Syntheses of Two Kinds of ^{14}C-Labelled 4-(N-Benzoyl-L-Tyrosyl)Aminobenzoic Acids

H. Yoshino, Y. Tsuchiya, T. Sato, K. Kinoshita and M. Uchiyama

Eisai Research Laboratory, Eisai Co., Ltd.

Koishikawa 4-6-10, Bunkyo-ku, Tokyo, Japan

Received June 7, 1977

Summary

4-(N-benzoyl-L-tyrosyl)aminobenzoic acid, a diagnostic agent for a pancreatic exocrine function test, was labelled with carbon-14. The synthetic procedure of 4-(N-benzoyl-L-tyrosyl)-aminobenzoic[carboxy-^{14}C] acid (I) and 4-(N-benzoyl[carbonyl-^{14}C]-L-tyrosyl)aminobenzoic acid (II) is described.

The overall radiochemical yields for (I) and (II) were 75.4% and 22.5%,respectively.

Key Words

4-(N-benzoyl-L-tyrosyl)aminobenzoic acid, Carbon-14, Pancreatic exocrine function test.

Introduction

4-(N-benzoyl-L-tyrosyl)aminobenzoic acid which comprises a sensitive peptide linkage to tracer aminobenzoic acid was shown in rat test to possess considerable discrimination for in vivo chymotrypsin activity when ingested [1] and it has therefor been developed as a diagnostic agent for a pancreatic exocrine function test. In order to study the metabolic, pharmacological and other biochemical aspects of this compound, its radioactive forms were required. We synthesized two kinds of ^{14}C-labelled compounds, 4-(N-benzoyl-L-tyrosyl)aminobenzoic[carboxy-^{14}C] acid and 4-(N-benzoyl[carbonyl-^{14}C]-L-tyrosyl)aminobenzoic acid.

0362-4803/78/0015-0001$01.00

Results

Synthesis of 4-(N-benzoyl-L-tyrosyl)aminobenzoic[carboxy-^{14}C] acid (I)

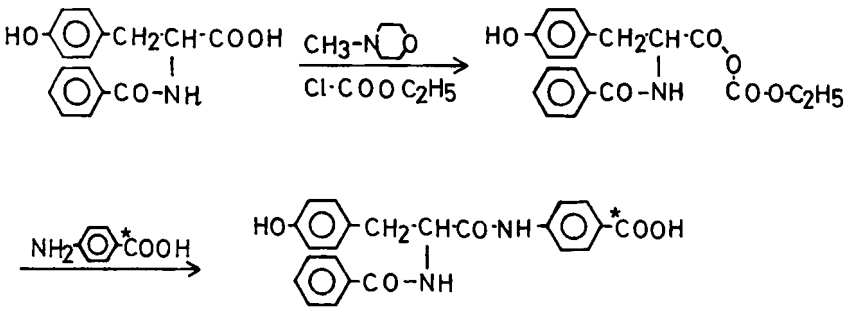
deBenneville reported 4-(N-benzoyl-L-tyrosyl)aminobenzoic acid was easily synthesized without racemization by the reaction of the mixed anhydride of N-benzoyl-L-tyrosine with p-aminobenzoic acid in the presence of a small amount of strong acid [1].

On the other hand, N-acyl-amino acids such as N-benzoyl-L-tyrosine generally show tendency to racemize more or less during peptide bond formation [2].
Therefor, the labelled compound (I) was synthesized by a method similar to that described above, after considerable investigations especially to confirm its optical purity (Scheme I). Table I shows the specific rotation of the products obtained from cold experiments on the same scale. The radio chemical yield of (I) thus obtained was 75.4%.

Table I Specific rotation of the products in the cold experiments on the same scale

$[\alpha]_D^{25}$ (C=1,DMF)
+82.2
+83.5
+85.8
+87 [lit. 1)]

Scheme I



(I)

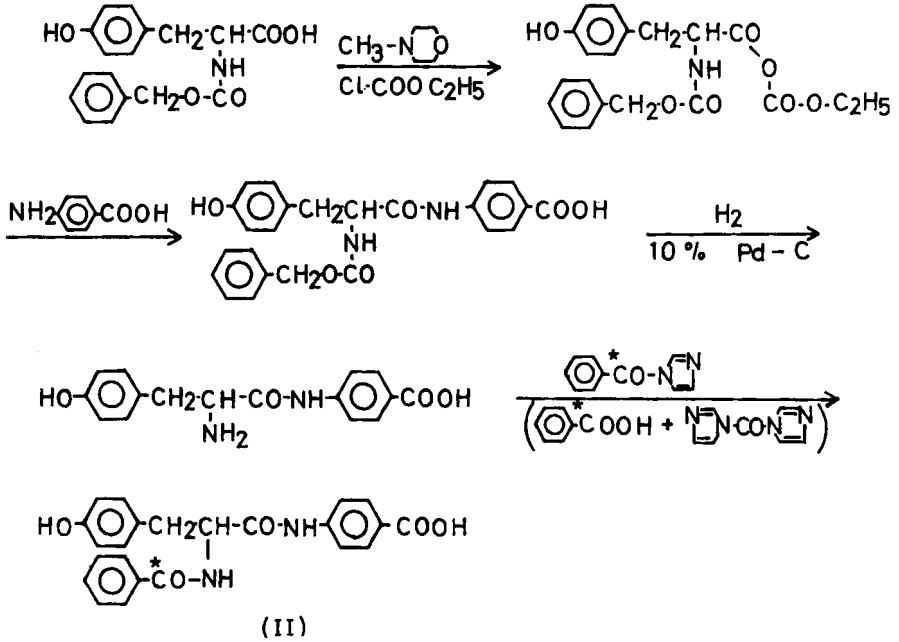
Synthesis of 4-(N-benzoyl[carbonyl-^{14}C]-L-tyrosyl)aminobenzoic acid (II)

The title compound (II) was synthesized by the reaction of reactive derivative of benzoic[carboxy-^{14}C] acid with L-tyrosyl-p-aminobenzoic acid since labelling of compounds is usually the most effective at the final step in the synthetic route. As a agent for activation of benzoic[carboxy-^{14}C] acid mixed anhydride, Woodward reagent K [3] and DCC-HOSu [4] were initially tried but difficulty was encountered in the purification of the products. Another agent N,N'-carbonyldiimidazole was then employed, and gave satisfactorily pure product (Scheme II). This provides a new method for synthesizing the title compound without racemization. The results of the cold experiments on the same scale are in Table II. The overall radiochemical yield was 22.5%.

Table II Specific rotation of the products in the cold experiments on the same scale

$[\alpha]_D^{25}$	(C=1, DMF)
+86.6	
+83.0	
+89.5	
+87	[lit. 1)]

Scheme II



(II)

Experimental

Materials and Methods

p-Aminobenzoic[carboxy-^{14}C] acid(55 mCi/mmole) and benzoic[carboxy-^{14}C] acid (60.1 mCi/mmole) were purchased from The Radiochemical Centre, Amersham, UK. Tetrahydrofuran and dimethylformamide were dried over molecular sieve 4A.

Identity and purity of the labelled products were determined by thin-layer chromatography on silica gel(Kiesel gel GF_{254}, Merck). The solvents were 1) $CHCl_3$-EtOH-AcOH (90:5:5), 2) $CHCl_3$-isoAmylOH-AcOH (50:50:1). The spots were detected by UV light(254nm) and a radiochromatogram scanner, Aloka model TLC-2B.

Radioactivity was measured on a liquid scintilation spectrometry, using Aloka model LSC-601.

4-(N-benzoyl-L-tyrosyl)aminobenzoic[carboxy-^{14}C] acid (I)

N-benzoyl-L-tyrosine(200 mg, 0.7 mmole) was dissolved in tetrahydrofuran(2 ml) and the solution was cooled below -10°. 7.7% N-methylmorpholine in tetrahydrofuran (0.99 ml, 0.7mmole) and 6.7% ethyl chloroformate in tetrahydrofuran(1 ml, 0.7 mmole) were added with stirring. After 15 min a solution of p-aminobenzoic[carboxy-^{14}C] acid (96 mg, 0.7mmole, 20 mCi) in tetrahydrofuran(2 ml) was added, along with a solution of p-toluenesulfonic acid monohydrate(13.3 mg,0.07 mmole) in tetrahydrofuran(2 ml). The mixture was stirred below -10° for 3 hr. After kept at 5° overnight, the solvent was removed under reduced pressure and 0.2N-HCl(7 ml) was added. The white precipitate was filtered, washed with water(5 ml) and ether (3 ml). TLC indicated the product was to be radiochemically and chemically pure. The radiochemical yield and the specific activity were 15.08 mCi(75.4%) and 79.5 μCi/mg, respectively.

L-tyrosyl-p-aminobenzoic acid

Benzyloxycarbonyl-L-tyrosine(31.5 g, 0.1 mole) was dissolved in tetrahydrofuran (200 ml),the solution was cooled to -15°,and N-methylmorpholine(11 ml, 0.1 mole)

and ethyl chloroformate(10.9g, 0.1 mole) were added. After 15 min a solution of p-aminobenzoic acid(13.7g, 0.1 mole) in tetrahydrofuran(50 ml) was added,along with a solution of p-toluenesulfonic acid monohydrate(1.9g, 0.01 mole) in tetrahydrofuran (10 ml). The mixture was stirred at 0 to -10° for 3 hr. After kept at 5° overnight, the mixture was poured into cold 0.1N-HCl(3 l) and filtered. Then hydrogenated in the presence of 10%Pd-C(1.0g,) in a mixture of MeOH(250 ml) and N-HCl(25 ml). After 5 hr the catalyst was filtered and N-NaOH(25 ml) was added. The solvent was removed under reduced pressure and recrystallized from water. The yield was 15.0g (50 %). m p 170-174°, $[\alpha]_D^{28}$ + 85.0 (C=1, N-NaOH). Anal. Calcd. for $C_{16}H_{16}N_2O_4 \cdot 3/2H_2O$: C,58.71; H,5.85 ; N,8.56 . Found : C,58.99; H,5.82 ; N,8.45 .

4-(N-benzoyl[carbonyl-^{14}C]-L-tyrosyl)aminobenzoic acid (II)

A mixture of benzoic[carboxy-^{14}C] acid(20.6 mg, o.169 mmole,10 mCi) and non-radioactive benzoic acid(101.4 mg, 0.83 mmole) was dissolved in tetrahydrofuran (2 ml). To the solution was added N,N'-carbonyldiimidazole(162 mg, 1 mmole). After stirring at room temperature for 3 hr,cooled to -15°, then a solution of L-tyrosyl-p-aminobenzoic acid(300 mg, 1 mmole) in dimethylformamide(4 ml) was added. After kept at -15° for 6 days the mixture was poured into 0.1N-HCl(60 ml). The white precipitate was filtered,washed with water(5 ml) and ether(3ml). TLC indicated the product was to be radiochemically and chemically pure. The radiochemical yield and the specific activity were 2.25 mCi(22.5%) and 23.4 μCi/mg, respectively.

Acknowledgement

We wish to thank Mr. A. Yamagishi, Director of Research & Development Division and Mr. S. Toyoshima, Director of Department of Organic Chemistry,for their support of this research.

References

1) deBenneville, P.L., Godfreg, W. J. and Sims, H. J.-J. Med. Chem. 15:1098 (1972).

2) Bodanszky, M. and Ondetti, M.A.-Peptide Synthesis, Interscience Publishers, New York, 1966, p. 137.

3) Woodward, R. B., Olofson, R. A. and Mayer, H.-J. Am. Chem. Soc. 83: 1010 (1961).

4) Weygand, F., Hoffmann, D. and Wunsch, E.-Z. Naturforsch. 21b:426 (1966).