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CHEMICAL ALTERATION OF NUCLEIC ACIDS AND THEIR COMPONENTS. XI.[1] HYDROGEN-DEUTERIUM EXCHANGE OF NUCLEOSIDES AND NUCLEOTIDES CATALYZED BY PLATINUM*

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The hydrogen-isotope labeling of nucleic acid bases is sometimes required in nucleic acid research. Many papers have already appeared on deuteration of 8-H in purines[2,3] and of 5-H in pyrimidines[4-6] through the ionic exchange processes. This paper describes catalytic hydrogen exchange of nucleosides and nucleotides using Adams platinum.[7] This catalytic method may provide a source of labeled compounds which are difficult to be obtained by other methods. All the materials used were purchased from Kojin Co., Tokyo, and Sigma Chem. Co., St. Louis. Platinum dioxide ($PtO_2 \cdot 2H_2O$) was purchased from Kawaken Fine Chemicals, Tokyo. Incorporated deuterium was determined by quantitative NMR analysis. Chemical purity of the products was checked by thin-layer chromatography and UV spectroscopy. Appreciable amount of any decomposed by-products was not detected in any of the cases examined in the present study.

General Procedure for Hydrogen Exchange An appropriate amount of platinum oxide (shown in the table) was suspended in 1 ml of D_2O in a test tube (10 × 150 mm) and mechanically vibrated in D_2 gas atmosphere for 1-3 hr at room temperature. After most of the solvent was removed with a small pipette, nucleoside or nucleotide dissolved in 1 ml of D_2O was added. Then the reaction mixture was frozen and degassed up to 10^{-3} Torr. After this procedure was repeated twice more to eliminate molecular hydrogen involved, the reaction tube was sealed under 10^{-3} Torr pressure. The tube was gently shaken in a water bath at 30±0.5° or kept standing in boiling water for 40 hr. After the catalyst was eliminated by filtration, the NMR spectrum of the filtrate was measured for quantitative analysis of the deuterium incorporated in the respective positions of the molecule.

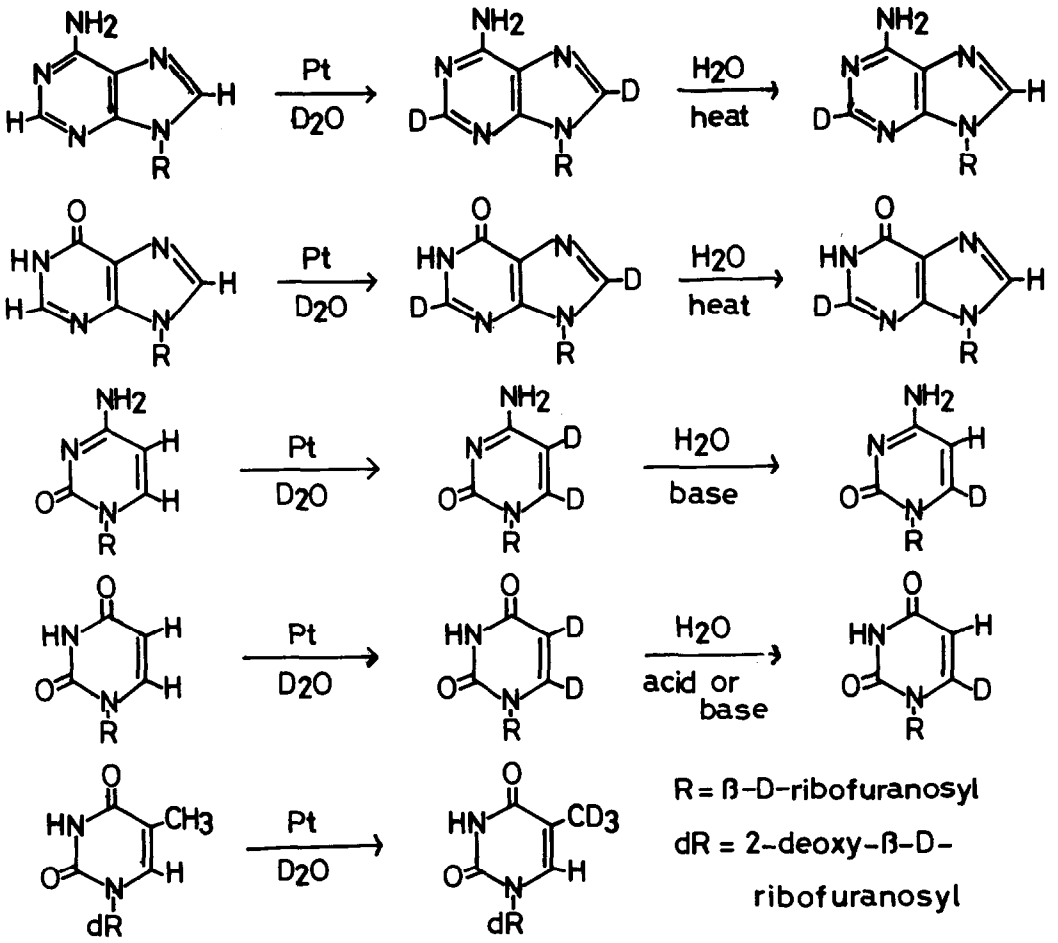
Results The results are shown in the table. Cytidine and uridine underwent deuteration at 30° in both 5- and 6-positions. The 5-H's were more readily

* This paper also constitutes Part XIII of a series of study entitled "Studies on Hydrogen Exchange. Part XII: ref. 3.

replaced than the 6-H's in both cases. It is of interest to note that raising of the temperature was not at all effective in these cases. No by-products were detected at 30° in both cases but at 100°, a trace amount of uridine was detected in the case of cytidine. The 5-H and 6-H of disodium cytidine-5'-monophosphate (5'-CMP[Na_2]) and disodium uridine-5'-monophosphate (5'-UMP[Na_2]) were also labeled without any unwanted side reaction at 30°. Thymidine underwent hydrogen exchange only in the substituent methyl group. The 6-H of thymidine was not exchanged even at 100°. This position is sterically hindered by the presence of the *ortho*-alkyl substituents on both sides, so that such a C-H hydrogen is completely inert in the catalytic exchange even at high temperatures.[8] The 5,6-dideuteriopyrimidine nucleosides and nucleotides thus obtained were derived to the corresponding 6-monodeuterio derivatives through

Table Catalytic Hydrogen-Deuterium Exchange of Nucleosides and Nucleotides after Treatment with Adams Platinum in D_2O for 40 hr

Compound	Amount (mg) of substrate	Amount (mg) of catalyst	Reaction temperature	Deuteration %		
Pyrimidines				5-H	6-H	5-Me
Cytidine	100	50	30°	100	100	-
Cytidine HCl	298	280	30°	100	0	-
Cytidine	55	10	100°	100	41	-
Cytidine	55	25	100°	100	70	-
Cytidine	55	50	100°	100	90	-
Uridine	262	112	30°	100	100	-
Uridine	55	10	100°	100	33	-
Uridine	55	25	100°	100	64	-
Uridine	55	50	100°	100	83	-
5'-CMP[Na_2]	348	112	30°	95	60	-
5'-UMP[Na_2]	348	112	30°	40	30	-
Thymidine	242	20	30°	-	0	0
Thymidine	242	50	30°	-	0	70
Thymidine	242	100	30°	-	0	100
Purines				2-H	8-H	
Adenosine	60	10	100°	100	100	
Inosine	60	10	30°	0	0	
Inosine	60	50	30°	15	15	
Inosine	60	10	100°	57	100	
Inosine	60	25	100°	82	100	
Inosine	60	50	100°	100	100	
5'-IMP[Na_2]	90	10	100°	80	90	
5'-IMP[Na_2]	90	25	100°	90	100	
5'-IMP[Na_2]	90	50	100°	100	100	



ionic exchange processes illustrated in the chart. Thus, 6-deuteriocytidine was obtained by treatment with sulfite[5] and 6-deuteriouridine was obtained by treatment with dil. HCl.[6] Adenosine was readily converted to the dideuterio compound at 100°, although the deuteration was incomplete at 30°, probably because of its poor solubility in D_2O. The same situation was demonstrated in the case of inosine. Disodium inosine-5'-monophosphate (5'-IMP[Na_2]) was also deuterated at 2- and 8-positions. At 100°, it was partially decomposed to inosine. Since its dephosphorylation occurred to the same extent in the absence of platinum, it may be an ionic hydrolysis and not the catalytic one. When the 2,8-dideuterated derivatives thus obtained were refluxed in H_2O for 1 hr, the corresponding 2-deuterated adenosine and inosine derivatives were produced in a quantitative yield.[3]

It is worth emphasizing that all the C-H hydrogens in the base moiety of

nucleosides and nucleotides can be replaced with deuterium, whereas none of the C-H in the sugar moiety is replaced under the reaction condition chosen in the present study. In general, the exchange seems to depend critically on the amount of the catalyst used and not so much on the reaction temperature, i.e., raising of the temperature from 30° to 100° accelerated the exchange only to a slight extent in the case of purines but not at all in the case of pyrimidines. It may be recommended that the catalyst should be used in a comparable amount to that of the substrate. The reaction temperature should be kept low because higher temperature tends to increase the by-product formation.

As far as the deuteration of nucleosides and nucleotides is concerned, catalytic method using Adams platinum described here seems to be better than that using platinum oxide.[9] The former can be done at lower temperature, even at room temperature, whereas the latter method requires the reaction temperature higher than 130~150°. In addition, the former is more selective for deuteration of the nucleic acid bases.

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