PREPARATION OF DEUTERIUM-LABELED NUCLEOSIDES BY PLATINUM-CATALYZED EXCHANGE AND REDUCTION

T. Kinoshita, Karl H. Schram and James A. McCloskey
Departments of Medicinal Chemistry and Biochemistry
University of Utah, Salt Lake City, Utah 84112

SUMMARY

Selective incorporation of deuterium by platinum-catalyzed exchange for six ribonucleosides and two 2'-deoxyribonucleosides has been studied as a function of catalyst/substrate ratio, temperature, time and concentration. In addition to exchange of carbon-bound hydrogens in purines, pyrimidines and pyrrolo[2,3-d]-pyrimidines, reactions involving D_2-saturated D_2O as solvent were used to effect extensive exchange of ribose hydrogens, principally at C-2'. Reduction-exchange of uridine was used to prepare 5,6-dihydrouridine-5,5,6,6,-d_4, and 4-amino-5,6-dihydro-7-(β-D-ribofuranosyl)pyrrolo[2,3-d]pyrimidine-2,5,5,6,6-d_5 from tubercidin. Sites and extent of deuteration were determined by mass spectrometry and proton magnetic resonance.

Key words: deuterium, nucleosides, platinum

INTRODUCTION

The preparation of selectively deuterated bases and nucleosides by acid- or base-catalyzed exchange at C-5,6 in pyrimidines and C-8 in purines has been widely used to provide substrates for a variety of chemical and biological experiments (1). Early work by Eidinoff and Knoll demonstrated the feasibility of catalytic exchange of H-2 and H-8 in adenine, and of H-8 in guanine (2). Maeda and Kawazoe further explored the use of platinum-catalyzed deuterium exchange in the base moieties of five nucleosides and two mononucleotides under a variety of conditions (3).

0362-4803/82/040525-10$01.00

Received August 17, 1981

We report here the results of experiments with nucleosides in which the catalytic exchange technique has been used to introduce deuterium into specific sites in the sugar skeleton in addition to the base, and into the base by double bond reduction. The variations in reaction conditions which were studied were catalyst/substrate ratio, temperature, time and concentration. Distribution of the deuterium label was measured by proton nuclear magnetic resonance, and mass spectrometry of the trimethylsilyl derivatives.

EXPERIMENTAL

Catalytic exchange and reduction reactions

Activated platinum was prepared by addition of an appropriate amount of PtO_2 (5-100 mg, Kawaken Fine Chemicals, Ltd., Tokyo, Japan) to 99% D_2O (1 ml, Aldrich Chemical Co., Milwaukee, WI) in a 10 ml two-neck round bottom flask. The suspension was magnetically stirred during passage of deuterium gas (Matheson, East, Rutherford, NJ) slowly through the solution for 1-2 hrs at room temperature. The solvent was removed by pipette and 1 ml of fresh D_2O was added prior to transfer of the residue for reaction by methods A or B (below).

Method A. Platinum black from the above suspension was transferred in a volume of approximately 0.5 ml to a 10 x 100 mm vacuum hydrolysis tube (No. K-739950, Kontes Scientific Glassware, Vineland, NJ). The solution was frozen in a dry ice-acetone bath, evacuated, then isolated from the vacuum line and allowed to thaw. Without reintroduction of atmosphere the solution was re-frozen, again connected to the vacuum line and the process continued as above for several cycles in order to remove dissolved oxygen and small amounts of molecular hydrogen. When frozen the tube was then opened and a D_2O solution (0.5 - 1.0 ml) of nucleoside (Tables 1 and 2), which had been degassed by a similar procedure, was added (total volume 1.0 - 1.5 ml). The freeze-thaw cycle

was again repeated for two cycles and the tube filled with a slight positive pressure of nitrogen gas, then sealed. The reaction tube was then heated in an oil bath for various times and temperatures as indicated in Tables 1 and 2. After centrifugation the aqueous phase was removed, dried in a rotary evaporator, and redissolved in H_2O (3 ml) to reexchange active hydrogen atoms. The product was again dried prior to mass spectrometry or nmr.

Method B. Freshly prepared platinum black was transferred in a volume of approximately 0.5 ml to a 10 x 100 mm tube as in Method A. Following addition of nucleoside (Tables 1 and 2) in D_2O (0.5 - 1.0 ml), the solution was purged by bubbling of deuterium gas for 10 min and the tube sealed. The resulting solution, saturated with deuterium, was then heated and the reaction products processed as in Method A.

Recoveries from representative reaction mixtures were estimated by hplc (C_{18} Microbondapak, 14% methanol, 0.01 M ammonium formate, pH 5.1) and found to lie generally within the range 70-90%. As an exception, a catalyst/substrate ratio of 10:1 in the case of 2'-deoxyadenosine caused significant decrease in the yield, to approximately 30%.

Mass spectrometry

Aliquots of reaction mixtures (ca. 100 µg) were dried over P_2O_5, then heated with N,O-bis(trimethylsilyl)acetamide (10 µl) and 1% trimethylchlorosilane (Pierce Chemical Co., Rockford, IL) in 90 µl of pyridine at 100° at 1 hr. The resulting trimethylsilyl (TMS) derivatives were introduced into an LKB 9000S mass spectrometer by gas chromatograph (4) under the following conditions: ionizing energy 70eV, ion source and carrier gas separator temperatures 270°. Deuterium distributions as shown in Tables 1 and 2 were calculated from M-CH_3 peaks; conclusions regarding deuterium distributions within the molecule were made from fragment ions from the base and sugar ion series (5,6).

Proton nuclear magnetic resonance

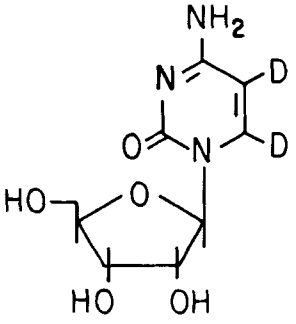
A proton nmr spectrum of adenosine which had been exchanged under conditions shown for experiment 10 in Table 2 was determined using a Varian SC-300 spectrometer at 300 MHz. A 5 mg sample was lyophilized from H_2O and redissolved in DMSO-$\underline{d}_6$. Chemical shifts (7) are reported relative to tetramethylsilane taken as 0 ppm: δ 7.38 (s, NH_2), 5.88 (s, H-1'), 5.46 (broad, H-2' and 3'-O$\underline{H}$), 4.16 (d, H-3'), 3.98 (m, H-4'), centered at 3.62 (H-5'); 5'-O$\underline{H}$ obscured by solvent; signals for H-2, H-8, H-2' not observed. A spectrum of 2'-deoxyadenosine (7 mg), which had been exchanged under conditions shown for experiment 16 in Table 2 was determined using a JEOL FX-270 spectrometer at 270 MHz, following lyophilization from 99.8% D_2O and solution in D_2O. Chemical shifts (8) are reported relative to tetramethylsilane (external standard) taken as 0 ppm: centered at δ 6.33 (H-1'), 4.58 (H-3'), 4.12 (H-4'), 3.76 (H-5'), 2.70 (H-2a'), and 2.50 (H-2b').

RESULTS AND DISCUSSION

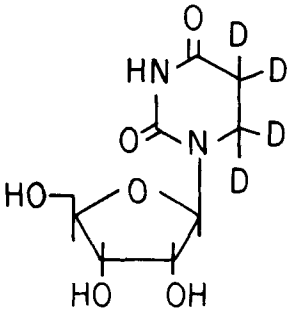
The results of platinum-catalyzed reactions of nucleosides in D_2O are given in Table 1. Method A is generally similar to that employed by Maeda and Kawazoe for the nucleosides, uridine, cytidine, thymidine and inosine (3), while Method B differs by addition of deuterium gas to the reaction medium.

The products obtained from uridine and cytidine by Method A are similar to those described previously (results not shown in Table 1) in which H-5 and H-6 have been exchanged (e.g., $\underline{1}$). However, by Method B uridine produces extensively base-labeled dihydrouridine ($\underline{2}$), in analogy to the reduction of uridine using rhodium catalyst (9). Dihydrouridine as a starting material produces only 36% $\underline{d}_1$ (3) and 8% of $\underline{d}_2$, which indicates that the extensive label in $\underline{2}$ results from exchange of the 5,6-double bond in uridine prior to reduction. On the other hand cytidine failed to undergo reduction, yielding only the dideuterio species $\underline{1}$.

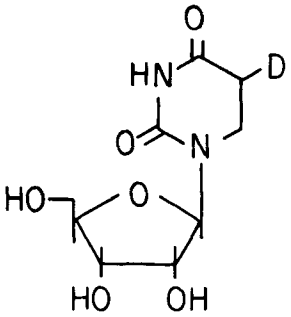
Structures of Principal Products Formed
by Catalytic Exchange and Reduction



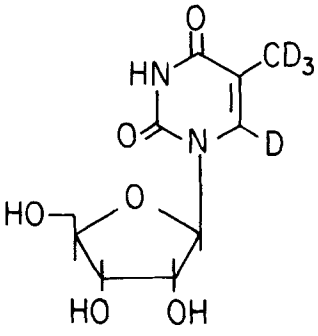
1



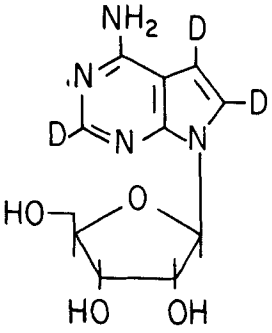
2



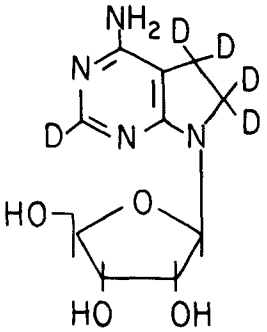
3



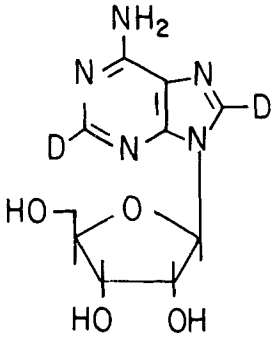
4



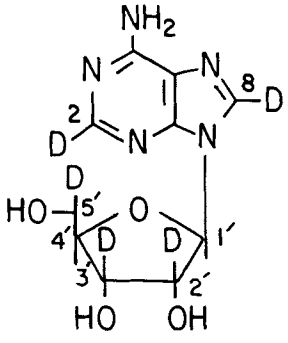
5



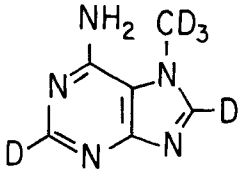
6



7



8



9

Table 1. Platinum-Catalyzed Exchange and Reduction Products of Nucleosides

Starting Material	Exchange Method	mg Pt/mg nucleoside	Temp °C	Reaction time hrs	Deuterium Distribution, Mole %[a]							Structure
					d_0	d_1	d_2	d_3	d_4	d_5	d_6	
Uridine	B	15/15	40	65			7	21	72			2
5,6-Dihydrouridine	B	16/8	80	120	56	36	8					3
Cytidine	B	15/30	80	45		7	93					1
1-β-D-(Ribofuranosyl)thymine	A	20/10	80	90			6	77	17			
1-β-D-(Ribofuranosyl)thymine	B	10/10	80	45		6	8	82	4			
1-β-D-(Ribofuranosyl)thymine	B	50/10	50	45			5	55	40			4
1-β-D-(Ribofuranosyl)thymine	B	50/10	80	120			2	47	48	3		
1-β-D-(Ribofuranosyl)thymine	B	50/10	95	45			3	47	45	5		
1-β-D-(Ribofuranosyl)thymine	B	50/5	50	45				47	53			
Thymidine	B	50/5	50	45				21	54	21	4	
Tubercidin	A	25/15	80	24	5	24	62	9				
Tubercidin	A	25/15	80	120			3	97				
Tubercidin	A	20/10	80	65			3	97				5
Tubercidin	B	10/5	40	24					8	92		
Tubercidin	B	20/10	40	65					6	94		6

[a]Corrected for presence of naturally-occurring heavy isotopes.

Table 2. Platinum-Catalyzed Exchange[a] of Adenosine and 2'-Deoxyadenosine

Reaction Number	mg Pt/mg Ado	mg Pt/mg dAdo	Temp °C	Time hrs	Deuterium Distribution, Mole %[b]						
					d_1	d_2	d_3	d_4	d_5	d_6	d_7
1	5/1		40	50	1	99					
2	10/10		60	90	3	97					
3	5/5		80	45	4	89	7				
4	50/5		80	110		11	37	33	14	5	
5	10/2		100	75		2	34	36	20	8	
6	10/10		100	65	3	75	18	4			
7	50/5		100	45		3	22	35	27	13	
8	50/10		100	45		21	47	23	9		
9	50/25		100	120		46	38	12	4		
10	100/10		100	65		4	35	36	21	4	
11	100/20		100	45		48	34	15	3		
12		30/5	100	120		12	28	36	19	5	
13		50/5	80	68			9	31	33	22	5
14		50/10	80	68		16	30	33	21		
15		50/30	80	68	8	81	11				
16		50/15	80	45		15	34	36	15		

[a]Method B. [b]Corrected for presence of naturally-occurring heavy isotopes.

1-β-D-(Ribofuranosyl)thymine (thymine riboside) failed to undergo reduction of the 5,6-double bond, reflecting steric hindrance offered by the C-5 methyl group, although Method A resulted in some labeling at C-6, contrary to earlier results with the deoxynucleoside thymidine (3). Exchange at C-6 appears to be sensitive to the catalyst/substrate ratio; 1:1 was least effective while ratios of 5:1 produced extensive exchange (4) which was not markedly altered by significant variations in reaction time and temperature. However at 80° and 95° Method B produced a small amount of labeling in the ribose moiety (3% and 5%, Table 1), as established by the mass spectrum of the TMS derivative (5).

The nucleoside antibiotic tubercidin undergoes extensive exchange in the pyrrolopyrimidine moiety using Method A, which becomes nearly quantitative (compound 5) at the longer reaction times studied. The reducing conditions of Method B resulted in essentially complete reduction and exchange of the 5,6-double bond to produce the labeled form (6) of the previously unreported compound 4-amino-5,6-dihydro-7-(β-D-ribofuranosyl)pyrrolo[2,3-d]pyrimidine.

Adenosine and 2'-deoxyadenosine were subjected to more extensive study of reaction variables, and selected products were examined by proton nmr to establish sites of deuteration in the sugar. When adenosine was subjected to Method B under conditions of reaction 4, Table 2, but using N_2 in place of D_2, essentially complete exchange of H-2 and H-8 was observed (7, data not shown in Table 1), as reasonably anticipated from earlier work on adenine, guanine (2) and inosine (3). Similar results were obtained from Method B (with D_2) under relatively mild conditions as shown in Table 2 (reactions 1-3). However at higher temperatures the unexpected incorporation of substantial amounts of deuterium into the ribose skeleton was observed. The labeling patterns from the base + 30 and base + 116 ions in the mass spectrum of the TMS derivative (6) showed quantitative exchange of the ribose hydrogen at C-2', suggesting that selective exchange of ribose

hydrogens may be possible. Therefore additional reactions under a variety of conditions were carried out, as represented by the data shown in Table 2.

The proton magnetic resonance spectrum taken of the reaction 10 product (Table 2) showed approximately 100% exchange of H-2, H-8, and H-2', 60% of H-3', and 20% of the methylene hydrogens at 5', as represented in structure 8. No evidence for change in anomeric configuration of the base, or in steric orientations of the hydroxyl groups, was found. In the case of adenosine, increased temperature appears to be the most important parameter in terms of promoting exchange of sugar hydrogens, as for example shown by comparison of results from reactions 2 and 6, Table 2. Beyond a minimum point no strong trend was noted from changes in reaction time, but higher ratios of catalyst to nucleoside (reactions 7 vs. 8, 7 vs. 11) were observed to be effective. The results of reactions 5 vs. 9 and 7 vs. 10 suggest that increasing molar concentrations of reactants tend to reduce the effectiveness of exchange under the conditions employed.

The reactions of 2'-deoxyadenosine (Table 2) were found to produce more extensive exchange in the sugar moiety than adenosine under similar conditions, as shown by comparison of reactions 4 and 13. Qualitatively similar results were observed for 1-β-D-(ribofuranosyl)thymine and thymidine (Table 1). In parallel to adenosine, increasingly higher catalyst to nucleoside ratios produced more extensive exchange in the sugar, as demonstrated by reactions 13, 14 and 15.

The proton nmr spectrum of the reaction 16 (Table 2) product showed complete exchange of H-2 and H-8 as expected, with approximately 50% exchange of H-2' and 35% of H-3'. Little or no exchange was observed at H-1', H-4', or H-5'. Interestingly, the extent of exchange at C-2' was nearly evenly divided between the two hydrogens bound to that carbon (H-2a' and H-2b') in spite of their different steric environments.

Attempts to incorporate significant levels of deuterium using Method B into the ribosyl moiety of guanosine were not successful. For example under conditions of 6 mg Pt/1 mg guanosine, 100°, 72 hrs, the isotopic distribution was 6% $\underline{d}_0$, 88% $\underline{d}_1$ (at C-8), 6% $\underline{d}_2$. No incorporation into methyl groups was obtained for 1-methyladenosine, N^6-methyladenosine, and 7-methylguanosine. However, 7-methyladenine showed a labeling distribution of approximately 3% $\underline{d}_2$, 6% $\underline{d}_3$, 30% $\underline{d}_4$ and 61% $\underline{d}_5$ (9) under conditions of 50 mg/10 mg, 100° and 65 hrs.

ACKNOLWEDGMENTS

The authors acknowledge financial support from the National Institutes of Health (GM 26892, CA 18024), and consultation and assistance from Dr. A. Srinivasan. K.H.S. was recipient of N.I.H. Fellowship CA 02466.

REFERENCES

1. For example: (a) Shapiro R. and Klein R. S. - Biochemistry 6: 3576 (1967); (b) Tomasz M., Olson J. and Mercado C. M. - Biochemistry 11: 1235 (1972); (c) Kochetkov N. K. and Budovskii E. I. - Organic Chemistry of Nucleic Acids, Part B, Plenum Press, New York, 1972, p. 282.
2. Eidinoff M. L. and Knoll J. E. - J. Am. Chem. Soc. 75: 1992 (1953).
3. Maeda M. and Kawazoe Y. - Tetrahedron Lett. 1643 (1975).
4. Hattox S. E. and McCloskey J. A. - Anal. Chem. 46: 1378 (1974).
5. McCloskey J. A., Lawson A. M. Tsuboyama K., Krueger P. M. and Stillwell R. N. - J. Am. Chem. Soc. 90: 4182 (1968).
6. McCloskey J. A. - Basic Principles in Nucleic Acid Chemistry, Vol. I, P.O.P. Ts'o, Ed., Academic Press, New York, 1974, Chap. 3.
7. Townsend L. B. - Synthetic Procedures in Nucleic Acid Chemistry, Vol. 2, Zorbach W. W. and Tipson R. S., Eds., Wiley-Interscience, New York, 1973, Chap. 7.
8. Slessor K. N. and Tracey S. - Carbohydr. Res. 27: 407 (1973).
9. Cohn W. E. and Green M. - J. Biol. Chem. 225: 397 (1957).