SYNTHESES OF [5-^{2}H]-URACIL, [5-^{2}H]-CYTOSINE, [6-^{2}H]-URACIL AND [6-^{2}H]-CYTOSINE

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SUMMARY

Facile syntheses of [5-^{2}H]-, [6-^{2}H]-uracil and [5-^{2}H]-, [6-^{2}H]-cytosine were investigated. The catalytic reaction of uracil or cytosine with $^2H_2$ gas in alkaline media gave rise to [6-^{2}H]-compounds almost exclusively. On the other hand, the reaction of 5-bromouracil or 5-bromocytosine with $^2H_2$ gas gave rise to a mixture of [5-^{2}H]-, [6-^{2}H]- and [5-^{2}H,6-^{2}H]-compounds depending on the experimental conditions. By controlling the temperature, the pressure of $^2H_2$ gas and the amounts of catalyst, [5-^{2}H]-uracil and [5-^{2}H]-cytosine were obtained. The isotopic distribution in each product was measured by ^{1}H NMR spectroscopy combined with an HPLC method.

Key words: Deuterium, 5D-uracil, 5D-cytosine, 6D-uracil, 6D-cytosine.

INTRODUCTION

ß-Decay of tritium incorporated in nucleic acid plays an important role in producing lethal and mutagenic effects$^{1-3)}$. These effects are critically influenced by the position of tritiation in the pyrimidine base of nucleic acid$^{4,5)}$. The chemical effects of the decay were also investigated in

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aqueous solution of tritiated uracil[6)]. In such experiments, bases tritiated at a specific position are used. Tritiation of 5-bromo-pyrimidine derivatives has been carried out[7-10)], but the isotopic distribution between 5 and 6 positions was not reported in these papers.

In the present work, ^{2}H-labelled uracil and cytosine were synthesized by the reaction of uracil (Ur), cytosine (Cy), 5-bromouracil (5-BrUr), or 5-bromocytosine (5-BrCy) with gaseous deuterium under various conditions. Similar procedures are likely to be useful in the syntheses of tritiated 5-Ur and 5-Cy, after taking into account the isotope effect.

EXPERIMENTAL AND RESULTS

Materials

Ur, Cy, 5-BrUr, 5-BrCy, Pd/$CaCO_3$, D_2O (99.55%) and D_2 gas were purchased from commercial sources and used without further purification. 1 N NaOD was prepared by dissolving NaOH in D_2O.

High Performance Liquid Chromatography

Products were analyzed by HPLC using a Model M-6000 A chromatograph (Waters Associates Inc. USA), a separation column of reversed phase type (Unisil Q-C_{18}, 25 x 4.0 cm, Gasukuro Kogyo) and a 0.01 M solution of NaH_2PO_4 as eluant. Peaks were observed at 210 or 260 nm and treated numerically (Chromatopac C-R1A, Shimadzu). The experimental errors were ± 2%.

^{1}H NMR Spectroscopy

^{1}H NMR spectra were taken under strongly alkaline conditions with a JEOL MH-100 spectrometer and TSP as an internal standard at room temperature. ^{1}H NMR (D_2O) Ur δ = 5.8 (1H,d. J = 7.0 Hz, 5-CH), 7.8 (1H,d. J = 7.0 Hz, 6-CH) Cy δ = 5.9 (1H,d. J = 7.0 Hz, 5-CH), 7.9 (1H,d. J = 7.0 Hz, 6-CH). The experimental errors were ± 10%.

<u>Reactions of Ur and Cy with D_2 gas</u>

A mixture of 58 mg of Ur (5.17×10^{-4} mol), (Run I-1, II-1) or Cy (5.22×10^{-4} mol), (Run I-2, II-2) and 120 mg of 10% Pd/$CaCO_3$, and 3 ml of 1N NaOD were placed in a 100 ml round-bottom flask with a magnetically stirring teflon rod. The flask was connected to a vacuum line and evacuated at -78 °C before admitting D_2 gas (178 mmHg, 1.05×10^{-3} mol (Run I) or 89 mmHg, 5.25×10^{-4} mol (Run II)). Each reaction mixture in the flask was stirred for 1 hr (Run I) or 20 hrs (Run II) at 15 °C, then the content was taken out and filtered. The filtrate was evaporated to dryness by a rotary evaporator. The precipitate was washed with distilled water, dried, then dissolved in 0.5 ml of D_2O, and put in a NMR tube with 10 mg (5.81×10^{-5} mol) of TSP. The distribution and amount of ^{2}H in Ur and Cy molecules were determined by ^{1}H NMR spectroscopy under alkaline condition. After the ^{1}H NMR analysis the solution was neutralized with 1N HCl and the products in the solution were analyzed by HPLC; their retention volumes were compared with those of the authentic samples. The peaks of Ur and Cy represent the sum total of the four isomers, i.e. 5H, 6H-, 5H, 6D-, 5D, 6H- and 5D, 6D-compounds. The yields of the first three were determined from the ^{1}H NMR measurements, and therefore that of 5D,6D-compound was estimated by simple substraction.

The ^{1}H NMR spectra in Run I showed that H-D exchange occurred only at the 6 position, although the yields of 6D-Ur and 6D-Cy were not high. The amount of 6D-Ur was larger than that of 6D-Cy. The products in Run II, for the most part, were 6D-compounds, and the formation of as little as 4% of 5D-Cy was detected. 5D,6D-Compounds were not formed in both Run I and II. 6OD-Ur, 6OD-Cy were not detected, either. The results are summarized in Table I.

Table I: The distribution of D in the products in the reaction of Ur or Cy with D_2 gas

Run No.	I-1	I-2	II-1	II-2
	Ur	Cy	Ur	Cy
Products:		%		
5-D 6-H	0	0	0	4 (trace)
5-H 6-D	38	25	96	79 (47)
5-D 6-D*	0	0	0	0 (52)
5-H 6-H (unreacted)	62	75	4	17 (0)
Conditions:				
D_2/Ur(Cy) (mol/mol)	2		1	
Pd-Cat/Ur(Cy) (w/w)	2		2	
reaction time, hr	1		20	
temperature, $^{\circ}C$	15		15	

*100 - (the sum of the other 3 isomers)
( ) without NaOD, dissolved in 7.5 ml D_2O

On the other hand, large amounts of 5D,6D-Cy together with 6D-Cy were obtained from the reaction in a neutral solution (numbers in parentheses in Table I). The result indicates that NaOD has a strong effect on H-D exchange of Ur and Cy. However, there was no reaction when Ur or Cy was mixed with 1N NaOD in the absence of D_2 and $Pd/CaCO_3$.

Reactions of 5-BrUr and 5-BrCy with D2 gas

In a second way to deuterate Ur and Cy molecules, 5-BrUr and 5-BrCy were used as starting material. They were allowed to react with D_2 gas in the presence of Pd catalyst under alkaline condition. A mixture of 100 mg of 5-BrUr (5.24×10^{-4} mol) (Run III-1) or 5-BrCy (5.26×10^{-4} mol) (Run III-2) and 200 mg of 10% Pd/$CaCO_3$, and 3 ml of 1N NaOD were placed in a 100 ml round-bottom flask with a teflon magnetic stirrer. D_2 gas (178 mmHg) was admitted into the flask as described above. The flask was sealed and then heated and stirred at 80 °C for 1 hr. The reactions at 15 °C were studied in Run IV. The results are shown in Table II.

In Run III, the H-D exchange occurred at both 5 and 6 positions. Although the starting materials were completely consumed, the total yield of Ur or Cy calculated from the HPLC peaks was as low as 45-50% indicating that some undesirable substances might have been produced. The results in Run IV (total yield 70-80%) indicated that 5D-Ur was produced with high yield, but that both 5D-Cy and 6D-Cy were produced together with 5D,6D-Cy in Run IV-2. Thus, we conclude that the reaction at room temperature is favorable for syntheses of 5D-Ur and 5D-Cy. The difference in the reactivity between 5-BrUr and 5-BrCy can be explained by the chemical effect of substituents at the 4 position.

Another series of experiments was carried out under conditions milder than those of Run III. In Runs V-VII the D_2 gas pressure and/or the amount of the catalyst were reduced as shown in Table II. Reduction in the quantities of D_2 gas and of the catalyst was found to be advantageous with respect to the production of the 5D-compounds. Although

Table II: The distribution of D in the products in the reaction of BrUr or BrCy with D_2 gas.

Run No.	III-1	III-2	IV-1	IV-2	V-1	V-2	VI-1	VI-2	VII-1	VII-2
	BrUr	BrCy	BrUr	BrCy	BrUr	BrCy	BrUr	BrCy	BrUr	BrCy
Products:	%									
5-D 6-H	15	9	81	39	77	75	39	33	54	64
5-H 6-D	45	19	2	32	3	0	0	0	13	8
5-D 6-D*	40	72	0	8	0	0	0	0	18	12
5-H 6-H	0	0	7	21	7	3	7	0	15	16
5-Br (unreacted)	0	0	10	0	13	22	54	67	0	0
Conditions:										
D_2/BrUr(BrCy)(mol/mol)	2		2		1		1		1	
Pd-Cat/BrUr(BrCy)(w/w)	2		2		2		0.5		0.5	
reaction time, hr	1		1		2		2		20	
temp.,°C	80		15		15		15		15	

* 100 - (BrUr(BrCy) + the other 3 isomers)

large amounts of unreacted 5-BrUr and 5-BrCy were recovered, especially in Run VI, D incorporation preferentially occurred at the 5 position.

In Run VII (total yield ∿ 85%), unreacted BrUr and BrCy were not detected, and the yields of 5D-compounds were 1.5-2 times larger than those in Run VI. However, 6D- and 5D,6D-compounds appeared. Apparently, a prolonged reaction results in a H-D exchange at their labile 6 positions. The reaction should be stopped within 2 hrs to avoid the formation of 6D-compounds. Thus, 6D-compounds could be conveniently obtained by a prolonged reaction of Ur and Cy.

In conclusion, the conditions of Run IV (for Ur) and Run V (for Ur and Cy) were found to be optimum for obtaining the 5D-compounds.

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