Synthesis of ^{14}C-labelled 2-[2-cyclohexylcarbonyloxy-3-{4-(2-pyridyl)piperazin-1-yl}propyl]-3aα,4β,7β,7aα-tetrahydro-4,7-methano-isoindole-1,3(2H)-dione dihydrochloride(E-0713)

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SUMMARY

A ^{14}C-labelled form of the antidiabetic compound E-0713 was synthesized for pharmacokinetic and metabolic studies in six steps, using maleic anhydride [2,3-^{14}C] as the labelled starting material.

Key words:Carbon-14, E-0713, Antidiabetic agent.

INTRODUCTION

E-0713[1] has been found to be effective in suppressing hyperglycemia caused by a glucose load in Streptozotocin-induced sub-diabetic animals (rats and mice) and genetically diabetic mice (obese-KK and non-obese-NSY). While sulfonylureas cause severe hypoglycemia, E-0713 does not alter the basal glucose level. Moreover, biguanides increase blood lactate levels, whereas E-0713 does not.[2]

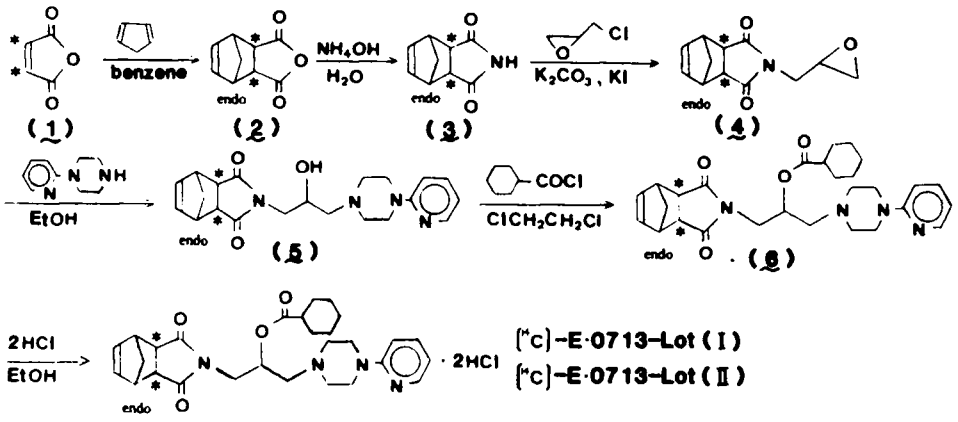
In order to fully investigate the pharmacokinetics and metabolism of E-0713, a radiolabelled form of it had to be synthesized.

0362-4803/85/080761-05$01.00

Received November 27, 1984
Revised January 7, 1985

METHODS AND RESULTS

The following scheme outlines the methods of synthesis employed:



*Indicates sites of labelled atoms of ^{14}C

Cyclopentadiene was added to maleic anhydride [2,3-^{14}C] (1) in benzene to give (2)[3], which was then transformed into (3) under reflux in ammonium hydroxide solution. Treatment of (3) with potassium carbonate in epichlorohydrin afforded (4), which was without purification reacted with 1-(2-pyridyl) piperazine in ethanol to yield (5). Reaction of (5) with cyclohexanecarbonyl chloride in 1,2-dichloroethane gave (6), which was treated with hydrochloric acid in ethanol to afford the corresponding dihydrochloride salt [^{14}C]-E-0713-Lot(I), which had a radiochemical purity of 97.9% and a specific activity of 4.87μCi per mg. The total chemical yield was 37.9%.

We also synthesized [^{14}C]-E-0713-Lot(II) with (3) as the starting material in 75.3% chemical yield. [^{14}C]-E-0713-Lot(II) had a radiochemical purity of 96.9% and a specific activity of 44.9μCi per mg.

The structure of [^{14}C]-E-0713 was confirmed by comparison (UV spectrum and TLC) with an unlabelled authentic specimen of E-0713.

EXPERIMENTAL

Maleic anhydride [2,3-^{14}C] (1) was purchased from New England Nuclear Corp.. Measurements of radioactivity were carried out using an Aloka LSC-700 type Liquid Scintillation Counter. The radiochromatograms were recorded using an Aloka Thin Layer Chromatogram Scanner Model TLC-101, and the UV spectra on a Shimazu MPS-5000 type Spectrophotometer.

3aα,4β,7β,7aα-Tetrahydro-4,7-methano-isobenzofuran-1,3-dione [3a,7a-^{14}C] (2)

Freshly distilled cyclopentadiene (0.5 ml, 6 mmol) was added gradually, with cooling, to a suspension of maleic anhydride [2,3-^{14}C] (1) (490 mg, 5 mmol, its specific activity was not measured, for it is apt to sublime) in benzene (10 ml). After stirring for 30 minutes, the reaction mixture was concentrated to about 3 ml, and n-hexane (16 ml) was added. The separated product was filtered, and then dried under vacuum (710 mg, 4.33 mmol).

3aα,4β,7β,7aα-Tetrahydro-4,7-methano-isoindole-1,3(2H)-dione [3a,7a-^{14}C] (3)

Ammonia water-28% (4 ml) and water (1.9 ml) were added to (2) (710 mg, 4.33 mmol), and heated under reflux for 5 hours. The reaction mixture was concentrated to about 1 ml, and the crystals produced were filtered, washed with a small amount of water, and then vacuum-dried (460 mg, 2.82 mmol).

2-(2,3-Epoxypropyl)-3aα,4β,7β,7aα-tetrahydro-4,7-methano-isoindole-1,3(2H)-dione [3a,7a-^{14}C] (4)

Potassium carbonate (2.8 g) and a catalytic amount of potassium iodide were added to (3) (460 mg, 2.82 mmol) in epichlorohydrin (14 ml), and heated at 60° - 64 °C for 5 hours. After the inorganic materials were filtered off, the filtrate was evaporated to give (4), which was used for the next stage.

2-[2-Hydroxy-3-{4-(2-pyridyl) piperazin-1-yl}propyl]-3aα,4β,7β,7aα-tetrahydro-4,7-methano-isoindole-1,3(2H)-dione [3a,7a-^{14}C] (5)

A mixture of 1-(2-pyridyl) piperazine (480 mg, 2.94 mmol) and crude (4) in ethanol (5.5 ml) was heated under reflux for 2 hours. The reaction mixture was cooled (which caused it to

crystallize) and then filtered. The crystals obtained were washed with a small amount of cold ethanol and dried under vacuum.(840 mg, 2.20 mmol)

2-[2-Cyclohexylcarbonyloxy-3-{4-(2-pyridyl) piperazin-1-yl} propyl]-3aα,4β,7β,7aα-tetrahydro-4,7-methano-isoindole-1,3 (2H)-dione[3a,7a-^{14}C] dihydrochloride ([^{14}C]-E-0713-Lot(I))

Cyclohexanecarbonyl chloride (1.5 g, 10.2 mmol), dissolved in 1,2-dichloroethane (20 ml), was added to (5) in 1,2-dichloroethane (60 ml) and heated under reflux for 8 hours. After the solvent was evaporated, ether (40 ml) and 0.0625N -hydrochloric acid (32 ml) were added to the residue, and the mixture was stirred for 10 minutes. The solution was then transferred to a separatory funnel, and the aqueous layer was basified with 1.75N-sodium hydroxide (20 ml). After extracting with chloroform (40 ml), the extract was washed with water, dried over $MgSO_4$, filtered and evaporated to give crude (6). Concentrated hydrochloric acid (500 mg) in ethanol (4 ml) was added to the residue dissolved in hot ethanol, and the solution was cooled. The separated crystals were collected, washed with a small amount of cold ethanol, and dried in vacuum to afford [^{14}C]-E-0713-Lot (I) [1.088 mg, 1.93 mmol, 37.9% yield from (1); specific activity 4.87 μCi per mg ; λ max MeOH:H_2O = 3:1, nm:252,320 (Fig.1) ; on TLC (Kieselgel 60 F_{254}, Merck), a single radioactive peak appeared at RF coincident with that of a fluorescent spot due to an unlabelled authentic specimen of E-0713 detected under an UV lamp (Fig.2) ; the radiochemical purity was found by TLC to be 97.9%]

[^{14}C]-E-0713-Lot (II) was prepared by the above method except that (5) was isolated by column chromatography (C-200,

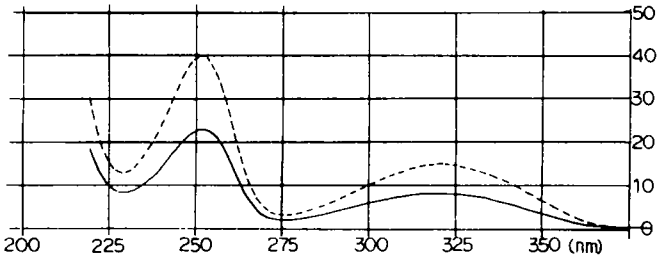


Fig.1 UV spectra of [^{14}C]-E-0713-Lot(I) ——— and unlabelled authentic E-0713 ------

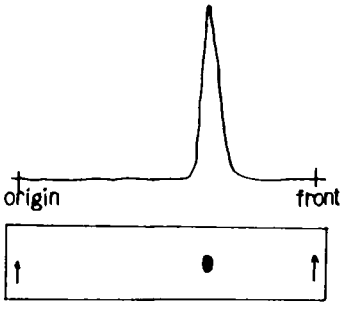


Fig.2 Radioscan of [^{14}C]-E-0713-Lot(I) on TLC developed with n-butanol : acetic acid : water = 5 : 2 : 2

Wako.; eluting with chloroform : ethanol = 97.2 : 2.8) with [3a, 7a-^{14}C]-(3) (specific activity was 171uCi/mg) purchased from Amersham Japan as the starting material. [^{14}C]-E-0713 weighed 304 mg and had a radiochemical purity of 96.9% and a specific activity of 44.9 μCi per mg. The chemical yield was 75.3% and the radiochemical yield was 68.0%.

ACKNOWLEDGMENTS

The authors wish to thank Mr.M.Yamanaka and Dr.T.Horie for their helpful advice and interest. We are also grateful to Miss C.Takashima for preparing this manuscript.

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