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論　文

〔2-^{14}C, 5-^{3}H〕シトシンの合成—III[1)]

朝野武美・桐谷玲子・藤田慎一

大阪府立放射線中央研究所
大阪府堺市新家町704

Synthesis of 〔2-^{14}C, 5-^{3}H〕 Cytosine using Bromine and Tritium Gas -III[1)]

Takeyoshi ASANO, Reiko KIRITANI and Shinichi FUJITA

Radiation Center of Osaka Prefecture
Shinke-cho, Sakai, Osaka, Japan.

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Abstract

A study for improving the yield in the synthesis of 〔2-^{14}C, 5-^{3}H〕 cytosine was made using a preferable condition for the reaction between 〔2-^{14}C, 5-Br〕 cytosine and tritium gas. Using 〔2-^{14}C〕 cytosine as the starting material, 29% of radioactive cytosine was recovered after bromination and tritiation. Tritium labelled in the product obtained was determined to 86%. Thus, the degree of double labelling was calculated as 70% on the basis of the radioactive content of the starting cytosine (81%).

1 Introduction

In the course of the studies on the chemical effects of the β-decay of tritium, the synthesis of double-labelled 〔2-^{14}C, 5-^{3}H〕 cytosine, a preliminary work, was previously reported in the presence of nonradioactive carriers[2)]. The tritium incorporated in cytosine, however, was only 3% of the expected yield. For our ultimate purpose, it was necessary to synthesize the double-labelled compound in a carrier free state. Recently, from the study on the Br-D exchange reaction[3)], we were able to know that pressure of tritium gas, relative amounts of Pd-catalyst and reaction temperature are important factors.

In the present work, we further tried to synthesize [2-^{14}C, 5-^{3}H] cytosine, giving consideration to those preferable conditions. Satisfactory results were obtained and the details are described in the following.

2 Experimental

Double-labelled [2-^{14}C, 5-^{3}H] cytosine was synthesized by bromination of [2-^{14}C] cytosine, followed by a catalytic exchange of Br-^{3}H. Principally the same technique as previously reported[2] was applied throughout the processes.

1. Synthesis of [2-^{14}C, 5-Br] cytosine.

[2-^{14}C, 5-Br] cytosine was synthesized as previously reported.[2] Commercial [2-^{14}C] cytosine (81% labelled, Moravek Biochemicals (California)) was used after purification by the HPLC method. 0.63mg (0.29mCi or 11 MBq) of the cytosine was mixed with 0.34 μl bromine dissolved in a mixture of CCl_4/water (73 μl/500 μl) and stirred for 1h at 0°C. Bromocytosine obtained was again purified by HPLC.

2. Synthesis of [2-^{14}, 5-^{3}H] cytosine.

0.49 mg of [2-^{14}C, 5-Br] cytosine (0.12mCi or 4.6MBq), and 0.39 ml (STP) of $^{3}H_2$ gas (1 Ci or 37 GBq, New England Nuclear Co.), 25 μl of 1N NaOH and 0.5 mg of Pd catalyst were introduced into a break seal ampoule (14 cm^3) and stirred for 1 hr at 15°C. After the reaction the catalyst was removed by centrifugation. The supernatant alkaline solution was neutralized, then evaporated to dryness. The recovered water contained 940 μCi (35 MBq) of tritium. Labile tritium in the products was removed by repetition of dissolution in water and subsequent evaporation.

Tririum gas remaining in the reaction vessel was removed by the adsorption on active charcoal at 77K. 0.053% of the radioactivity initially used was still remained in the vessel, which was observed by a tritium monitor.

3. Purification and analysis of products.

In each step of the synthesis, products were purified by an HPLC method (Column : reverse-phase Unisil Q C_{18} 7.6 × 300 mm (Gasukuro Kogyo Co.) ; mobile phase : 25 - 50% methanol).

Products were analyzed as previously reported.[2] Calculation of the data from the scintillation spectrometer was conveniently carried out by a deviced on-line system.

3 Rssults and Discussion

The study of the reaction of Br-D exchange[3] revealed that a somewhat mild

condition was desirable for obtaining a good yield of 〔5D-6H〕 cytosine. The present condition was chosen taking account of the result.

Figure 1 shows radio- and UV-chromatograms on HPLC obtained for products after bromination (A) and tritiation (B). Amounts of by-products were relatively large in bromination and very small in tritiation. The details are presented in Table I. The percentage distributions of bromocytosine and recovered cytosine were principally simillar to the previous data.[2)]

Table II represents amounts and radioactivities of products obtained in each process.

Recovery of cytosine after bromination and tritiation was 29%. The specific activity of ^{14}C (51 mCi/mmol) was not changed through out the experiments.

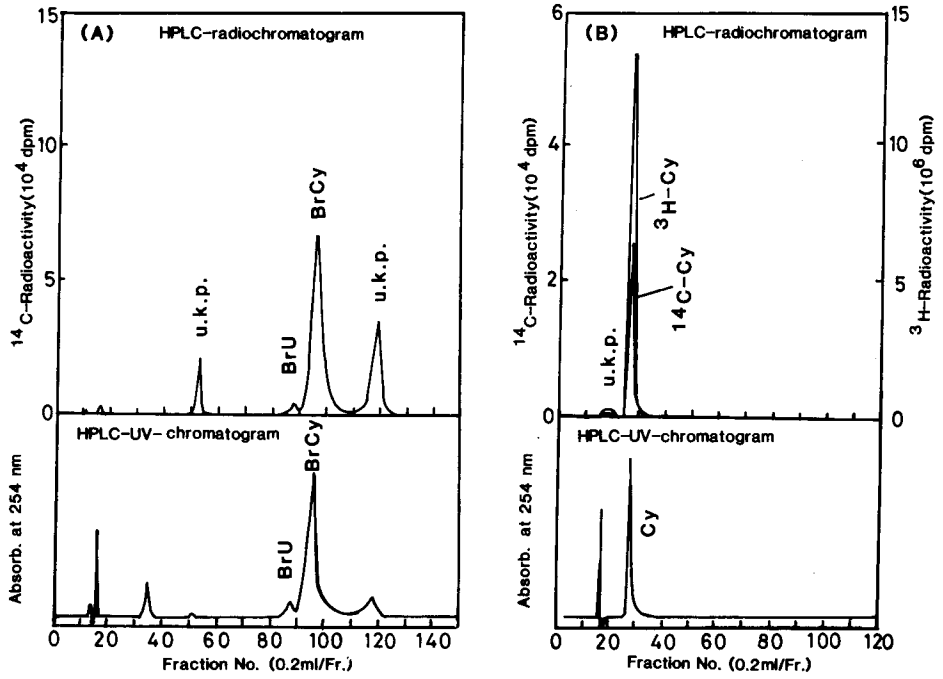

Fig. 1 Radio- and UV-chromatograms on HPLC

(A) : Bromination of 〔2-^{14}C〕 cytosine

(B) : Br-^{3}H exchange reaction between 〔2-^{14}C,5-Br〕cytosine and $^{3}H_2$ gas

Table I. Products distribution in bromination of [2-^{14}C] cytosine and in Br-^{3}H exchange reaction between [2-^{14}C,5-Br] cytosine and $^{3}H_2$ gas.

Analysis		Distribution, %			
		Cy	BrCy	BrU	uncapt. or u.k.p.
HPLC	UV	0	59	2	39
	^{14}C	0	59	3	35
HPLC	UV	90	0	—	10
	^{14}C	86	0	—	14
	^{3}H	90	0	—	10

Table II. Yields and radioactivities of products in bromination and Br-^{3}H exchange reaction.

Products	Wt./mg	^{14}C Act./mCi	^{3}H Act./Ci
[2-^{14}C,5-Br]Cy	0.63	0.17	———
[2-^{14}C,5-^{3}H]Cy	0.18	0.080	0.040

[2-^{14}C] cytosine (0.63mg, 0.29mCi) was used as starting material.

With ^{3}H, the specific activity of the end product was calculated to be 25 Ci/mmol, which corresponded to 86% of the labelling yield. Since the percentage of ^{14}C-labelling was 81%, the degree of double labelling was calculated to be 70%. This value is much better than that reported previously (~3%)[2]. The differnce in ^{3}H-labelling yield between these two experiments may be attributed to two reasons. The first reason is that the mixing of carrier free tritium gas with hydrogen gas may have been

insufficient in the previous study. Another reason is as follows. In the study of Br -D exchange reaction using D_2 gas by means of ^{1}H-NMR spectroscopy[3], appreciable amount (~15%) of 〔5-H, 6-H〕 cytosine was observed under an excessive reaction condition, although the origin of hydrogen atom was not evident. Unfavorale substance, 〔5-D,6-D〕 cytosine was further observed at high reaction temperature (80°C). Reduction in pressure of D_2 gas and in quantity of catalyst, and decrease of reaction temperature resulted in producing 〔5-D,6-H〕 cytosine effectively. In present study, tritium gas pressure was diminished by half (from 39 mmHg to 21 mmHg) in comparison with the previous study. The weight ratio of catalyst to 〔2-^{14}C,5-Br〕 cytosine was diminished by one third (from 3/1 to 1/1) . The reaction temperature decreased from 80°C to 15°C. The change of these reaction conditions may be the reason which brought about good results. The sample here will be supplied to our next plan for the study on the chemical effects of the tritium decay.

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