SYNTHESIS OF [2-^{14}C,5-^{3}H]CYTOSINE AND [2-^{14}C,5-^{3}H]URACIL VIA BROMINATION AND CATALYTIC BROMINE-TRITIUM GAS EXCHANGE

Takeyoshi ASANO and Reiko KIRITANI

Research Institute for Advanced Science and Technology,
University of OSAKA Prefecture.

1-2 Gakuen-cho, Sakai, Osaka 593, JAPAN.

SUMMARY

In micro-scale experiments, [2-^{14}C,5-^{3}H]cytosine and [2-^{14}C,5-^{3}H] uracil were synthesized via bromination and catalytic Br-^{3}H exchange reaction with the use of [2-^{14}C]-cytosine and -uracil and tritium gas. The double labelling percentages of these products were 70 and 26, respectively. It was assumed that [2-^{14}C,5-Br] uracil was subjected to reaction with hydrogen atom originally adsorbed on a palladium catalyst. This is to a lesser extent valid for [2-^{14}C,5-Br]cytosine. The percentages of ^{3}H labelling at 5 position of pyrimidine ring of cytosine and uracil were proved to be 96 and 73, respectively. For the analysis and purification of products, the HPLC eluting conditions using C18 reverse column and NaH_2PO_4 aqueous solution or H_2O/methanol mixture as eluent were studied. Unreacted tritium gas was recovered with the use of adsorbents such as active charcoal and Zr-V-Fe getter.

KEYWORDS: Double-labelling;[2-^{14}C,5-^{3}H]Cytosine;[2-^{14}C,5-^{3}H]Uracil;Tritium.

1. INTRODUCTION

The transmutation effects of tritium incorporated in nucleic acid bases have been studied from the viewpoint of hot atom chemistry and biological hazards [1,2]. In the present work we describe the synthesis of double labelled [2-^{14}C,5-^{3}H]pyrimidine bases.

The synthesis of tritium labelled compounds have been carried out by 1) hydrogen-lithium exchange using butyl-lithium and tritiated water [3], 2) hydrogen-lithium exchange using $LiAl^{3}H_4$ [4], 3) hydrogen exchange by tritium water promoted by $AlCl_3$ [5] and 4) catalytic halogen-tritium exchange using $CH_3COO^{3}H$ and Pt catalyst [6]. The micro-synthesis of carrier free labelled compound with radioactivity of mCi order is rather difficult when tritiated water or tritiated reagents is used.

On the other hand, catalytic halogen-tritium exchange using tritium gas and palladium on calcium carbonate catalyst [6] is easier in handling tritium. Another advantage of this method is in use of tritium gas which is readily available at high specific activity.

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Previously, the synthesis of [2-^{14}C,5-^{3}H]uracil, followed by bromination of [2-^{14}C]uracil was reported [7]. The method is not suited for the synthesis of [2-^{14}C,5-^{3}H]cytosine because of by-products formation (5-bromouracil is produced together with 5-bromocytosine). Furthermore, it was revealed that 5-bromocytosine reacted with deuterium gas to give the mixture of 5-D and 6-D cytosine [8]. In order to avoid such side-reactions and work out the best reaction condition for obtaining carrier free [5-^{3}H]cytosine, we examined in the present study the method of bromination and tritiation of [2-^{14}C]cytosine and [2-^{14}C]uracil under lower temperature than used in previous experiment [7]. As technical subjects, the HPLC separation procedure for the analysis and purification and the recovery of unreacted tritium gas on active charcoal were studied.

2. EXPERIMENTAL

2.1. Materials

[2-^{14}C]Cytosine (0.5 mCi, 51 mCi/mmol) was obtained from Moravek Biochemicals (Industry, Cal.), [2-^{14}C]uracil (1 mCi, 52 mCi/mmol) was from Amersham International plc (Amersham, England), and tritium gas (1 Ci) from Du Pont New England Nuclear Co. Ltd. (Boston, Mass.). The methanol used was HPLC grade and all other chemicals were analytical grade. All of these were commercially available from Wako Chemicals.

2.2. Bromination

The bromination in CCl_4 was compared with that in CCl_4/H_2O of mixed solvent by using nonlabelled cytosine. Detailed experimental conditions are shown in Table 1 (Runs 1-1 ~ 1-4). For the bromination of nonlabelled uracil, some micro-scale experiments were carried out at the same condition as Run 1-4 (Runs 2-1 ~ 2-3 in Table 1). Runs 1-5 and 2-4 in Table 1 were radioactive experiments. Pyrimidine base (cytosine, [2-^{14}C]cytosine, uracil or [2-^{14}C]uracil) and an equimolar amount of bromine dissolved in CCl_4 or CCl_4/H_2O were placed in an ampoule (1.5 ml) with a magnetic spin bar. The ampoule was cooled at -78 °C, and sealed off with flame. The reaction mixture was allowed to react under stirring with a magnetic stirrer. Then, the solvent was evaporated to dryness under reduced pressure. The solid remaining was washed with water 3 times and finally dissolved in water.

2.3. Hydrogenation

The Br-H, -D or -^{3}H exchange reaction for 5-bromocytosine, [2-^{14}C,5-Br]cytosine, 5-bromouracil or [2-^{14}C, 5-Br]uracil was carried out. The procedure of exchange reaction between 5-bromopyrimidine base and hydrogen gas (or deuterium gas) was similar to that described in previous paper [8]. Here a diminution in experimental scale of exchange reaction was furthermore studied. Detailed experimental conditions are shown in Table 2.

At the reaction between [2-^{14}C,5-Br]cytosine or [2-^{14}C,5-Br]uracil and tritium gas (Runs 3-7 and 4-5), a reaction vessel was specially designed as

shown in Fig. 1. Two break-seal ampoules containing tritium gas (1 Ci) and tritium adsorbent [in Run 3-7, granular active charcoal (Tsurumi-HC 30F) (1 g), in Run 4-5, Zr-V-Fe alloy deposited on the constantan (SAES Getters Co. Japan-St707) (1g), [9]] were attached to the reaction tube. The charcoal in

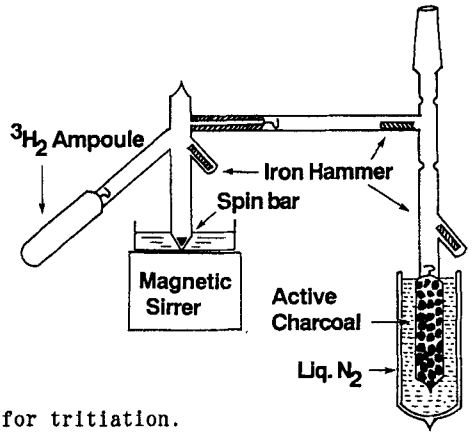

Figure 1. Reaction vessel for tritiation.

ampoule was previously degassed at 400 °C in vacuo (1 X 10^{-4} Torr) and the Zr-V-Fe getter was degassed at 780 °C in vacuo (5 X 10^{-6} Torr). [2-^{14}C, 5-Br]cytosine or [2-^{14}C, 5-Br]uracil dissolved in 0.3 N sodium hydroxide aqueous solution (25 µl) and 10 % palladium on calcium carbonate (10 % $Pd/CaCO_3$) catalyst which was also pre-degassed at 600 °C in vacuo (1 X 10^{-4} Torr) were placed in reaction tube with magnetic spin bar. This branched vessel was connected to a vacuum line, and sealed off with flame under reduced pressure at -78 °C. Then a break-seal tip was opened to lead tritium gas to the tube. After the reaction was completed, the vessel was connected again to a vacuum line with ground joint shown in Fig. 1 and sealed off with flame at the lower part of the joint under reduced pressure. The ampoule packed with active charcoal was cooled by liquid N_2 (using of Zr-V-Fe getter, adsorption was performed at room temperature) and two break-seal tips were opened. Unreacted tritium gas was adsorbed on the adsorbent for 15 min. Finally the adsorbent tube was sealed off.

Preliminary an absorptive capacity of charcoal was examined for hydrogen. Adsorption isotherm on charcoal of 1 g at 77 °K showed an equilibrium pressure of 3.2 - 0.015 Torr for the adsorption volum of hydrogen gas of 17 - 0.43 cm^3 STP, respectively. The experimental data were substituted to Dubinin-Astakhov equation [10]:

$$(W/W_o) = \exp(-A/E)^n \qquad (1)$$

where W : field volume of adsorption space (ml/1 g of adsorbent);
$W=q/\rho$; q is amount of adsorbate (g /1 g of adsorbent);
ρ is density of adsorbed phase (0.310g/cm^3),
W_o: volume of the adsorption space (ml/1 g of adsorbent),
A : RT ln P_s/P,
P_s: saturation vapor pressue (Torr); 200 atm at 77 °K,
P : equilibrium vapor pressure (Torr),
E : adsorption characteristic energy related to latent heat of vaporization (cal/mol),
n : a constant which is related to relative diameter of pore to molecule.

Fig. 2 shows Dubinin-Astakhov plot. The values W_o, E and n could be computed as follows: W_o=0.20±0.06 ml/g, E=1500±150 cal/mol, n=4.0±1.4. The values are fairly good approximate ones to the data published in literature [11], and suggest the adsorption of hydrogen and tritium gas was accomplished by the Dubinin-Astakhov relationship.

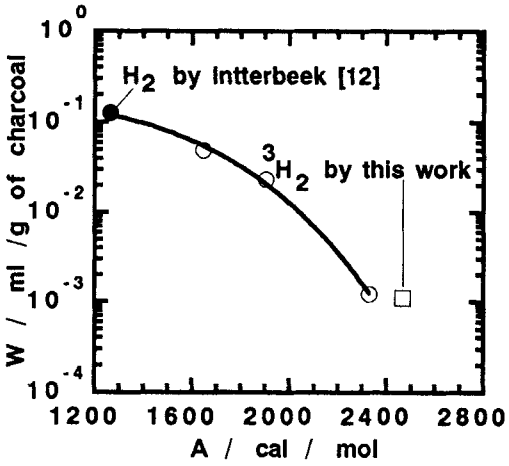

Figure 2. The Dubinin-Astakhov plot for adsorption of hydrogen and tritium gas on active charcoal.

On the occasion of opening the reaction vessel, an amount of the residual tritium gas in vessel was measured by a tritium monitor. The reaction mixture was dissolved in water. The catalyst in solution was removed by centrifugation. Supernatant was evaporated to dryness. The product was repeatedly washed with water three times and finally dissolved in water.

2.4. Bromination of synthesized [2-^{14}C,5-^{3}H]cytosine and [2-^{14}C,5-^{3}H]uracil

The [2-^{14}C,5-^{3}H]cytosine (0.0024 μg, ^{3}H 0.54 μCi, ^{14}C 0.001 μCi) or [2-^{14}C,5-^{3}H]uracil (0.011 μg, ^{3}H 0.92 μCi, ^{14}C 0.0055 μCi) obtained above (section 2.3) were added to nonlabelled cytosine or uracil of 1 mg, respectively, and allowed to react with bromine. The procedure of bromination was similar to Run 1-4 described in section 2.2.

2.5. ^{1}H NMR spectroscopy after deuteration of [5-Br]cytosine

The alkaline aqueous solutions of deuterated cytosine obtained in the reaction between [5-Br]cytosine and deuterium gas (Runs 3-1 ~ 3-3) were evaporated to dryness. The solids remaining were dissolved in deuterium oxide without neutralization (cytosine is slightly soluble in water but readily soluble in alkaline solution) and their ^{1}H NM spectra were measured at room temperature with JEOL MH-100 spectrometer. Internal TSP (3-(Trimethylsilyl)-propionic acid-d4-sodium salt, $C_6H_9D_4NaO_2Si$), 10 mg in

NMR tube, was used as a reference. The δ values for [5-Br,6-H]Cy, [5-D,6-H]Cy and [5-H,6-D]Cy were given by 7.9, 7.6 and 5.4, respectively.

2.6. HPLC analysis and purification

The analysis of products was carried out with HPLC (Model 520 Gasukuro Kogyo Co Ltd.) using a reverse phase column packed with Unisil Q C18 (particle size: 5 μm, Gasukuro Kogyo Co.). Peaks were monitored with a UV detector at 254 nm. In the experiments using radioactive materials, the purification of product was carried out with the use of a separation column of same packing. As eluting agents, primary sodium phosphate (NaH_2PO_4) aqueous solution was used in analysis, and methanol aqueous solution was used in purification. HPLC apparatus was connected with a fraction collector (LKB2111 Multi Rac, Bromma, Sweden). In the analysis of radioactivity, the eluate was fractionated into scintillation vials by every 0.2 ml.

Retention volume of pyrimidine bases is altered by the change of concentration of eluent. In the elution with NaH_2PO_4 aqueous solution of 10^{-6} M, the pyrimidine bases were eluted in the order, cytosine, 5-bromocytosine, 5-bromouracil and uracil. The retention volumes of BrCy, BrUr and Ur were approximately constant with 18 ml (BrCy), 16 ml (BrUr) and 5 ml (Ur) in the whole range of concentration of NaH_2PO_4 solution examined. However, that of Cy decreased extremely from 25 ml to 4 ml with increasing concentration of eluent from 10^{-6} M to 10^{-4} M, and became the smallest volume and constant in the 10^{-4} M to 10^{-1} M region. In the elution with H_2O/methanol mixture, the pyrimidines were eluted in the same order as in the case of NaH_2PO_4 solution. In the elution with pure water, the retention volumes depending on the pyrimidine were as large as 350 ~ 50 ml. The their retention volumes decreased with increasing concentration of methanol, and gave approximately constant as large 30 ~ 10 ml at the concentration above 70 % of methnol.

The columns and eluting agents were follows:

[analysis of pyrimidine bases]
column: Unisil Q-C18 4.6φ x 250 mm
eluent: 10^{-2}M or 10^{-1}M NaH_2PO_4 aqeous solution.
[purification of pyrimidine bases]
column: Unisil Q-C18 7.6φ x 300 mm
eluent: 25 % methanol for 5-bromocytosine
eluent: 70 % methanol for cytosine
eluent: 10 % methanol for 5-bromouracil
eluent: 10 % methanol for uracil

2.7. Radioactivity measurement

The ^{14}C and ^{3}H radioactivities were measured by the mode of double label counting with liquid scintillation counter (Packard, Tri-Carb 460 CD). A mixture of toluene-based scintillation solution (12.5 ml) and ethanol (2.5

ml) was used as the counting medium. To avoid overlapping of β-ray spectra of ^{3}H and ^{14}C in the channel B, discrimination point of channel A and B was set at 25 keV (:channel A for ^{3}H is 0 ~ 25 keV, channel B for ^{14}C is 25 ~ 156 keV). Analysis of radioactivity data of was carried out using Hewlett-Packard calculator 9815 A and plotter 9862 A.

3. RESULTS AND DISCUSSION

3.1. Bromination

The synthesis of 5-bromouracil in CCl_4 has been reported [7,13]. This synthesis of 5-bromouracil requires a period of 10 h at 90 °C to complete the bromination. Hilbert et al. synthesized 5-bromouracil and 5-bromocytosine at room temperature in water [14] where the bromination proceeds more rapidly.

In the present work the bromination of cytosine in CCl_4 was compared with that in CCl_4/H_2O of mixed solvent where CCl_4 played a role in holding a slight amount of bromine. The results are shown in Table 1 (Runs 1-1 ~ 1-4). 5-Bromocytosine was formed slowly in CCl_4. Even if the reaction was prolonged, the yield was rather low (26 ~ 28 % for 10 ~ 48 h). However, cytosine was rapidly converted to 5-bromocytosine with relatively high yields (63 ~ 73 % for 1 h) in CCl_4/H_2O at 25 °C and 0 °C. Significant amounts of by-products, 5-bromouracil and unknown products, were formed in the both solvents.

Table 1. Products yields in bromination of cytosine and uracil.

Run	Cy	Br_2	CCl_4/H_2O	Temp	Time	Meas	Yield %			
	mg	μl	ml	°C	hr		BrCy	BrUr	Cy	uk.p.[c]
1-1	1	0.48	0.5/0.0	85	48	UV	28	17	10	(45)
1-2	1	0.48	0.5/0.0	85	10	UV	26	0	35	(39)
1-3	1	0.48	0.1/0.5	25	1	UV	73	17	0	(10)
1-4	1	0.48	0.1/0.5	0	1	UV	63	18	0	(19)
1-5	0.6[a]	0.34	0.1/0.5	0	1	UV	56	3	0	(41)
						^{14}C	59	3	0	38
Run	Ur	Br_2	CCl_4/H_2O	Temp	Time	Meas	Yield %			
	mg	μl	ml	°C	hr		BrUr	Ur		uk.p.[c]
2-1	1.1	0.53	0.1/0.5	0	1	UV	57	21		(22)
2-2	1.1	0.53	0.1/0.5	0	1	UV	64	21		(15)
2-3	0.7	0.36	0.1/0.5	0	1	UV	54	13		(33)
2-4	1.5[b]	0.72	0.1/0.5	0	1	UV	60	19		(21)
						^{14}C	63	20		17

Volume of reaction ampoule : 1.5 ml

[a] [2-^{14}C]cytosine 0.62 mg 0.29 mCi 51 mCi/mmol

[b] [2-^{14}C]uracil 1.5 mg 0.69 mCi 52 mCi/mmol

[c] unknown products

For the synthesis of 5-bromouracil, the results are shown in Table 1 (Runs 2-1 ~ 2-3). The reaction aspect of uracil was somewhat different. The yield of desired product was similar to that of 5-bromocytosine, but some uracil still remained unreacted up to 1 h.

The brominations of [2-^{14}C]cytosine and [2-^{14}C]uracil were performed under the same condition as Run 1-4. The [2-^{14}C]cytosine having 99.6 % of radiochemical purity and [2-^{14}C]uracil having 99.7 % of radiochemical purity were used for the synthesis. The products yields on HPLC-UV- and HPLC-radiochromatograms are shown in Table 1 (Runs 1-5 and 2-4) and Figs. 3 and 5. On the HPLC chromatograms, some by-products observed with UV detector did not appear in radiochromatograms or vice versa. The difference of percentage yields between UV absorbance and ^{14}C activity measurements is due to the method of estimation. At UV spectrophotometric determination, a mole yield which was equal to a mole amount of starting material was regarded as 100 percent. The yield of uk.p. was estimated by subtraction of total mole yields of identified products from 100 percent. On the other hand, at estimation by radioactivity measurement, a radioactivity yield which was equal to that injected into HPLC column was regarded as 100 percent. The radiochemical yields of [2-^{14}C,5-Br]cytosine and [2-^{14}C,5-Br]uracil were 59 % and 63 %, respectively. The crude [2-^{14}C,5-Br]cytosine and [2-^{14}C,5-Br]uracil were purified with HPLC by repetition of injections of 35 μg / 10 μl each of sample 18 times and 750 μg / 750 μl each of sample 2 times. The HPLC chromatogram peak of 5-bromocytosine is adjacent to that of 5-bromouracil and hence in the HPLC purification of 5-bromocytosine the products from cytosine must be injected into the HPLC column by small portions with care. The radiochemical purities of [2-^{14}C,5-Br]cytosine and [2-^{14}C,5-Br]uracil were 97 % (recovery: 76 %) and 99 % (recovery: 86 %), respectively.

3.2. Hydrogenation

In a previous paper, it was revealed that the reaction of 5-bromocytosine or 5-bromouracil with deuterium gas produced a mixture of [5-D]-, [6-D]- and [5-D,6-D]compounds depending on the experimental conditions [8]. By controlling temperature, pressure of deuterium gas and amount of Pd/$CaCO_3$ catalyst, [5-D]cytosine and [5-D]uracil were effectively obtained. The purpose of the present study was to reduce the scale for the tritiation. At first, the synthesis of [5-D]cytosine was carried out with the use of several tens mg of 5-bromocytosine and deuterium gas in the Runs 3-1 ~ 3-3. The products yields and NMR spectra of the products are shown in Table 2 and Figs. 7 (a) and 7 (b), respectively. In Run 3-1 at 80 °C and catalyst/5-bromocytosine wt. ratio of two and in Run 3-2 at 15 °C and that ratio of two, 5-bormocytosine reacted completely, but besides [5-D]cytosine, [6-H], [5-H,6-H], [5-D,6-D] and [5-H,6-D]cytosine were produced. In the NMR spectrum (a) of Run 3-1, the [5-D,6-H], [5-H,6-D] and [5-H,6-H]cytosine were

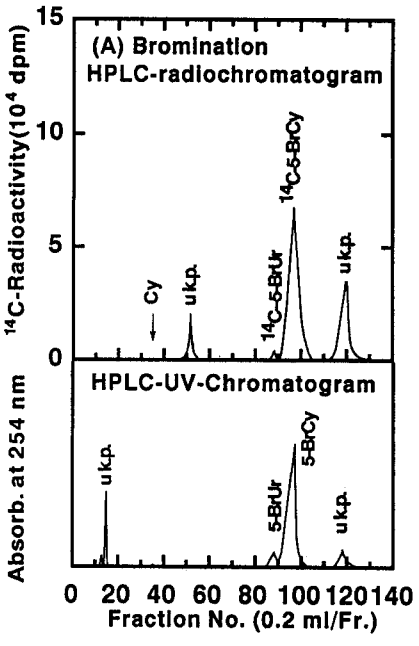

Figure 3. HPLC UV- and radiochromatogram for bromination of [2-^{14}C]cytosine.

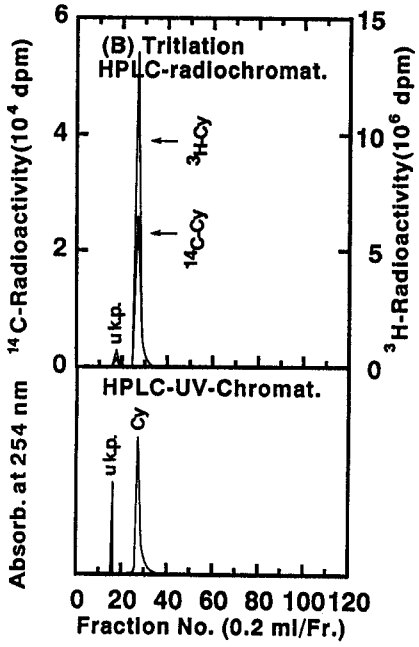

Figure 4. HPLC UV- and radiochromatogram for tritiation of [2-^{14}C,5-Br]cytosine.

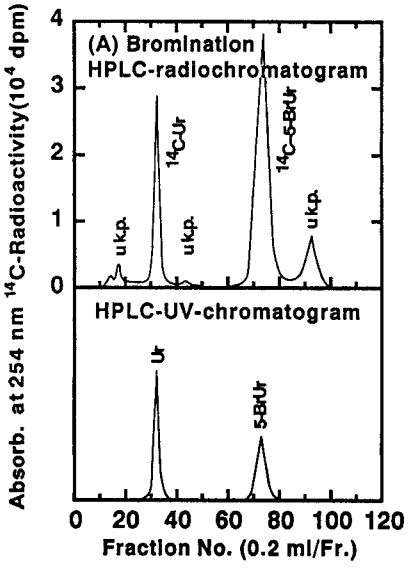

Figure 5. HPLC UV- and radiochromatogram for bromination of [2-^{14}C]uracil.

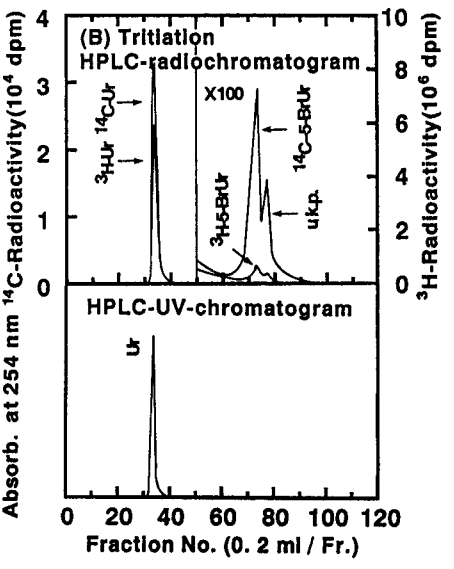

Figure 6. HPLC UV- and radiochromatogram for tritiation of [2-^{14}C,5-Br]uracil.

observed with the yield of 19, 24 and 30 %, respectively. The 5-H product was found to be formed with increase in amount of the catalyst. Therefore it was assumed that 5-H product was formed partly by the action of hydrogen originally absorbed in Pd/$CaCO_3$ catalyst and that 6-D product was formed by the raction of [5-D,6-H]cytosine with deuterium gas [8]. The Run 3-3 is the reaction at 15 °C and catalyst/bromocytosine wt. ratio of one. Although a fraction of bromocytosine remained without reaction, [5-D,6-H]cytosine was formed effectively as shown in NMR spectrum (b).

Next, the synthesis of [5-H]cytosine was carried out with the use of 5-bromocytosine of ca. 1 mg and hydrogen gas as shown in the Runs 3-4 ~ 3-6 where the catalyst/5-bromocytosine wt. ratio one. In the case of 1 mg of 5-bromocytosine (Run 3-4), a fraction of 5-bromocytosine remained constant, even when the reaction time was prolonged for 2 h. In the case of 0.5 mg of 5-bromocytosine (Run 3-5), the reaction was completed within 2 h. It needed a special care to maintain a small volume of alkaline solution of 25 μl during the reaction. Although an attempt to increase the volume of solvent by 200 μl was made, the reaction did not proceed even at reaction time of 5 h (Run 3-6).

The synthesis of [5-H]uracil was studied with an alteration in volume of alkaline solution. The conditions of the others were similar to Run 3-5.

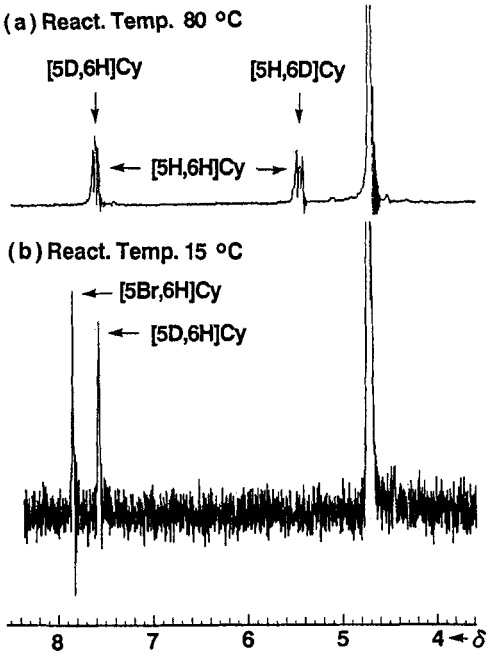

Figure 7. ^{1}H NMR spectra of products by deuteration of 5-bromocytosine in Runs 3-1 and 3-3 (in alkaline D_2O; 60 MHz; internal standard : $C_6H_9D_4NaO_2Si$).

Table 2. Products yields in hydrogenation of [5-Br]cytosine and [5-Br]uracil.

Run	BrCy	H_2	Vessel	Pd/$CaCO_3$	0.3N NaOH	Temp	Time	Meas	Yield %		
	mg	Torr	ml	mg	μl	°C	hr		Cy	BrCy	uk.p.[e]
3-1	120	210[c]	110	235	3500	80	1	UV	80	0	(20)
3-2	50	164[c]	140	100	1500	15	1	UV	90	0	(10)
3-3	20	121[c]	140	20	300	15	1	UV	29	55	(16)
3-4	1.0	27	40	1	25	15	3	UV	66	18	(16)
3-5	0.5	21	14	0.5	25	15	2	UV	82	0	(18)
3-6	0.3	8	40	0.3	200	15	5	UV	0	81	(19)
3-7	0.5[a]	22[d]	13	0.5	25	15	3	UV	61	0	(39)
								^{14}C	86	0	14
								^{3}H	90	0	10

Run	BrUr	H_2	Vessel	Pd/$CaCO_3$	0.3N NaOH	Temp	Time	Meas	Yield %		
	mg	Torr	ml	mg	μl	°C	hr		Ur	BrUr	uk.p.[e]
4-1	0.6	21	14	0.8	75	15	3	UV	0	86	(14)
4-2	1.2	21	14	1.4	50	15	3	UV	5	91	(4)
4-3	0.6	21	14	0.8	25	15	3	UV	63	0	(37)
4-4	1.2	21	14	1.2	25	15	3	UV	84	0	(16)
4-5	1.3[b]	22[d]	13	1.3	25	15	3	UV	46	1	(53)
								^{14}C	94	2	4
								^{3}H	97	v.s.	3

[a] [2-^{14}C,5-Br]cytosine 0.48 mg 0.13 mCi 51 mCi/m mol

[b] [2-^{14}C,5-Br]uracil 1.29 mg 0.35 mCi 52 mCi/m mol

[c] deuterium gas [d] tritium gas of 1 Ci [e] unknown products

The results are shown in Table 2 (Runs 4-1 ~ 4-4). It became evident that the reaction did not proceed at a large volume of alkaline solution of 75 μl or 50 μl (Runs 4-1 and 4-2). At 25 μl the hydrogenation proceeded favorably as shown in Runs 4-3 and 4-5.

The Br-^{3}H exchange reaction between [2-^{14}C,5-Br]cytosine (or [2-^{14}C,5-Br]uracil) and tritium gas was therefore carried out under the same condition as Run 3-5. The [2-^{14}C,5-Br]cytosine and [2-^{14}C,5-Br]uracil purified as described above, were used. The products yields and HPLC-radiochromatograms are shown in Table 2 (Runs 3-7 and 4-5) and Figs. 4 and 6. The amounts of by-products were relatively small. The radiochemical yields of [2-^{14}C,5-^{3}H]cytosine and [2-^{14}C,5-^{3}H]uracil were 86 and 94 % for ^{14}C and 90 and 97 % for ^{3}H, respectively. The difference of percentage yields between UV absorbance and ^{14}C radioactivity measurements is due to the same reason as described above.

The recovery of tritium after reaction was as follows:

	Run 3-7	*Run 4-5*
in exhaust gas	*0.52 mCi*	*3.6 mCi*
in washing water	*0.95 mCi*	*0.06 mCi*
in adsorbent	*961 mCi*[a]	*967 mCi*[b]

[a] *active charcoal(77°K)*; [b] *Zr-V-Fe getter(Room Temp.)*

The radioactivities of gaseous tritium in the reaction vessels of Runs 3-7 and 4-5 were 1/1850 and 1/270 of tritium remaining unreacted. The data suggest that the adsorbents such as active charcoal and Zr-V-Fe getter are useful for the safe handling of tritium gas in laboratory.

The ^{14}C and ^{3}H radioactivities of starting materials, that of synthesized compounds and the labelling percentages of final products are summarized in Table 3. For the tritium labelling of cytosine a good result (:^{3}H label. 86 %) was obtained. On the other hand, the tritium labelling percentage of uracil was relatively poor (:^{3}H label. 32 %) and was similar to the previous result (:^{3}H label. 39 %). This means that Br-H exchange reaction occurred in at least 14 % of 5-bromocytosine and 68 % of 5-bromouracil (:in taking into account of presence of 6-^{3}H pyrimidine,

Table 3. The radioactivities and ^{14}C,^{3}H labelling percentage of [2-^{14}C,5-Br]cytosine, [2-^{14}C,5-^{3}H]cytosine, [2-^{14}C,5-Br]uracil and [2-^{14}C,5-^{3}H]uracil.

	weight mg	^{14}C Act. mCi	Sp. Act. mCi/mmol	^{3}H Act. Ci	Sp. Act. Ci/mmol
Bromination of [2-^{14}C]cytosine					
used Cy	0.62	0.29	51.4		
produced BrCy	0.63	0.17	50.5		
purified BrCy	0.48	0.13	51.0		
Tritiation of [2-^{14}C,5-Br]cytosine					
used BrCy	0.48	0.13	50.7		
produced Cy	0.17	0.077	50.9	0.038	25.1
^{14}C	labelling percentage (51/63) x 100				= 81 %
^{3}H	labelling percentage (25/29) x 100				= 86 %
^{14}C,^{3}H double	labelling percentage (0.81 x 0.86) x 100				= 70 %
Bromination of [2-^{14}C]uracil					
used Ur	1.50	0.69	51.6		
produced BrUr	1.50	0.41	52.2		
purified BrUr	1.30	0.35	51.6		
Tritiation of [2-^{14}C,5-Br]uracil					
used BrUr	1.29	0.35	51.5		
produced Ur	0.35	0.16	51.5	0.029	9.40
^{14}C	labelling percentage (52 /63) x 100				= 82 %
^{3}H	labelling percentage (9.4/29) x 100				= 32 %
^{14}C,^{3}H double	labelling percentage (0.82 x 0.32) x 100				= 26 %

these are minimum values). To diminish the reaction of hydrogen absorbed in $Pd/CaCO_3$ catalyst, the catalyst was degassed prior to use in the synthesis of radioactive compounds. We conclude that, in the case of 5-bromouracil, the reaction of hydrogen absorbed in catalyst occurred easily even at low reaction temperature as 15 °C, whereas the tritium gas reacted preferentially with 5-bromocytosine. In conclusion, the ^{14}C and ^{3}H double labelling percentages of cytosine and uracil were 70 and 26 %, respectively.

3.4. Bromination of [2-^{14}C,5-^{3}H]cytosine and [2-^{14}C,5-^{3}H]uracil

A confirmation of ^{3}H labelling at 5 position of pyrimidine ring was made by the bromination of [2-^{14}C,5-^{3}H]cytosine and [2-^{14}C,5-^{3}H]uracil. The purified labelled samples were used for the reaction. Product yield was measured by UV detector and radioactivity measurement of tritium. The results are shown in Table 4. The 51 % of bromocytosine produced contains the 2 % of ^{3}H. This means that the 4 % of ^{3}H is labelled at 6 position of pyrimidine ring of cytosine. Therefore it was proved that the 96 % of ^{3}H was labelled at 5 position of pyrimidine ring in [2-^{14}C,5-^{3}H]cytosine obtained in Run 3-7. Similarly, 73 % of ^{3}H was labelled at 5 position in [2-^{14}C,5-^{3}H]uracil obtained in Run 4-5. A large ^{3}H radiochromatogram peak which was

Table 4. Products yields in bromination of [2-^{14}C,5-^{3}H] cytosine and [2-^{14}C,5-^{3}H]uracil; Percentage of tritium labelling at 5 and 6 position of pyrimidine ring of original labelled compounds.

Bromination of [2-^{14}C, 5-^{3}H]cytosine					
	yield %				
measurement	BrCy	BrUr	Cy	water[a]	uk.p.[c]
UV	51	27	2		(20)
^{3}H	2	1	32[b]	54	11
^{3}H labelling at 6 position of pyrimidine ring : (0.2/0.51)X100 = 4 %					
^{3}H labelling at 5 position of pyrimidine ring : 96 %					
Bromination of [2-^{14}C, 5-^{3}H]uracil					
	yield %				
measurement	BrUr		Ur	water[a]	uk.p.[c]
UV	66		18		(16)
^{3}H	18		60[b]	10	12
^{3}H labelling at 6 position of pyrimidine ring : (0.18/0.66)X100 = 27 %					
^{3}H labelling at 5 position of pyrimidine ring : 73 %					

[a] ^{3}H in washing water

[b] Unreacted compound plus unknown ^{3}H-products

[c] unknown products

not detected with UV monitor was observed in both bromination of [2-^{14}C,5-^{3}H]cytosine and [2-^{14}C,5-^{3}H]uracil. This peak occurs between the UV-chromatogram peaks of cytosine and uracil and this ^{3}H peak and cytosine peak (or uracil peak) partly overlap each other. The amount of tritium is shown in Cy- or Ur-column in Table 4. The sum of percentages of ^{3}H distribution in Cy (or Ur) and water nearly equal to the sum of percentage yields of brominated products. Accordingly it is assumed that this ^{3}H-peak contains a product possibly an oxyacid such as ^{3}HBrO formed by the reaction between bromine and tritium released from pyrimidine ring and that ^{3}H in washing water is dissolved in the chemical form of ^{3}HHO.

4. CONCLUSION

1) The micro-scale synthesis of carrier free doubly labelled [2-^{14}C,5-^{3}H]-cytosine and -uracil were carried out via bromination and catalytic Br-^{3}H exchange reaction with the use of [2-^{14}C]-cytosine and -uracil and tritium gas as starting materials. In order to avoid side-reactions, the synthesis were made under lower temperature (15 °C) than that used in previous paper. The radiochemical yield of tritium labelling at 5 position of pyrimidine ring was examined by bromination of the tritium labelled products.

2) As technical subjects, for the analysis and purification of products, the HPLC procedures using C18 reverse column and aqueous solution of NaH_2PO_4 or methanol as eluents were studied. The recovery of unreacted tritium gas was carried out with the use of adsorbents. It was revealed that the adsorbents such as active charcoal and Zr-V-Fe getter were useful for the safe handling of tritium.

3) From [2-^{14}C]cytosine of 0.6 mCi and tritium gas of 1 Ci, [2-^{14}C,5-^{3}H]cytosine having ^{14}C, ^{3}H double labelling percentage of 70 (^{14}C: 0.077 mCi; ^{3}H: 0.038 Ci) was synthesized. From [2-^{14}C]uracil of 1.5 mCi, [2-^{14}C,5-^{3}H]uracil having ^{14}C, ^{3}H double labelling percentage of 26 (^{14}C: 0.16 mCi; ^{3}H: 0.029 Ci) was synthesized. It was assumed that the low yield of tritium labelling for [2-^{14}C,5-^{3}H]uracil would be due to the competitive reaction between tritium gas used and hydrogen atom originally adsorbed on palladium catalyst. By the bromination of the doubly labelled compounds, the percentages of ^{3}H labelling at 5 position of pyrimidine ring of [2-^{14}C,5-^{3}H]-cytosine and -uracil were proved to be 96 and 73, respectively.

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