until the filtrate was colorless. Drying was achieved at reduced pressure in a desiccator with P_2O_5. Final yields of II were usually about 50%. The molecule displays ultraviolet absorption peaks at 325 nm (ε 7900), 294 (5300), and a shoulder at 240 (8500).

Anal. Calcd for $C_{17}H_{22}N_3O_8P \cdot H_2O$: C, 45.84; H, 5.39; N, 9.44; O, 32.36; P, 6.97. Found: C, 45.93; H, 5.62; N, 9.32; O, 32.17; P, 6.96.

The pmr and circular dichroic spectra of II are shown in Figures 3 and 5b. Electrophoretic and chromatographic analyses show the product to be a single migrating species.

"Cyclic" *N*-(5-Phosphopyridoxyl)-3′-amino-L-tyrosine (III). Ten millimoles of PLP (2.47 g) and 10 mmol of 3-nitro-L-tyrosine (2.26 g) were dissolved in 100 ml of H_2O with the addition of 4 ml of 10 *N* KOH. Upon addition of 2 g of PtO_2, H_2 reduction was carried out as described for compound II. The reaction stopped after theoretical H_2 uptake, and upon removal from the reduction apparatus N_2 was used to retard coloration. An Amberlite CG-50 column, 67 cm × 2.7 cm, was employed and elution was carried out with H_2O. After detection of the proper fractions,[27,29] and flash evaporation of these to 5–10 ml, cooling on ice gave crystals suitable for collection. These were retained on a sintered glass filter and washed with small volumes of cold H_2O until the filtrate was colorless. Yields of 80–85% were obtained after drying over P_2O_5. Compound III shows a uv absorption maximum at 325 nm (ε 9600) with shoulders at 305 (7000) and 240 (11,400).

Anal. Calcd for $C_{17}H_{20}N_3O_8P$: C, 48.10; H, 4.72; N, 9.90; O, 30.02; P, 7.30. Found: C, 47.70; H, 4.75; N, 10.03; O, 29.80; P, 7.72.

Figures 3 and 6a show the pmr and circular dichroic spectra of compound III.

Spontaneous Synthesis of III. A mixture of pyridoxal phosphate, 0.25 mmol (61.8 mg), and 0.25 mmol of 3-amino-L-tyrosine (49 mg) was formed in 1.5 ml of H_2O. Upon the addition of 0.1 ml of 10 *N* KOH the pH rose to 10.5. Analysis of the reaction mixture showed a uv spectrum corresponding to the cyclic compound III as described previously. The material was loaded onto an Amberlite XE-64 column, 70 cm × 2.5 cm, eluted with H_2O, and collected in 10-ml fractions. Fractions absorbing at 325 nm eluted as a major peak. These fractions were pooled and crystals were obtained upon reduction to a small volume. This product was identical with that obtained in the previous synthesis as judged by uv, CD, and pmr spectra, as well as its behavior in the various chromatographic and electrophoretic systems. The two cyclic preparations also acted in an indistinguishable manner when employed in the immunological studies.

Compound VI. Two millimoles of 3-amino-L-tyrosine, 392 mg, was added to 2 ml of H_2O and addition of 1–2 small drops of 10 *N* KOH brought the pH of the stirred solution to approximately 10. Two millimoles of formaldehyde (0.165 ml of 37% solution in H_2O) was added and the pH was maintained at 9–10 with KOH solution. The reaction was permitted to proceed for 15 min after which concentrated HCl was added to lower the pH to 5–6. The precipitate was left to sit on ice for 1 hr and was then collected and washed with cold H_2O. Even after reprecipitation, chromatography showed the preparation to contain some minor contaminants.

The compound displayed a uv peak at 293 nm (ε 4000) and a shoulder at 235 (6600). Upon chromatography, the major component demonstrated an ability to react with aromatic amine reagents. It did not react with ninhydrin to give the blue color typical of an amino acid, but gave a yellow spot. This result is indicative of α-imino acids such as proline and is consistent with structure VI. The pmr analysis shown in Figure 6b confirms the product.

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Nucleosides. LXXIX. Facile Base-Catalyzed Hydrogen Isotope Labeling at Position 6 of Pyrimidine Nucleosides[1]

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Abstract: Quantitative or nearly quantitative incorporation of deuterium at C-6 in all common pyrimidine nucleosides, including ara-C, has been achieved. The method involves the treatment of such nucleosides with a mixture of deuterated base and DMSO-d_6. In those cases where the sugar hydroxyls can easily participate in the saturation of the 5,6-double bond, quantitative incorporation of deuterium at C-5 was also obtained. In these latter cases, 6-deuterated nucleosides were obtained from 5,6-dideuterated derivatives by treating them under conditions in which only the 5 position exchanges. The mechanism of H-6 exchange is thought to involve the direct abstraction of H-6 by base. Experimental evidence supporting this mechanism is given. The method for isotope labeling at C-6 of nucleosides described herein should be eminently adaptable for the incorporation of tritium label into pyrimidine nucleosides for use in biochemical studies.

One of the important aspects of the chemistry of pyrimidines and their nucleosides is the susceptibility of the 5,6-double bond to 1,4-nucleophilic addition reactions. A manifestation of this reactivity is the observed hydrogen isotope exchange at the 5 position when reversible addition reactions are performed in deuterated media. Some of the most prominent examples of H-5 exchange are: of cytidine in acidic buffers,[2] of certain pentofuranosyluracils in basic media,[3-6] and of uridine with catalysis by glutathione.[7] On the other hand, a facile base-catalyzed exchange at

(1) This work was supported in part by funds from the National Cancer Institute, National Institutes of Health, U. S. Public Health Service (Grant No. CA 08748), and by a postdoctoral fellowship to J. A. R. from the Gimbel Foundation.

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position 6 has been found to occur in various 5-halogenouridines[4,8] and slower in 5-unsubstituted nucleosides.[6] During a preliminary study of the base-catalyzed H-5 exchange of 2′,3′-*O*-isopropylideneuridine in a mixture of DMSO-d_6–D_2O, we observed that both H-5 *and* H-6 exchanged at approximately equal rates. In view of the facility of the method and the applications that nucleosides labeled at C-6 would have in many chemical and biochemical studies, we have explored further the base-catalyzed reactions of the most common pyrimidine nucleosides in mixtures of DMSO-d_6–D_2O (or DMSO-d_6–MeOD). At the outset, we also hoped that a study of these exchanges would give insight into the mechanism of H-6 exchange. The present paper is an account of this investigation.

Experimental Section

Materials and Methods. Uridine, 2′-deoxyuridine, cytidine, and thymidine were purchased from Cyclo Chemical Corp., Los Angeles, Calif.; Ip-U[9] was from Waldhof, West Germany; ara-C[9] was supplied by Upjohn Co., Kalamazoo, Mich. Dimethyl-d_6 sulfoxide (DMSO-d_6), deuterium oxide (D_2O), methanol-d_1, sodium deuterioxide, and tetramethylsilane were obtained from Stohler Isotope Chemicals, Rutherford, N. J. Melting points were determined by the capillary method on a Thomas-Hoover apparatus. Evaporations were carried out *in vacuo* with bath temperature below 40°. Proton magnetic resonance (pmr) spectra were recorded on a Varian A-60 spectrometer using tetramethylsilane as internal standard (δ 0.00). All reactions, unless otherwise stated, were performed in closed vials and the reaction mixture was stirred magnetically. The extent of the H → D exchange reactions was measured by comparison of the integral of the protons being exchanged with that of the anomeric proton which did not exchange. Before the base-catalyzed reaction, all nucleosides were first treated with D_2O to exchange the N–H and sugar hydroxyl protons. This was achieved by dissolving the nucleoside in D_2O followed by evaporation of the solution to dryness; this operation was repeated twice. Unless otherwise stated, after the base-catalyzed exchange reactions were completed, the nucleosides were recovered from the reaction mixtures by charcoal chromatography. Activated charcoal, G-60 (Fisher Scientific Co., Fair Lawn, N. J.), was used as such. The procedure[5] consisted in making a slurry of a 1:1 (w/w) mixture of charcoal and cellulose powder in water and allowing this slurry to settle on a cellulose bed in a glass column (~4 cm internal diameter). The ratio of charcoal/nucleoside was ~15 w/w. The reaction mixtures were first neutralized with 1 *N* HCl and then diluted to a specified volume in each case (*vide infra*). These solutions were passed through the charcoal column. After all the solution had passed through, the column was washed with water until the eluate was chloride ion negative as detected by reaction with silver nitrate solution. The nucleosides were eluted from the charcoal with 50% EtOH–H_2O and the effluents concentrated, dissolved in a small amount of water, and filtered through a Millipore filter (0.5 μ) to eliminate charcoal particles (Millipore Corp., Bedford, Mass.). In all cases, the absorption of the nucleoside on the charcoal was quantitative and the elution of nucleoside from it was almost quantitative as determined by uv spectroscopy. Ultraviolet absorption properties as well as melting points found for deuterated nucleosides were in good agreement with those exhibited by commercial undeuterated samples or those reported in the literature.

5,6-Dideuterio-2′,3′-*O*-isopropylideneuridine. Ip-U (1.0 g, 3.5 mmol) was dissolved in 8.5 ml of DMSO-d_6, and 3 ml of 10% NaOD in D_2O was added. The resulting solution was heated at 66° for 20 hr, after which time the complete mixture was diluted with water and neutralized with 1 *N* HCl and the volume adjusted to 100 ml with water. After charcoal chromatography and concentration of the eluates (360 ml), the product crystallized giving 0.80 g of Ip-U-5,6-d_2: mp 163–165°; the pmr spectrum (DMSO-d_6) showed the absence of both H-5 and H-6 signals (Figure 1); the remainder of the spectrum was identical with that of pure Ip-U.

6-Deuterio-2′,3′-*O*-isopropylideneuridine. Ip-U-5,6-d_2 (0.10 g) was dissolved in 1 ml of 1 *N* NaOH and the resulting solution kept at 60° for 15 hr. The reaction mixture was then diluted with water and neutralized with Dowex 50-H^+. The solvent was evaporated and the residue crystallized from acetone–petroleum ether to give 0.09 g of Ip-U-6-*d*: mp 162–164°; the pmr spectrum (DMSO-d_6) showed that 90% of D-5 and 10% of D-6 had exchanged (Figure 1); the remainder of the spectrum was identical with that of Ip-U. A similar reaction of Ip-U with NaOD–D_2O gave Ip-U which contained at least 95% deuterium at C-5 and ~5% deuterium at C-6 (Figure 1).

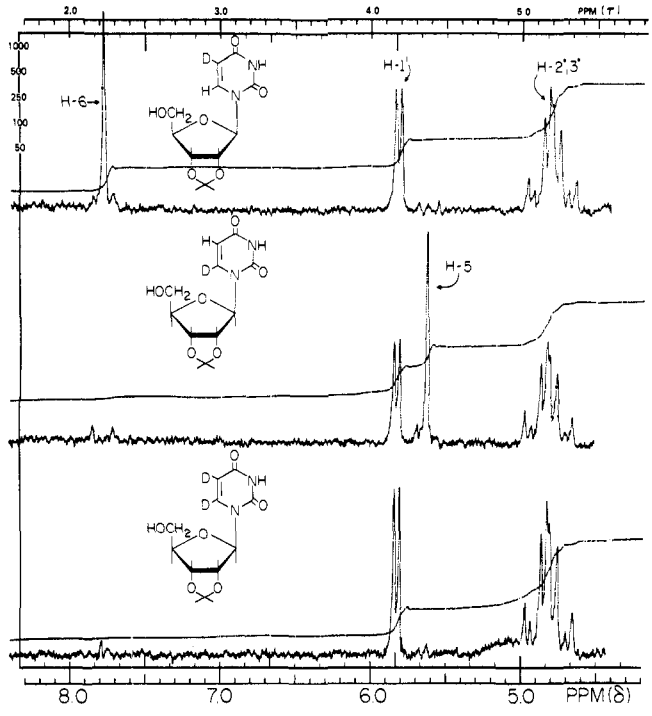


Figure 1. The proton magnetic resonance spectra of labeled Ip-U nucleosides in DMSO-d_6: Ip-U-5,6-d_2 bottom; Ip-U-6-*d*, middle; and Ip-U-5-*d*, top.

6-Deuterio- and 5,6-Dideuteriouridine. Uridine (0.48 g, 2 mmol) was dissolved in 4.5 ml of DMSO-d_6, and 1.7 ml of a 10% solution of NaOD in D_2O was added. The cloudy mixture cleared on heating, and after 16 hr at 65° the pmr spectrum of the mixture showed that about 17% of H-6 had been exchanged. The temperature was raised to 75° and, after a total of 6 days, ~85% of H-6 had exchanged for deuterium. The reaction mixture was diluted with water and neutralized. The volume was adjusted to 50 ml and a quantitative determination of the optical density of the solution revealed that ~25% of the chromophore had been destroyed. After charcoal chromatography, the effluent was concentrated to an oily residue which was dissolved in ~5 ml of hot methanol. On cooling, 0.10 g of labeled uridine was obtained: mp 164–166°, identical with that given by a commercial sample of uridine; the pmr spectrum (D_2O) showed that C-6 was ~80% deuterated, whereas C-5 contained only ~30% deuterium. The remainder of the spectrum was identical with that of uridine.

2′-Deoxy-6-deuterio- and 5,6-Dideuteriouridine. 2′-Deoxyuridine (0.46 g, 2 mmol) was dissolved in 4 ml of DMSO-d_6 and 1.5 ml of a 16% solution of NaOD in D_2O was added. After ~22 hr at 75°, H-6 had exchanged almost completely, whereas only ~20% deuterium had been incorporated at C-5. The reaction mixture was kept at 75° for an additional 5 hr and then diluted with water and neutralized. The volume was adjusted to 50 ml and the optical density determined. It was found that 76% of the pyrimidine chromophore was still present. After charcoal chromatography and concentration of the effluent, the residue was crystallized from hot methanol to give 0.205 g, mp 161–163°. Recrystallization from methanol gave 0.165 g, mp 164°; the pmr spectrum (D_2O) showed that C-6 was 95% deuterated whereas C-5 contained only ~15% deuterium. The remainder of the spectrum was identical with that of 2′-deoxyuridine.

Under the same conditions, thymidine exchanged only about 30% of H-6 in 25 hr.

(8) C. T. Cori, Ph.D. Thesis, Washington University, St. Louis, Mo., 1970.

(9) Abbreviations used are: Ip-U, 2′,3′-*O*-isopropylideneuridine; ara-C, 1-β-D-arabinofuranosylcytosine.

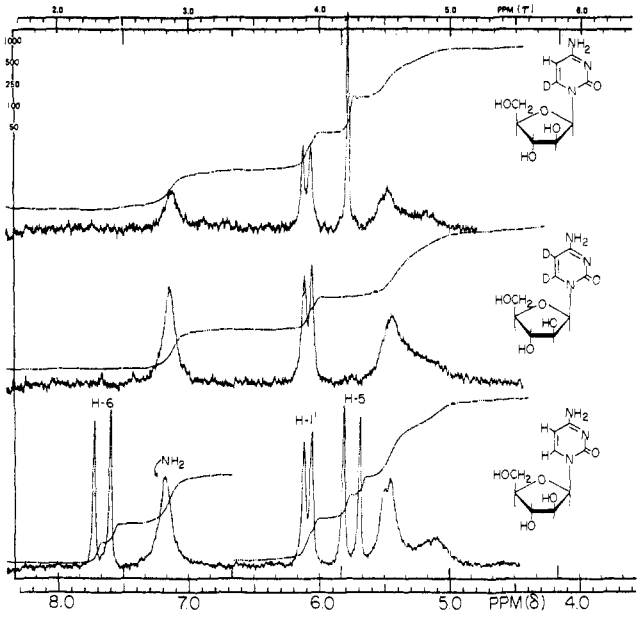


Figure 2. The proton magnetic resonance spectra of ara-C nucleosides in DMSO-d_6. Ara-C, bottom; ara-C-*5,6-d*$_2$, middle; and ara-C-*6-d*, top

6-Deuteriothymidine. Thymidine (0.484 g, 2 mmol) was dissolved in 6 ml of DMSO-d_6, and 2 ml of a 10% solution of NaOD in D_2O was added. The mixture was poured into a tube and frozen, and the tube was sealed. The mixture was allowed to reach room temperature and then heated in an oven at ~135° for 48 hr. The tube was cooled and opened. The pmr spectrum of the brown mixture showed that H-6 had exchanged to the extent of ~90%. The mixture was diluted with water and neutralized. The volume of the solution was adjusted to 100 ml and the optical density measured. It was found that 88% of the expected total optical density was still present. After charcoal chromatography, the effluent (~450 ml) was evaporated to give a white crystalline product which was triturated with cold ethanol and filtered to give 0.365 g, mp 186–187°; the pmr spectrum (DMSO-d_6) was identical with that for thymidine except that ~90% of deuterium was incorporated at C-6.

5,6-Dideuteriocytidine. Cytidine (0.48 g, 2 mmol) was dissolved in 4.5 ml of DMSO-d_6 and 1.7 ml of a 2.5 *M* solution of MeONa in MeOD was added.[10] After 17 hr at 60°, the pmr spectrum of the mixture showed that H-5 and H-6 were completely replaced by deuterium. The mixture was diluted with water and rapidly neutralized. The volume was adjusted to 100 ml and a quantitative determination showed that 94% of the total optical density was still present. The λ_{max}^{pH1} 280 nm showed that no deamination had occurred. After charcoal chromatography, the effluent (425 ml) was concentrated and the residue was crystallized from ethanol–water to give 0.33 g, mp 212–213°; the pmr spectrum (DMSO-d_6) showed the complete absence of H-5 and H-6, whereas the remainder of the spectrum was identical with that for cytidine.

1-β-D-Arabinofuranosyl-5,6-dideuteriocytosine (ara-C-*5,6-d*$_2$). The procedure was identical with that described for the synthesis of 5,6-dideuteriocytidine. The yield was 0.31 g of ara-C-*5,6-d*$_2$, mp 215–216°; the pmr spectrum (DMSO-d_6) showed complete absence of H-5 and H-6 (Figure 2).

1-β-D-Arabinofuranosyl-6-deuteriocytosine (ara-C-*6-d*). The above dideuterated nucleoside (0.122 g, 0.5 mmol) was dissolved in 10 ml of MeOH containing 1.31 mmol of MeONa. The solution was kept at 50° for 10 hr and then diluted and neutralized. A quantitative recovery based on optical density determination was obtained. The solution was concentrated and the residue (0.15 g, mp 205–206°) was crystallized from ethanol–water to give 75 mg, mp 209–211°. This product still contained a small amount of NaCl, but it was not further purified. The pmr spectrum (DMSO-d_6) showed the absence of H-6 and a singlet for H-5; the remainder of the spectrum was identical with that for ara-C (Figure 2).

Exchange of 1,3-Dimethyl-5-substituted Uracils. To a solution of the 5-substituted uracil derivative (0.14 mmol) in 0.3 ml of DMSO-d_6, 20 μl of a 1.25 *M* NaOD solution (0.05 mmol) was added and the exchange of H-6 determined by pmr spectroscopy at ~38°. The approximate times for 50% H-6 exchange were: 5-bromo-, <10 sec; 5-methoxy-, ~30 sec; 5-hydrogen, ~1 min, and 5-methyl-, ~20 min. 1,3-Dimethyluracil exchanged 50% of its H-5 in ~50 min.[11]

Discussion

Exchange of H-5. The base-catalyzed hydrogen isotope exchange at position 5 of various pyrimidine nucleosides has been studied previously by Santi and Brewer,[3] Cushley, *et al.*,[4] Heller,[5] and Wechter.[6] In all these cases, methanolic base[3] or aqueous base[4,5,6] was employed. They have suggested that the mechanism of this exchange involves the 1,4-addition of a nucleophile to the 5,6-double bond of the pyrimidine moiety. A similar mechanism has been proposed by Kalman[7] for the H-5 glutathione catalyzed exchange of uridine. Furthermore, it has been observed that base-catalyzed exchange at position 5 proceeds at a faster rate in those nucleosides having a hydroxyl group which can easily participate in the saturation of the 5,6-double bond.[3,4,6] Our results obtained in mixtures of deuterated base with DMSO-d_6 fully agree with these findings as shown by the fact that 2′,3′-*O*-isopropylideneuridine, in which anchimeric assistance by the 5′-hydroxyl group is expected to be facilitated by the isopropylidene group, exchanges *both* H-5 and H-6 at approximately the same rate. In contrast, uridine and 2′-deoxyuridine, in which the 5′-hydroxyl group participates to a lesser extent,[3,4] exchange H-5 at a rate much slower than they exchange H-6. In the case of cytosine nucleosides, exchange of H-5 does occur readily as shown by the formation of 5,6-dideuteriocytidine and 1-β-D-arabinofuranosyl-5,6-dideuteriocytosine. This difference in the labeling at position 5 between uracil and cytosine nucleosides can be explained by consider-

(10) When NaOD in D_2O was used instead of MeONa in MeOD, exchange at C-5 and C-6 took place but hydrolytic deamination to uridine was observed. Thus, when an equivalent amount of NaOD in D_2O (1.7 ml of a 2.5 *M* solution of NaOD) was added to a solution of cytidine (2 mmol) in 4.5 ml of DMSO-d_6 and the reaction mixture heated at 65° for 14 hr, 33% of the uv-absorbing material was uridine as detected by electrophoresis in borate buffer pH 9.2. From the pmr spectrum of the mixture it could be determined that H-6 was almost completely exchanged indicating that the rate of exchange of H-6 of cytidine was faster than that of its hydrolysis to uridine. Further evidence that no deamination is observed in cytosine derivatives by using nonaqueous base was the observed complete stability of ara-C to treatment with 3 equiv of MeONa in MeOH under reflux for 2 days.

(11) (a) That the loss of the H-6 signal in 1,3-dimethyl-5-substituted uracils is due to exchange by deuterium rather than to conversion to other products is supported by the following. (i) For the decomposition of 1,3-disubstituted uracils in alkali, saturation of the 5,6-double bond is required. It is known[11b] that 1,3-dimethyluracil undergoes slow decomposition overnight in 1 *N* NaOH at room temperature with loss of selective absorption. It was shown recently[11c] that 5′-*O*-Me-3-Me-Ip-U remains essentially unchanged in 1 *N* KOH at 37° for at least 90 min whereas 3-Me-Ip-U loses 50% of its selective absorption in the ultraviolet in 0.3 *N* KOH within 3 min. In the latter compound, anchimeric assistance by the 5′-oxyanion is provided for saturation of the 5,6-double bond. With the 1,3-dimethyl-5-substituted uracils which we investigated, anchimeric assistance is not possible. Further, the ratio of pyrimidine/alkali is ~3 which would not favor intermolecular nucleophilic addition reactions to any significant extent in the relatively short time span required for the loss of the H-6 signal. (ii) During the exchange reactions of 1,3-dimethyl-5-substituted uracils no other signals are detected which could be attributed to side products. Instead, specific pmr spectral changes due to H-6 exchange by deuterium are observed. For example, with 1,3-dimethyluracil loss of the H-6 signal was accompanied by the collapse of the H-5 doublet into a singlet. In the case of 1,3-dimethylthymine, the loss of the H-6 signal occurred concomitantly with the collapse of the 5-methyl doublet into a sharp singlet. The remainder of the spectra was unchanged. (b) D. Shugar and J. J. Fox, *Biochim. Biophys. Acta*, **9**, 199 (1952); (c) Y. Kondo, J. L. Fourrey, and B. Witkop, *J. Amer. Chem. Soc.*, **93**, 3527 (1971).

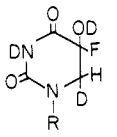
ing the effects that a negatively charged uracil moiety[12] imposes on the reactivity of the 5,6-double bond. Firstly, a negative charge, as in the case of uracil nucleosides, will oppose the approaching oxyanion (intra- or intermolecular reaction) by electrostatic repulsion;[13] secondly, the distribution of the negative charge in the pyrimidine ring increases the electron density at the 5,6-double bond which diminishes its susceptibility toward 1,4 addition. The total shielding effect associated with the ionization of the uracil moiety of uridine was found to be 0.20–0.21 ppm.[7]

Exchange of H-6. Exchange at position 6 in 5-halogeno nucleosides has been reported. For example, 5-fluorouridine exchanges H-6 for deuterium when treated with 0.5 *N* NaOD in D_2O ($t_{1/2} \sim 20$ min at 60°);[4] 5-chloro-, 5-bromo-, and 5-iodouridine also undergo H-6 exchange when treated with $(CH_3)_4NOD \cdot 5D_2O$ in DMSO-d_6 ($t_{1/2} < 0.5$ hr at room temperature, ratio: base/nucleoside ~ 5).[8] The H-6 exchange of unsubstituted nucleosides has also been observed.[6]

Thus, when uridine was treated with NaOD in D_2O (alkali/uridine = 3) at 95° for 2.5 hr, a trace of 6-deuteriouridine could be detected by pmr spectroscopy. In a similar run in which the product was actually isolated, Wechter determined that incorporation of deuterium at C-6 had occurred to the extent of 4% (pmr determination).[6] Under these conditions, 20% of the starting material was decomposed before isolation and the 5 position had incorporated 24% deuterium. The same procedure applied to cytidine (alkali/cytidine = 5) for 18 hr followed by isolation of the undecomposed cytidine still present gave 5,6-dideuteriocytidine (55%), 5-deuteriocytidine (25%), 6-deuteriocytidine (10%), and unlabeled cytidine (10%). It is to be noted that, under these conditions, the rate of hydrolysis to uridine was estimated to be about one-half of the rate of exchange of H-5 or H-6. On the other hand, ara-C exchanged H-5 exclusively under identical conditions. Thus after 18 min at 95° (alkali/ara-C = 3.5), ara-C-*5-d* comprised 64% of the mixture. In this case, however, the rate of exchange at position 5 was 20 times faster than the hydrolysis to arabinofuranosyluracil.[6]

In contrast to the results of Wechter, we were able to obtain quantitative, or nearly quantitative, incorporation of deuterium at C-6 in *all* of the common pyrimidine nucleosides, and also in ara-C, when the corresponding nucleoside was treated with a mixture of deuterated base and DMSO-d_6. As stated previously, in those nucleosides in which an oxyanion of the sugar moiety can participate easily in a 1,4 addition across the 5,6-double bond, we also observed exchange of H-5. This latter property allowed us to obtain selectively labeled nucleosides on C-6 by treating the dideuterated nucleoside under conditions in which only exchange at C-5 is observed (NaOH in H_2O or MeONa in MeOH). In this manner we were able to obtain 6-deuterio-2′,3′-*O*-isopropylideneuridine (Figure 1) and ara-C-*6-d* (Figure 2).

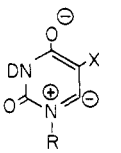
Several mechanisms have been proposed to account for the exchange of H-6 for deuterium in nucleosides. Thus, Cushley, *et al.*,[4] proposed that the observed exchange of H-6 in 5-fluorouracil derivatives, which also occurs under neutral conditions, proceeded through the formation of a saturated intermediate (A) which could



A

R = H, CH_3, β-D-Ribofuranosyl

eliminate HOD to give 6-deuterio-5-fluorouracil derivatives. Two experimental results seem to rule out this mechanism. (1) During the exchange at C-6, 5-hydroxyuracil derivatives were not formed from 5-fluorouracil derivatives nor from the other 5-halogenouridines.[8] (2) Structures such as intermediate A have been found to resist dehydration. For example, 5-trifluoromethyl-5-hydroxy-5,6-dihydrouracil does not undergo dehydration even under the most drastic acidic or basic conditions;[14] 5-hydroxy-5,6-dihydrothymine was also found to be stable in acidic or neutral media.[15] On the other hand, the exchange of 5-halogenouridines (*i.e.*, chloro, bromo, and iodo) was postulated to occur through the direct abstraction of H-6 by base to yield intermediate anion B stabilized by the adjacent positively charged nitrogen.[8]

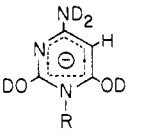


B

R = β-D-Ribofuranosyl

X = Cl, Br, I

A third mechanism has been proposed by Wechter[6] to account for the rapid labeling of cytidine in NaOD–D_2O. This proposal assumes the formation of a homocyclopentadienyl anion intermediate C.[6] It was claimed[6]



C

R = β-D-Ribofuranosyl

that "the undetectable rate of exchange at C-6 relative to C-5 in ara-C is consistent with the inability of the previously proposed anhydro intermediate D to form an intramolecular base such as C." Our results clearly do not support the existence of intermediate C as being responsible for the exchange at C-6. Exchange of H-6 in ara-C proceeded readily in DMSO-d_6–MeOD containing MeONa, a medium which could not possibly

(12) In the media employed (deuterated base with DMSO-d_6) for the exchange reactions, the uracil moiety is surely completely ionized (pK_a of 1-substituted uracils is ~9). The greatly enhanced activity of the oxyanions present in these media is such that we often observed the concomitant exchange of the methyl deuterons of DMSO-d_6 as shown by the increase of the integration of its signal at $\delta \sim 2.50$. The pK_a of DMSO is ~40: H. O. House, "Modern Synthetic Reactions," W. A. Benjamin, New York, N. Y., 1965, p 164.

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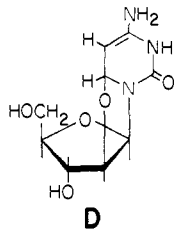
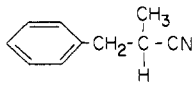


D

support the formation of C.[16] Furthermore, in the case of uridine and 2′-deoxyuridine, exchange of H-6 is much faster (in DMSO-d_6–D_2O using NaOD as a base) than the exchange of H-5; since formation of an intermediate of the type C requires first the 1,4 addition of OD^- at C-6, it would be expected that this addition would also result in exchange of H-5. Clearly this is not the case, and it must be concluded that exchange at C-6 does not involve the 1,4 addition of a nucleophile to the 5,6-double bond.

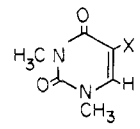
The most plausible mechanism of H-6 exchange for deuterium in DMSO-d_6–D_2O (MeOD) containing NaOD (NaOMe) is through the formation of an anion at C-6 by direct abstraction of H-6 by base as suggested by Beak[17] and Cori[8] for H-6 exchange in 5-halogenonucleosides. The greater facility of dissociation of H-6 in the presence of DMSO-d_6 can be explained by the known dependency on the solvent of ionization of carbon acids. For example, the rates of racemization of 2-methyl-3-phenylpropiononitrile (E) reflect the rates of



E

carbanion formation.[18] With potassium methoxide as base, the rate of racemization of E in 1.5% methanol–98.5% DMSO is 10^7 times greater than the rate in pure methanol. The greatly enhanced activity of the methoxide anion (or the hydroxide anion) in DMSO compared with methanol (or water) is well documented.[19]

Formation of anion on C-6 is also indicated by the difficulty encountered in the synthesis of 6-deuteriothymidine (~90% exchange at 135° for 48 hr) as compared with 2′-deoxyuridine (>95% exchange at 75° after 22 hr). In the case of thymidine, the inductive effect of the methyl group would not favor the development of a negative charge on C-6. Furthermore, a qualitative estimate of the effect of 5-substituents on the rate of exchange of H-6 in 5-substituted 1,3-dimethyluracils (F) showed that the ease of exchange follows the



F

X = H, CH_3, CH_3O-, Br

order: 5-Br > 5-MeO > 5-H > 5-Me. This result is to be expected on the basis of inductive effects and is consistent with the formation of a transition state with an appreciable degree of carbanion character. These results are also in agreement with the large substituent effects observed for the base-catalyzed H–D exchange of N-substituted pyridinium ions.[20]

Anionic or ylide intermediates similar to B have also been postulated to explain the hydrogen–deuterium exchange of the C-2 and C-6 protons of *N*-methyl-4-pyridone[21] and the C-2 proton of 1-methyl-4-pyrimidone.[22] In the former case, too, substitution of the 3 and 5 position by methyls resulted in a decreased rate of H-2 and H-6 exchange.[21]

The ease of H-6 exchange in 1,3-dimethyluracil (50% exchange in ~1 min at 38°; see Experimental Section) illustrates the inhibitory effect that a negatively charged uracil has on the rate of H-6 exchange (Ip-U, for example, shows 50% exchange in ~3 hr at 66°).

In the case of the uracil nucleosides studied, the reactive species of the heterocycle would be almost certainly the monoanion[12] and the intermediate carbanion would be a dianion. It is likely therefore that the relatively slow exchange of H-6 of Ip-U compared to 1,3-dimethyluracil is a consequence of the increased energy required to produce a doubly charged species.

It must be emphasized that the isotopic labeling at C-6 of nucleosides described herein should be eminently adaptable for the incorporation of tritium label into pyrimidine nucleosides for use in biochemical studies. Furthermore, it is obvious that by the use of *simple*, well-known procedures it is now possible to convert some of the labeled nucleosides discussed above into others. For example, hydrolysis of Ip-U-*5,6-d*$_2$ and Ip-U-*6-d* with 90% trifluoroacetic acid at room temperature for 5–10 min[23] should give 5,6-dideuteriouridine and 6-deuteriouridine, respectively. Furthermore, selective conditions are available to transform 5,6-dideuteriocytidine into 6-deuteriocytidine or into 6-deuteriouridine by the methods described by Shapiro and Klein.[2]

Acknowledgment. The able assistance of Mr. Marvin Olsen in recording the pmr spectra is greatly appreciated.

(16) According to Wechter,[6] the formation of intermediate C requires first the formation of a 6-hydroxy-5,6-dihydropyrimidine derivative followed by ionization of the 6-OH group to the 6-oxyanion-5,6-dihydropyrimidine. The latter *anion* abstracts H-6 intramolecularly to produce C. If Wechter's proposed mechanism holds, then in the case of our DMSO-d_6–MeOD–NaOMe medium, one should obtain the 6-methoxy-5,6-dihydro derivative which obviously could not form the 6-oxyanion necessary for H-6 abstraction. Moreover, in such a 6-methoxy-5,6-dihydro structure, the proton at C-6 is similar in environment to the anomeric proton (H-1′) which does not undergo exchange for deuterium. The fact is that H-6 does exchange in MeOD–NaOMe–DMSO-d_6 which renders the proposed mechanism[6] untenable.

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