SYNTHESIS OF N-[N-[(S)-1-ETHOXY[^{14}C]CARBONYL-3-PHENYL[1-^{14}C]PROPYL]-L-ALANYL]-N-(INDAN-2-YL)GLYCINE HYDROCHLORIDE ([^{14}C]CV-3317)

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SUMMARY

N-[N-[(S)-1-Ethoxycarbonyl-3-phenylpropyl]-L-alanyl]-N-(indan-2-yl)glycine hydrochloride (CV-3317), a new potent angiotensin converting enzyme inhibitor, was labeled with carbon-14. Diethyl [U-^{14}C]oxalate was condensed with ethyl 3-phenylpropionate, subsequently decarboxylated to yield ethyl [1,2-^{14}C]-2-oxo-4-phenylbutyrate. The reductive condensation of the latter with tert-butyl N-L-alanyl-N-(indan-2-yl)glycinate followed by hydrolysis afforded [^{14}C]CV-3317 in an overall radiochemical yield of 17.4 %.

Key Words: labeled compound, carbon-14, CV-3317, angiotensin converting enzyme inhibitor

INTRODUCTION

N-[N-[(S)-1-Ethoxycarbonyl-3-phenylpropyl]-L-alanyl]-N-(indan-2-yl)glycine hydrochloride (CV-3317) is a potent angiotensin converting enzyme inhibitor presently under development as an antihypertensive agent.[1-3] Carbon-14 labeled CV-3317 was prepared for studying its metabolic transformations and distribution in test animals.

RESULTS

Diethyl [U-^{14}C]oxalate (1) was condensed with ethyl 3-phenylpropionate using sodium ethoxide to yield ethyl 3-ethoxycarbonyl-[1,2-^{14}C]-2-oxo-4-phenylbutyrate (2). In our trial runs using

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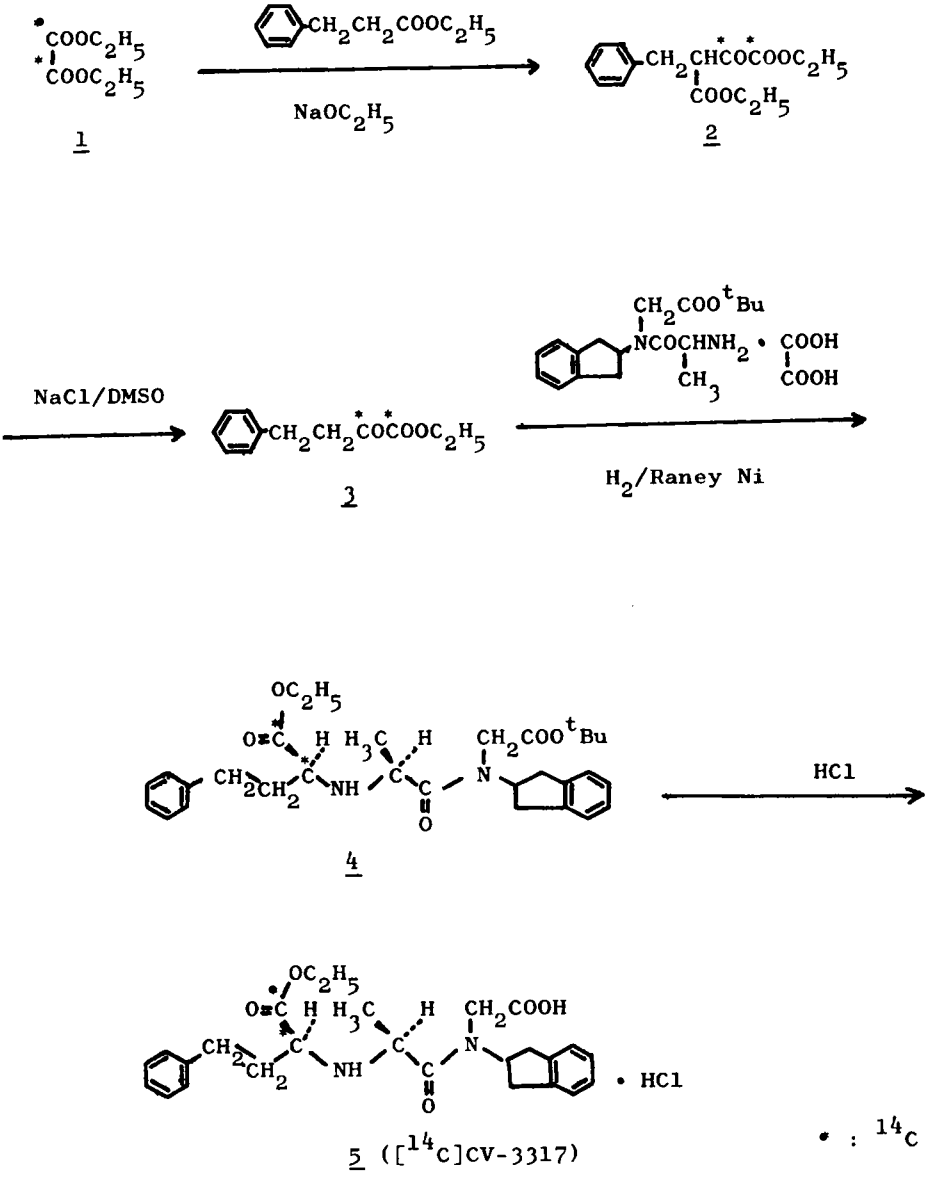

1 2 3 4 5 ([14C]CV-3317)

Scheme 1

unlabeled materials, ethyl 2-oxo-4-phenylbutyrate was synthesized by the hydrolysis and decarboxylation of ethyl 3-ethoxycarbonyl-2-oxo-4-phenylbutyrate and subsequent esterification of resulting 2-oxo-4-phenylbutyric acid. However, one-step decarboxylation of 2 with sodium chloride in aqueous dimethylsulfoxide[4] was found

to be superior for the synthesis of ethyl [1,2-^{14}C]-2-oxo-4-phenylbutyrate (3). The reductive condensation of 3 with tert-butyl N-L-alanyl-N-(indan-2-yl)glycinate in the presence of Raney Ni gave a diastereoisomeric mixture of tert-butyl N-[N-[1-ethoxy[^{14}C]carbonyl-3-phenyl[1-^{14}C]propyl]-L-alanyl]-N-(indan-2-yl)glycinate (4) in which S form was produced predominantly. Each isomer could be separated by column chromatography. In the hot run, the diastereoisomeric mixture of 4 was subjected to the next step without further purification. The tert-butyl group in 4 was removed with saturated solution of hydrogen chloride in ethyl acetate at room temperature. The resulting diastereoisomeric mixture (R/S=7/93) was recrystallized three times to give a pure S form of [^{14}C]CV-3317 (338 MBq). This compound had a specific activity of 839 MBq/mmol and 99.6 % of radiochemical purity. The content of R isomer of [^{14}C]CV-3317 was less than 0.4 % by high performance liquid chromatography (HPLC) analysis.

EXPERIMENTAL

Diethyl [U-^{14}C]oxalate with a specific activity of 1.37 GBq/mmol was purchased from Amersham Japan Ltd. The unlabeled intermediates and final product, used as references for comparison with the corresponding labeled compounds, were synthesized by the same division of Takeda Chemical Industries Ltd. HPLC analyses were performed on a system consisting of a Nucleosil 5C_{18} column (4x150 mm), a Shimadzu PCP-841 pump and UV detector (254 nm) using a mobile phase of 0.1 M KH_2PO_4:CH_3CN (55:45). A t_R value for CV-3317 was 9.09 min at a flow rate of 0.47 ml/min. Liquid scintillation counting was done with an Aloka LSC-671 Liquid Scintillation Spectrometer.

Ethyl 3-ethoxycarbonyl[1,2-^{14}C]-2-oxo-4-phenylbutyrate (2)

Diethyl [U-^{14}C]oxalate (1, 1.94 GBq, 1.4 mmol) was diluted with unlabeled diethyl oxalate (160 mg, 1.1 mmol). Ethyl 3-phenyl-

propionate (515 mg, 2.9 mmol), dry benzene (10 ml) and 1 M NaOEt in EtOH (3.0 ml, 3.1 mmol) were added to the above mixture. The reaction mixture was stirred at 90°C for 0.5 h. The solvent was removed in vacuo at 70°C. The resulting residue was diluted with cold water. The aqueous solution was acidified (pH 3) with 2 N HCl and extracted with CH_2Cl_2 (25 ml). The organic phase was separated and concentrated in vacuo to yield yellow oil of 2 (729 mg).

Ethyl [1,2-^{14}C]-2-oxo-4-phenylbutyrate (3)------- A mixture of 2 (729 mg), NaCl (370 mg) and 90 % DMSO (7 ml) was heated at 135°C for 1.3 h with stirring. After cooling, the reaction mixture was diluted with AcOEt (20 ml). The AcOEt solution was washed with saturated aqueous NaCl and dried over Na_2SO_4. After evaporating off the solvents in vacuo, benzene (10 ml) was added to the resulting residue and the solvents were removed azeotropicaly in vacuo to yield brown oil of 3 (502 mg).

tert-Butyl N-[N-[1-Ethoxy[^{14}C]carbonyl-3-phenyl[1-^{14}C]propyl]-L-alanyl]-N-(indan-2-yl)glycinate (4)------- A mixture of 3 (502 mg), tert-butyl N-L-alanyl-N-(indan-2-yl)glycinate oxalate (1.37 g, 3.15 mmol), powdered molecular sieve 3A (2 g), anhydrous AcONa (0.52 g, 6.34 mmol) in AcOH (0.5 ml) and EtOH (20 ml) was subjected to catalytic reduction using Raney Ni (wet weight 4 g) at room temperature under one atmosphere of H_2 gas. After completion of absorption of the theoretical amount of H_2 gas, EtOH solution was decanted and the residue was washed three times with each 30 ml of EtOH. The combined EtOH solution was concentrated in vacuo to 10 ml. To the resulting residue, water (30 ml), AcOEt (30 ml) and $NaHCO_3$ (1.5 g) were added. The mixture was filtered through Celite (5 g) to remove insoluble materials. The AcOEt phase was separated and dried over Na_2SO_4. The solvent was removed

in vacuo to give greenish brown oil of a diastereoisomeric mixture of 4 (1.3 g).

N-[N-[(S)-1-Ethoxy[^{14}C]carbonyl-3-phenyl[1-^{14}C]propyl]-L-alanyl]-N-(indan-2-yl)glycine Hydrochloride (5, [^{14}C]CV-3317)----

A solution of 4 in HCl-AcOEt (1.533 g of HCl gas bubbled in 7 ml of AcOEt) was allowed to stand for 2.5 h at room temperature. The insoluble materials were removed by filtration and the filtrate was diluted with Et_2O (30 ml). The resulting precipitates were filtered to give pale green powder (682 mg). The powder was recrystallized twice from a mixed solvent of MeOH (1 ml) and AcOEt (10 ml) to yield colorless powder (258 mg). Finally, this powder was dissolved in a mixture of acetone (1 ml), water (2.5 ml) and 2 N HCl (2.5 ml) and allowed to stand for 1 h in a refrigerator. The crystals separated were filtered to afford colorless plates of 5 ([^{14}C]CV-3317, 197 mg, 338 MBq). The radiochemical yield of 5 was 17.4 % based on diethyl [U-^{14}C]oxalate. The radiochemical and chemical purities of 5 were 99.6 % and 99.3 %, respectively, by HPLC analyses. The specific activity of 5 was determined as 839 MBq/mmol.

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