME, 4°C, 18h) in essentially quantitative crude yield[4]. This compound did not show decomposition (as determined by TLC) after several days of standing at room temperature. Active ester 3 was then coupled with L-[4,5-^{3}H]proline (3.8 mCi/mmol) (EtOH-H_2O, $NaHCO_3$, 20°C, 18h)[4] to give crude 4 (84%) as a diastereomeric mixture. The desired SSS epimer was selectively precipitated from acetonitrile as its maleate salt (5), and isolated in 27% yield (based on compound 2), with a specific activity of 4.0 mCi/mmol.

The radiochemical purity of 5 was tested by HPLC analysis. Thus, collection and counting of the fractions corresponding to enalapril free base gave a radiochemical purity value for this sample of 99.7%. Furthermore, this analysis indicated less than 1% of the undesired RSS isomer.

EXPERIMENTAL

Methods. ^{1}H NMR spectra were recorded on a Varian T-60 spectrometer or on a Varian XL-200 spectrometer. Infrared spectra were recorded on a Perkin-Elmer 983 Infrared Spectrophotometer. Combustion analyses were performed with a Carlo Erba 1106 Elemental Analyzer. Analytical thin layer chromatography (TLC) was performed using Merck silica gel 60 F_{254} plates. L-[4,5-^{3}H]proline (5 mCi) was purchased from the "Service des Molécules Marquées" (CEN Saclay). HPLC analyses were performed on a Waters apparatus, equiped with a UV detector using a C-18 μ-bondapak column heated at 54°C, with a solvent mixture consisting of 40% methanol and 60% of a 0.005M tetra-n-butylammonium phosphate buffer (pH 7.2). Scintillation counting was carried out using a Beckman Model 3800 Scintillation Counter fitted with automatic external standardisation.

Ethyl 2-oxo-4-phenylbutanoate (1). An oven-dried, 3-necked, 1-L round-bottom flask equiped with a mechanical stirrer, a constant pressure addition funnel, and a condenser connected to a nitrogen bubbler, was charged with magnesium turnings (7.29 g, 0.3 mol) . Nitrogen was flushed through the system and dry tetrahydrofurane (150 ml) was introduced, followed by few more crystals of iodine. A solution containing 2-phenylethyl bromide (55.5 g, 0.3 mmol) in dry tetrahydrofurane (150 ml) was then added. After addition of ca 5 ml of this solution, the flask was heated until the reaction was initiated, as monitored by the disappearance of the orange coloration. The rest of the solution was added at a rate that allowed gentle refluxing of the mixture. When the addition was complete, the

mixture was stirred at 60°C for 30 min. The clear solution was alllowed to cool to room temperature and then it was cannulated to a cooled (-10°C) solution containing diethyl oxalate (87.6 g, 0.6 mmol) in dry tetrahydrofurane (180 ml) under a nitrogen atmosphere.

After the addition was complete, the mixture was stirred at -10°C for 30 min, and then quenched by the addition of 3N hydrochloric acid until pH 3 was achieved. The mixture was concentrated in vacuo and the residue partitioned between water (200 ml) and dichloromethane (300 ml). The organic phase was dried over anhydrous sodium sulfate, filtered and concentrated in vacuo to a yellowish liquid.

Distillation under vacuum (0.1 mm Hg) gave two fractions : the first (42.13 g, bp 35°C) consisting of diethyl oxalate, and the second (42.43 g, 68%, br 99°-102°C) being the desired product as a yellowish liquid : Rf 0.33 (20% ethyl acetate:hexane); IR (film) 2984, 1750, 1727, 1249, 1067 cm^{-1}; ^{1}H-NMR (60 MHz, CCl_4) δ (TMS) 7.3 (s, 5H, arom.), 4.2 (q, J=7, 2H, OCH_2CH_3), 3.1-2.8 (m, 4H, CH_2CH_2), 1.3 (t, J=7, 3H, OCH_2CH_3).
Elemental analysis calculated for $C_{12}H_{14}O_3$: C, 69.88; H, 6.84. Found : C, 69.45; H, 6.90.

N-(1(R,S)-(ethoxycarbonyl)-3-phenylpropyl)-L-alanine (2). A dried, two-necked, 100 ml flask fitted with a calcium chloride tube was charged with L-alanine (891 mg, 10 mmol) and ethyl 2-oxo-4-phenylbutanoate (10.3 g, 50 mmol). Glacial acetic acid (20 ml), anhydrous ethanol (8 ml) and finely powdered 4A molecular sieves (5 g) were added and the mixture was stirred at room temperature for 30 min. Then, a solution of sodium cyanoborohydride (1.25 g, 20 mmol) in anhydrous ethanol (5 ml) was added dropwise, during 5 h. The resulting mixture was stirred vigorously at room temperature for 17 h, and then filtered. The filtrate was concentrated in vacuo. Toluene (40 ml) was added and the solvents were azeotropically removed in vacuo. This last operation was repeated a second time, and the resulting oil was treated with water (140 ml) and chloroform (120 ml). The mixture was cooled to 0°C and the pH adjusted to 8.5 with 6N sodium hydroxide solution. The organic phase was decanted (emulsions tend to appear) and the aqueous phase acidified at 0°C to pH 4.3 with 5N hydrochloric acid solution. The aqueous phase was then extracted with chloroform (5x75 ml) and the combined organic layers were dried over anhydrous sodium sulfate, filtered and concentrated to afford a white solid (2.65 g). HPLC analysis under the above stated conditions indicated a SS:RS diastereomeric ratio of 62:38 (retention times 6.72 and 8.79 min

respectively) (Note 1). This solid was recrystallized from hot ethyl acetate-hexane. The white precipitate was filtered, washed with hexane (50 ml) and dried (vacuum, 10h) to yield 1.59 g (57%) of the title compound as white crystals. HPLC analysis indicated a SS:RS ratio of 65:35 : mp 121.1-124.4°C; IR (KBr) 3465, 2938, 1743, 1606, 1452, 1396, 1360, 1342, 1216, 1169, 858, 755 and 561 cm^{-1}; ^{1}H NMR (60 MHz, $CDCl_3$) δ (TMS) 8.2 (br s, 2H), 4.2 (q, J=7, 2H), 4.4-3.2 (m, 2H), 3.0-2.5 (m, 2H), 2.5-1.8 (m, 2H), 1.45 (br d, 3H), 1.27 (t, J=7, maj. isom.), 1.24 (t, J=7, min. isom.). An analytical sample of the SS isomer (98 + %) was obtained after two recrystallizations in hot acetonitrile : pf 148.1-148.9°C; ^{1}H NMR (200 MHz, $CDCl_3$) δ (TMS) 7.3-7.1 (m, 5H, arom), 4.4 (br s, 2H, NH, CO_2H), 4.21 (q, J=7.1, 2H, $C\underline{H}_2$ CH_3), 3.36 (dd, J=5.8, J=7.0, 1H, $CHCO_2Et$), 3.24 (q, J=7.2, 1H, $C\underline{H}CH_3$), 2.8-2.7 (m, 2H, $PhC\underline{H}_2CH_2$), 2.0-1.6 (m, 2H, $PhCH_2C\underline{H}_2$), 1.44 (d, J=7.2, 3H, $CHC\underline{H}_3$), 1.29 (t, J=7.1, 3H, $CH_2C\underline{H}_3$).
Elemental analysis calculated for $C_{15}H_{21}NO_4$: C, 64.49; H, 7.58; N, 5.01. Found : C, 63.94; H, 7.63; N, 5.04.

Note 1 : The SS absolute configuration of the major isomer of 2 was established by correlation with that of the major isomer of 4, which is known to be SSS.

N-(1(R,S)-(ethoxycarbonyl)-3-phenylpropyl)-L-alanine, N'-hydroxysuccinimide ester (3). To a cold (0°C) suspension of N-(1(R,S)-(ethoxycarbonyl)-3-phenylpropyl)-L-alanine (500 mg, 1.79 mmol) and N-hydroxysuccinimide (207 mg, 1.8 mmol) in 1,2-dimethoxyethane (5 ml) was added N,N'-dicyclohexylcarbodiimide (DCC) (371 mg, 1.8 mmol) at once. The mixture was stirred a 0°C for 1.5 h and let stand at 4°C overnight. The mixture was filtered through a column containing celite and the filtrate diluted with chloroform (35 ml). The organic phase was washed with a 10% solution of sodium carbonate (3x15 ml), dried over anhydrous sodium sulfate and concentrated in vacuo to afford of a colorless oil (664 mg, 98%) : Rf 0.63 (ethyl acetate); ^{1}H NMR (60 MHz, $CDCl_3$) δ (TMS) 7.3 (s, 5H), 4.18 (q, J=7, 2H), 4.0-3.6 (m, 1H), 3.6-3.2 (m, 1H), 3.0-2.5 (m, 2H), 2.80 (s, 4H), 2.3-1.6 (m, 2H), 1.50 (d, J=7, CH_3 major isomer), 1.47 (d, CH_3, minor isomer), 1.23 (t, J=7, 3H).

1-(N-(1(S)-(ethoxycarbonyl)-3-phenylpropyl)-L-alanyl)-L-[4,5-^{3}H]proline, maleate salt (5). Into a 25-ml, round-bottom flask was placed L-proline (149 mg, 1.3 mmol) and sodium bicarbonate (218 mg, 2.6 mmol). By means of a syringe, a solution containing L-[4,5-^{3}H]proline (5 mCi) in water (5 ml) stabilized with 2% ethanol was added. The resulting clear solu-

tion was treated with a solution containing 3 (489 mg, 1.3 mmol) in ethanol (2 ml) (+1 ml ethanol rinse) and the resulting cloudy mixture stirred for 20 h at room temperature. The solvents were evaporated (rotavapor, 30-40°C, ca 10 min) and water (15 ml) was added. The solution was transferred to a constant pressure separation funnel containing chloroform (15 ml) and equiped with a mechanical stirrer. The mixture was stirred and the aqueous phase was collected in a 50 ml dish containing a magnetic bar. The *pH* was then adjusted to 2.7 by the slow addition of 1N hydrochloric acid solution and the solution was saturated with sodium chloride. The clear solution was then transferred to the separation funnel and extracted with chloroform (5x15 ml). The collected organic phases were dried over anhydrous sodium sulfate, filtered, concentrated in vacuo and dried (0.1 mm, 2.5 h, 20°C) to afford of a colorless paste (420 mg, 84%).

This paste was dissolved in acetonitrile (0.8 ml) and treated with a warm solution containing maleic acid (138 mg, 1.2 mmol) in acetonitrile (1.5 ml). A white solid precipitated after 10 min. The mixture was stirred overnight at room temperature and then filtered, washed with cold acetonitrile (1 ml) and ether (2 ml), and dried (0.1 mm, 2 h, 20°C) to afford 5 as a white, crystalline powder (174 mg, 27%).

Determination of the chemical and radiochemical purity of 5. Analysis by HPLC indicated 5 to be 99.2% chemically pure by comparison with a sample of enalapril maleate recrystallized two times from hot acetonitrile. The eluent conditions were chosen such that separation of the two diastereomers (SSS and RSS) was guaranteed, as previously determined for an authentic sample. Under these conditions, no trace of the RSS isomer was detected in the radioactive sample. The retention times for these epimers were 8.25 and 10.00 min respectively. In order to determine the radiochemical purity of the sample, fractions were collected from the chromathograph. The fractions corresponding to the enalapril peak added 99.7% of the total initial radioactivity.

References

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