

# *O*<sup>6</sup>,5'-Cyclo-5,6-dihydro-2'-deoxyuridine. Novel Deoxyuridine Photoproducts

Jean Cadet,<sup>†1a</sup> Lou-Sing Kan,<sup>‡1b</sup> and Shih Yi Wang\*<sup>1b</sup>

Contribution from the *Laboratoire de Radiobiologie, Departement de Recherche Fondamentale, Grenoble, France, and the Program in Environmental Chemistry, Department of Environmental Health Sciences, The Johns Hopkins University, School of Hygiene and Public Health, Baltimore, Maryland 21205. Received March 31, 1978.*

**Abstract:** Irradiation (254 nm) of deoxyuridine (dUrd)<sup>8</sup> in dilute aqueous solutions resulted in the formation of two isomeric photohydrates, 6-hydroxy-5,6-dihydro-2'-deoxyuridine, as major products and two diastereoisomers of *O*<sup>6</sup>,5'-cyclo-5,6-dihydro-2'-deoxyuridine as minor products. The former were produced by 1,4-addition of water to dUrd and the latter presumably by intramolecular cycloaddition of OH(5') of the sugar moiety to C(6) of the pyrimidine rings. Most probably the two processes compete for the same zwitterionic intermediate. The photoproducts were characterized by spectral data and elemental analyses, conformational analyses based on NMR data are discussed, and absolute configurational assignments are made. In addition, the photohydrates were synthesized by bromination of dUrd and followed by zinc/acetic acid reduction. Rate differences observed in the reverse or dehydration reactions of the photoproducts are rationalized in terms of basicity and ring strain. Formation of these new photoproducts may be significant to the understanding of the photobiology of nucleic acids.

## Introduction

Irradiation (mainly 254 nm) of uracil (Ura) derivatives in dilute aqueous solutions yields predominantly photohydrates, 5,6-dihydro-6-hydroxyuracil (ho<sup>6</sup>hUra) derivatives, whose structures have been unambiguously established.<sup>2,3</sup> These photohydrates readily reverse to the originals when treated with acid, base, or heat under mild conditions.<sup>2-5</sup> 1,4-Addition of water to the conjugated enone system of Ura has been suggested as a possible photochemical mechanism.<sup>2b,3,6,7</sup> NMR analysis of a *crude* solution of photolyzed uridine (Urd) indicated the presence of two diastereoisomers of ho<sup>6</sup>hUrd<sup>8</sup> which have also been shown to be  $\gamma$ -radiation products identified by chemical synthesis.<sup>9</sup> Likewise, photoproducts of 2'-deoxyuridine (dUrd) have been resolved into two bands by chromatography and tentatively assigned as the two stereoisomeric photohydrates.<sup>10</sup> However NMR analysis of one of these bands showed that it alone comprises the stereoisomeric pairs; therefore, the other with such a large difference in the *R<sub>f</sub>* values must consist of a different class of compounds.<sup>11</sup> Contrary to a report by others<sup>12</sup> we have found that the latter, like the photohydrates, are readily reversible to the originals. For the purpose of clarification efforts were made to characterize these new photoproducts, and the following reports our findings.

## Experimental Section

Infrared (KBr pellets) and UV absorption spectra were recorded on a Perkin-Elmer infrared spectrophotometer, Model 21, and a Beckman recording spectrophotometer, Model DK-1, respectively. NMR spectra in D<sub>2</sub>O at ~18 °C were obtained on a Varian HA-100 with FT unit or Cameca 250-MHz spectrometer operating in the field-sweep mode and using sodium 3-trimethylsilyl[2,2,3,3-<sup>2</sup>H<sub>4</sub>]-propionate as internal standard (TSP = 0.0 ppm). Optical rotation and circular dichroism (CD) spectra were recorded in methanol with a Quick Jouan polarimeter and a Jobin-Yvon spectrometer. A Waters Model R1 401 instrument equipped with a loop injector (Model U6K) and a 25-cm column of 5 $\mu$ -reversed phase RP-8 (Merck) was used for the LC separations. Radioactive samples were counted in a Packard Tricarb Model B 2450 liquid scintillation counter.

**Ultraviolet Irradiations.** Two kinds of irradiation apparatus were

used in this study. One, with seven Sylvania G15T8 germicidal lamps, has been described previously.<sup>2b</sup> The other, with one G8T5 lamp, was used mainly for experiments with radioactive compounds; the dose rate was 47 J/(m<sup>2</sup>/s).

**Irradiation of dUrd (1) in Aqueous Solution.** A 1 mM aqueous solution of dUrd (pH 6.5, unbuffered) was purged first with N<sub>2</sub> for ~15 min and then was photolyzed with (mainly) 254-nm light until there was no further absorbance decrease in the 260-nm region. After concentration under reduced pressure, the solution was applied on precoated cellulose thin layer plates (Merck 5510-9H). For isolation purposes, preparative plates were used. The eluent (A) was the lower layer of a solution of CHCl<sub>3</sub>/CH<sub>3</sub>OH/H<sub>2</sub>O (4:2:1 by volume) to which 5 mL of absolute CH<sub>3</sub>OH had been added for each 100 mL of the organic phase. After the plate was heated for 1 h at 110 °C, four photoproducts in addition to unchanged dUrd were detected by fluorescence in short-wavelength UV light. Spraying a plate with cysteine-sulfuric acid and heating with a hair dryer also produced five purple spots. Each of the four compounds was eluted from the chromatograms with methanol, evaporated until dryness, recrystallized from absolute ethanol, washed with ether, and dried over P<sub>2</sub>O<sub>5</sub>.<sup>13</sup>

Mixtures obtained by irradiation of [2-<sup>14</sup>C]dUrd (~10<sup>-3</sup> M, 20 mCi/mM, New England Nuclear) were applied to plates for two-dimensional TLC (eluent A described above, and eluent B, EtOAc/2-PrOH/H<sub>2</sub>O, 75:16:9) followed by autoradiography. The radioactive spots were each scraped from the plates into scintillation vials containing 10 mL of Aquasol and counted.

**Characterization of Photoproducts.** Four photoproducts, 6*S*-( $-$ ) and 6(*R*)-( $+$ )-6-hydroxy-5,6-dihydrodeoxy-2'-uridine [2 and 3, ( $-$ ) and ( $+$ )-ho<sup>6</sup>hUrd], 6(*R*)- and 6(*S*)-*O*<sup>6</sup>,5'-cyclo-5,6-dihydrodeoxy-2'-uridine [4 and 5, 6(*R*)- and 6(*S*)-cdUrd], were characterized. Some of their pertinent features are presented in Table I.

**( $-$ ) and ( $+$ )-2'-Deoxyuridine Bromohydrin (6 and 7).** A solution of dUrd (500 mg, 2 mmol, Sigma) in 5 mL of H<sub>2</sub>O was placed in an ice bath and 300  $\mu$ L of bromine was added. After 10 min at ~0 °C, the excess Br<sub>2</sub> was removed by air current and the mixture was separated by LC using a mixture of H<sub>2</sub>O/CH<sub>3</sub>OH (9:1 v/v) as eluent, at a head pressure of 3200 psi and a flow rate of 0.8 mL/min. Two fractions with retention times of 4.2 (3.5 mg) and 6.3 min (6.5 mg) were collected from each 100- $\mu$ L sample. The former was a crystalline product shown to be ( $-$ )-Br<sup>5</sup>ho<sup>6</sup>hdUrd (6) and the latter ( $+$ )-Br<sup>5</sup>ho<sup>6</sup>hdUrd (7) (see below).

**( $-$ ) and ( $+$ )-6-Hydroxy-5,6-dihydro-2'-deoxyuridine (2 and 3).** Samples of 6 and 7 (200 mg) in 2 mL of H<sub>2</sub>O were each treated with 200 mg of zinc powder and 50  $\mu$ L of acetic acid. The reaction mixtures were stirred for 5 min at 0 °C and then centrifuged. The supernatant was filtered and evaporated to dryness and the residue was subjected to preparative TLC (cellulose plates, eluent A). Products were isolated as described for ( $-$ )-ho<sup>6</sup>hdUrd (6*S*) and ( $+$ )-ho<sup>6</sup>hdUrd (6*R*) in the above irradiation reaction procedure.

**Kinetic Studies of the Reverse Reaction.** Solutions (0.1 mM) of compounds 2-5 were prepared in either 1 N HCl or 0.01 N NaOH.

<sup>†</sup>J. C. was a Visiting Associate at Hopkins.

<sup>‡</sup>L. S. K. is a member of the Division of Biophysics, Department of Biochemistry of the School.

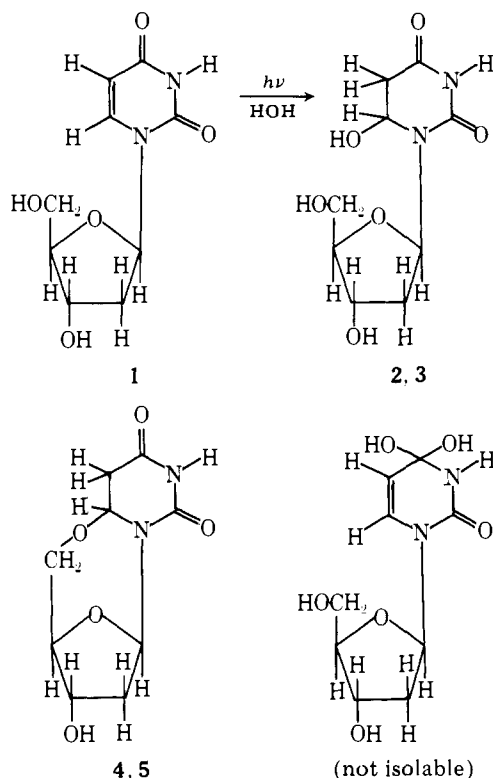
<sup>8</sup>Abbreviations used are the approved IUPAC-IUB symbols [*Biochemistry*, 49, 4022 (1970)] and the linkage symbols proposed by W. E. Cohn, N. J. Leonard, and S. Y. Wang, *Photochem. Photobiol.*, 19, 89(1974).

Table I. Pertinent Characteristics of Various Products

compd	$R_f$ value [ $R_{dUrd}$ ]	mp, °C	$[\alpha]_D^{23}$ in $CH_3OH$ (concn) <sup>3</sup>	infrared band, $cm^{-1}$ C=O	C-OH
dUrd (1)	0.28 [1.00]				
(-)-ho <sup>6</sup> hdUrd (2)	0.12 [0.43]	$a[56]^b$	+10.1 (0.06)	1685	1049, 1089
(+)-ho <sup>6</sup> hdUrd (3)	0.17 [0.59]	$a[56]^b$	+21.2 (0.06)	1686	1049, 1090
6( <i>R</i> )-c-dUrd (4)	0.34 [1.20]	126 [126] <sup>c</sup>		1677, 1703	1070
6( <i>S</i> )-c-dUrd (5)	0.44 [1.58]	128 [126] <sup>c</sup>	-55.0 (0.07)	1675, 1705	1072
(-)-Br <sup>5</sup> ho <sup>6</sup> hdUrd (6)		265	+40.7 (0.06)	1736, 1685	1035, 1040
(+)-Br <sup>5</sup> ho <sup>6</sup> hdUrd (7)		<i>a</i>	-61.1 (0.05)	1735, 1685	1044, 1060

<sup>a</sup>The hygroscopic nature of the compound did not allow the accurate determination of the melting point. <sup>b</sup>The melting point given in brackets was reported<sup>10</sup> for the mixture of 2 and 3. <sup>c</sup>The melting point given in brackets was reported<sup>10</sup> for the mixture of 4 and 5.

Scheme I



The solutions were allowed to stand at 22 °C and the appearance of absorbance in the range 210–360 nm was automatically recorded. The absorbance maxima at time intervals were used for kinetic analyses.

## Results and Discussion

**Isolation of the Photoproducts.** The difficulties encountered previously in the isolation and characterization of the products of this photoreaction (see Scheme I) were not so much due to poor separations but to sensitivity of these photoproducts to the conditions of chromatography. For instance, the early use of silica gel<sup>10</sup> for TLC may have caused decomposition or reverse reactions of the new compounds thus obscuring their identities for characterization. Optimal separation of the four sufficiently stable photoproducts was achieved by preparative TLC on cellulose plates using eluent A. [Addition of water at the C(4)-carbonyl may also occur, but such products would be too unstable to isolate.<sup>14</sup>] The products were located as either fluorescent spots by taking advantage of their reversibility on heating or by the color reaction specific for the deoxyribose moiety.<sup>15</sup>  $R_f$  values and yields are given in Table I. The two major products, 2 and 3, were formed in similar amounts but the two minor ones, 4 and 5, were of approximately 3:1 ratio.

The results were corroborated by autoradiography of the

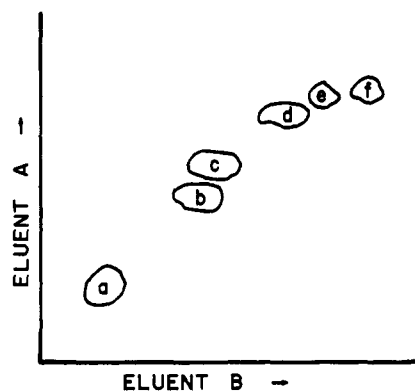
reaction mixture from irradiation of [2-<sup>14</sup>C]dUrd in aqueous solution. Figure 1 shows the five radioactive spots found in addition to dUrd. Four of them are monomolecular pyrimidine photoproducts, 2, 3, 4, and 5 as indicated. A scintillation counting study (Table II) showed that the formation of the spot nearest the origin decreased with increasing periods of irradiation, having reached a maximum prior to 11 min, while yields of the other products increased with increasing time. This photoreversibility of (a) photoproduct(s) initially formed with high efficiency is characteristic of pyrimidine dimers.<sup>16</sup> Indeed, four isomers of dUrd(↔)dUrd (8) have been identified and the details will be reported elsewhere.

**Conformational Analysis by NMR Studies. Pyrimidine Moiety.** From the chemical shift and coupling constant data (Table III), the characteristic ABX pattern<sup>7,17</sup> for H<sub>5a</sub>, H<sub>5e</sub>, and H<sub>6</sub> in the NMR spectra of 2, 3, 4, and 5 showed that the 5,6-double bond had been reduced. Similarly, the chemical shifts of H<sub>5</sub> and H<sub>6</sub> in 6 and 7 (synthetic intermediates for 2 and 3, see below) revealed a saturated C(5)–C(6) bond. Therefore, the pyrimidine moiety in these compounds exists as a puckered half-chair ring. The downfield shift of the H<sub>5</sub> proton for 6 and 7 as compared to the other four is expected owing to the presence of an electron-withdrawing C(5)–Br atom.

**2'-Deoxyribose Moiety.** The conformation of the 2'-deoxyribose moiety in 2, 3, 6, and 7 may be analyzed in terms of pseudorotation or a dynamic equilibrium between *N*[C(2')-exo, C(3')-endo] and *S*[C(2')-endo, C(3')-exo] conformers (Figure 2) which allows unequivocal determination of the conformation of each furanoid ring in these nucleosides.<sup>18,19,21</sup> This method cannot be applied to the cyclohydrates 4 and 5, where a third ring is present, and they will be examined separately.

Based on coupling constants, the degree of pucker ( $\tau_m$ ) and the phase angle of pseudorotation (*P*) of the lowest potential energy conformation of *N* and *S* conformers,  $N_{P_0}$  and  $S_{P_0}$ , were estimated by a simple graphic method<sup>20</sup> (Table IV). Percentage of *N*-type conformer for 1, 2, 3, 6, and 7 is 41, 27, 38, 25, and 39, respectively. Thus there appears to be a slight shift of the *N* ⇌ *S* equilibrium in favor of *S* conformers of the (+) diastereoisomers, 3 and 7, in comparison to dUrd (1), but shifts for the (–) diastereoisomers, 2 and 6, are greater. In addition, the degree of puckering ( $\tau_m$ ) of the sugar moiety of the (–) diastereoisomers is larger than that of the (+) isomers and dUrd.

The conformation of the exocyclic C(5')H<sub>B</sub>H<sub>C</sub>OH group normally interconverts in solution between various gauche (*g*) and trans (*t*) conformers.<sup>22</sup> Three predominant conformations with minimal potential energy are related to the rotation about the C(4')–C(5') bond and their percentages (*P<sub>gg</sub>*) can be estimated (Table IV). Apparently (*gt* + *tg*) conformers exist to a greater extent than the *gg* conformer, particularly in the case of compound 2. These results together with the absence of a pronounced deshielding effect on H(2'), which was observed previously for syn compounds or compounds mimicking the

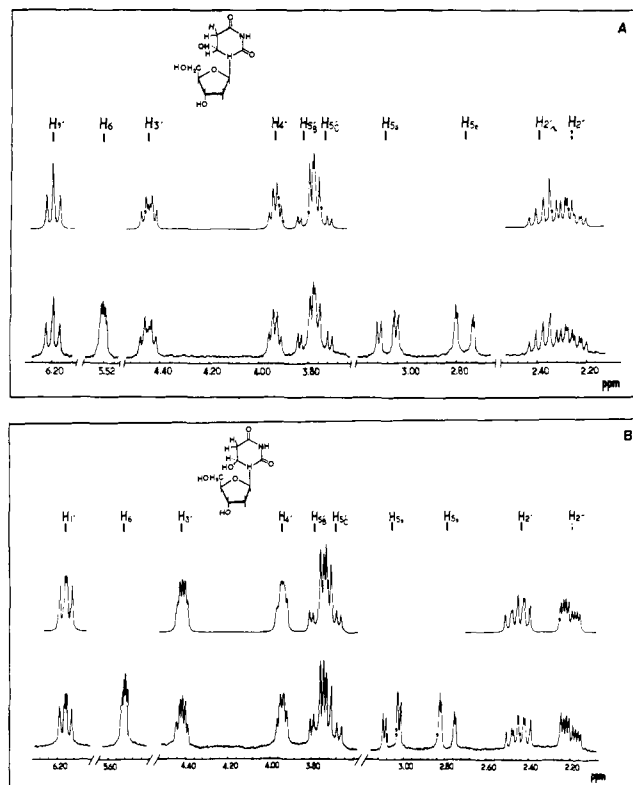


**Figure 1.** Autoradiograph of the photoproducts from the irradiation of [2-<sup>14</sup>C]dUrd in an aqueous solution developed with eluents A [the lower layer of a solution of CHCl<sub>3</sub>/CH<sub>3</sub>OH/H<sub>2</sub>O (4:2:1 by volume) to which 5 mL of absolute CH<sub>3</sub>OH has been added for each 100 ml] and B [EtOAc/2-PrOH/H<sub>2</sub>O (75:16:9)]. Spot a corresponds to dUrd (>)dUrd, b (-)-ho<sup>6</sup>hdUrd, c, (+)-ho<sup>6</sup>hdUrd; d, dUrd; e, 6(*R*)-c-dUrd; and f 6(*S*)-c-dUrd.

**Table II.** Quantitative Analyses of Photoproducts at Various Times of Irradiation<sup>a</sup>

compd	radioactivity, %		
	11 min	20 min	30 min
dUrd (>)dUrd (8)	25.7	22.0	13.6
(-)-ho <sup>6</sup> hdUrd (2)	9.9	20.9	29.3
(+)-ho <sup>6</sup> hdUrd (3)	14.3	31.0	39.9
dUrd (1)	46.5	18.3	8.3
6( <i>R</i> )-c-dUrd (4)	1.0	1.4	1.9
6( <i>S</i> )-c-dUrd (5)	2.5	6.4	7.0

<sup>a</sup>The dose rate is  $2.62 \times 10^3$  J/m<sup>2</sup>·min.



**Figure 2.** (A) Spectra for (+)-ho<sup>6</sup>hdUrd and (B) for (-)-ho<sup>6</sup>hdUrd measured in D<sub>2</sub>O (20 mM, pD 7.0, ~20 °C). The upper are the computer-simulated spectra and lower ones are <sup>1</sup>H 250-MHz Fourier transform NMR spectra, respectively.

**Table III.** Chemical Shifts ( $\delta$ ) and Coupling Constants ( $J$ ) of Reaction Products<sup>a</sup>

compd	2	3	4 <sup>b</sup>	5 <sup>b</sup>	6	7
H <sub>5a</sub>	3.053	3.073	3.324	3.274	4.542	4.555
H <sub>5e</sub>	2.796	2.768	3.087	3.113	(Br) <sup>c</sup>	(Br) <sup>c</sup>
H <sub>6</sub>	5.516	5.520	5.277	5.519	5.550	5.512
H <sub>1'</sub>	6.162	6.196	6.780	6.590	6.250	6.256
H <sub>2'</sub>	2.451	2.364	2.847	2.793	2.411	2.393
H <sub>2''</sub>	2.207	2.254	2.522	2.590	2.240	2.284
H <sub>3'</sub>	4.419	4.442	4.971 <sup>a</sup>	4.744	4.440	4.425
H <sub>4'</sub>	3.944	3.931	4.644	4.630	3.968	3.958
H <sub>5'B</sub>	3.771	3.799	4.227	4.053	3.761	3.803
H <sub>5'C</sub>	3.706	3/746	4.227	4.335	3.761	3.740
Coupling Constants						
5a,5e	-17.3	-17.3	-17.7	-17.1	<i>c</i>	<i>c</i>
5e,6e	2.0	1.9	2.8	8.9(a,a)	2.4	2.5
5a,6e	4.0	3.7	4.9	5.5	<i>c</i>	<i>c</i>
1',2'	8.5	6.7	1.1	2.3	8.5	7.2
1',2''	6.2	6.9	8.0	7.7	6.1	6.5
1',3'	<i>c</i>	<i>c</i>	<i>c</i>	-0.4	<i>c</i>	<i>c</i>
2',2''	-14.1	-14.3	-15.6	-15.0	-14.1	-14.2
2',3'	6.6	6.7	7.6	7.0	6.3	6.7
2'',3'	3.2	4.5	3.2	2.1	2.9	4.1
3',4'	2.9	4.1	0.6	0.6	2.7	4.0
4',5'B	5.3	4.6	3.7	2.4	4.4	4.9
4',5'C	4.2	3.6	3.7	0.7	4.4	3.7
5'B,5'C	-12.3	-12.3	<i>c</i>	-13.0	<i>c</i>	-12.3

<sup>a</sup>The chemical shifts (ppm) and the coupling constants (Hz) are measured at 250 MHz and are estimated to be accurate to within 0.003 ppm and 0.2 Hz. <sup>b</sup>These values are measured at 100 MHz and from internal reference *tert*-butyl alcohol (*t*-BuOD) in D<sub>2</sub>O at 20 °C, pD 6.8. <sup>c</sup>Not observed or not applicable.

syn conformation, are indicative of an anti conformation for these dihydro derivatives.<sup>23</sup>

**Structural Analysis of Cyclohydrates (c-dUrd).** Both experimental and simulated NMR spectra of 4 and 5 have been determined (Figure 3) and a complete set of chemical shift and

coupling constant data is presented in Table III. The magnitude of trans coupling constants  $J_{1',2'}$ ,  $J_{2'',3'}$ , and  $J_{3',4'}$  is small. The dihedral angles calculated according to the modified Karplus equation<sup>18</sup>

$${}^3J_{\text{HH}} = 10.5 \cos^2 \phi_{\text{HH}} - 1.2 \cos \phi_{\text{HH}}$$

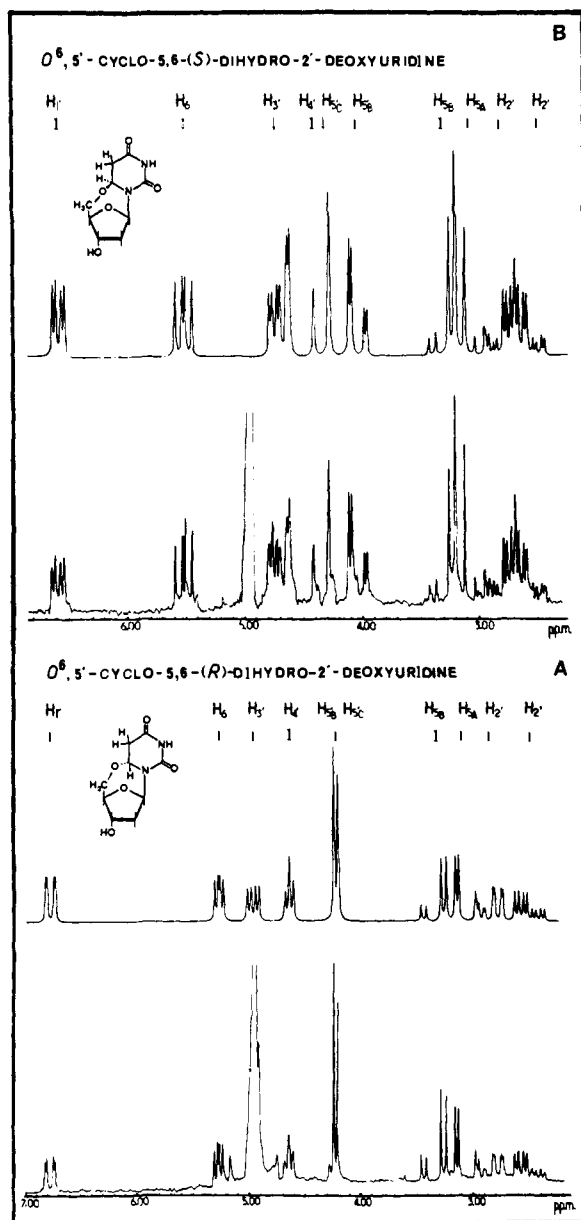


Figure 3. (A) Spectra for 6(*R*)-c-dUrd and (B) for 6(*S*)-c-dUrd measured in D<sub>2</sub>O (20 mM, pD 7.0, ~18 °C). The upper are the computer-simulated spectra and lower ones are <sup>1</sup>H 100-MHz fast Fourier transform NMR spectra, respectively.

where  $\phi_{HH}$  is the torsion or dihedral angle are between 100 and 110° indicating a rigid ring, part of which is also involved in a seven-membered ring. As expected from examination of molecular models, the magnitude of the trans coupling constants  $J_{1',2'}$  and  $J_{2',3'}$  is influenced more by the conformation within the puckered pyrimidine ring than by the C(6) absolute configuration. The apparent decrease in the value of  $J_{1',2'}$  with the concomitant increase in  $J_{2',3'}$  for **4** indicates a rotation about the C(2')-C(3') bond in the direction of the C(2')-exo conformer when the C(6)-O(5') anhydro bond is in the pseudoaxial position. An opposite trend is observed for **5** which shows an unusual C(6)-O(5') equatorial conformation for a pyrimidine "hydrate" type of compound. Further confirmation of the assignment of **5** is given by the observed magnitude of 2.4 and 0.7 Hz for the corresponding  $J_{4',5'B}$  and  $J_{4',5'C}$  coupling constants. Identical values were predicted for the pure *gg* conformer of the exocyclic hydroxymethyl group about the C(4')-C(5') bond.<sup>24</sup>

Large differences were observed in the respective magnitudes of the chemical shifts of H(6) and H(1') for **4** and **5**

Table IV. Determination of Sugar Moiety Conformation of Reaction Products by a Simple Graphic Method

compd	1 <sup>a</sup>	2	3	6	7
$J_{1',2'}$ <sup>b</sup>	5.6	7.4	5.8	7.4	6.2
$J_{3',4'}$	4.0	2.9	4.1	2.7	4.0
$J_{2',3'}$ <sup>b</sup>	5.9	5.7	5.8	5.5	5.8
sum $J_{1',2'} + J_{3',4'}$	9.6	10.3	9.9	10.1	10.2
$\tau_m$ <sup>oc</sup>	38.5	42	40	42	41
$N_{P_0}$ <sup>c</sup>	17	24	20	21	23
$S_{P_0}$ <sup>c</sup>	163	156	160	159	157
$J_{4',5'B}$	5.1	5.2	4.6	4.4	4.9
$J_{4',5'C}$	3.4	4.1	3.6	4.4	3.7
sum	8.5	9.3	8.2	8.8	8.6
$P_{gg}$ , % <sup>d</sup>	45	37	48	42	44
$P_N$	41	27	38	25	39

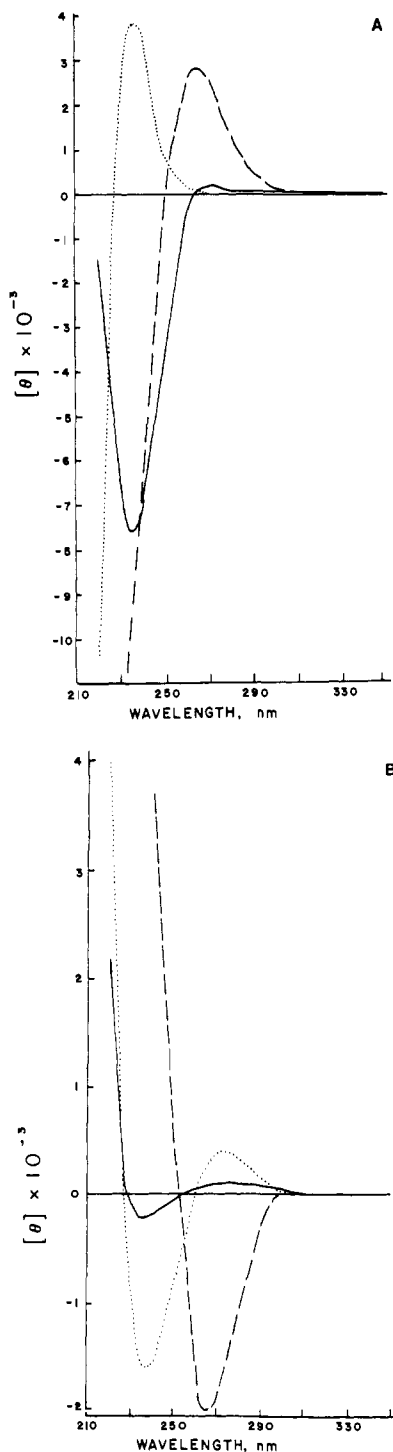
<sup>a</sup>These values are taken from Schleich et al. (1972) of a 220-MHz spectrum. <sup>b</sup>Coupling constants are corrected for electronegativity effects according to  $J_{cor} = J_{expt} (1 - 0.07\Delta\epsilon)$  where  $\Delta\epsilon = 1.30$ . <sup>c</sup>These values are extrapolated from Guschlbauer and Son<sup>20</sup> with Karplus parameters:  $A = 10.2$ ;  $B = -0.8$ ;  $C = 0$ . <sup>d</sup>The dynamic conformer distribution about the C4'-C5' bond of the sugar moiety is calculated according to  $P_{gg} = (13 - \Sigma)/10$ , where  $\Sigma$  is the sum of the coupling constants of  $J_{4',5'B} + J_{4',5'C}$ .<sup>22</sup>

(Table III). The appreciable downfield shift (0.3 ppm) of  $\delta H(6)$  for **5** may be accounted for by an electric field and/or a magnetic anisotropic effect due to the close proximity of the H(6) atom to the furanose ring oxygen.<sup>25</sup> Models indicate that this condition is fulfilled for (6*S*)-cdUrd (**5**) irrespective of the C(6) conformation. The 6*S* assignment to **5** is reinforced by H(6) deshielding and H(1') upfield shift for the 5-iodo-*O*<sup>6</sup>,5'-cyclo-5,6(*S*)-dihydrothymine with respect to the *R* diastereoisomer.<sup>26</sup>

As seen in the circular dichroic (CD) spectra (Figure 4), the sign of the Cotton effects at 230 nm for photohydrates and cyclophotohydrates, presumably due to  $n \rightarrow \pi^*$  transition,<sup>27</sup> depends mainly on the configuration and the conformation of the asymmetric C(6). The CD curves of both 6*R*-**2** and **4** which have a C(6)-O bond in a pseudoaxial conformation display a positive Cotton effect. As expected, the CD spectra of 6*S*-**3** show a negative Cotton effect. The positive Cotton effect which is observed for the 6*S*-cyclophotohydrate **5** can be related to the change in the C(6)-O conformation.

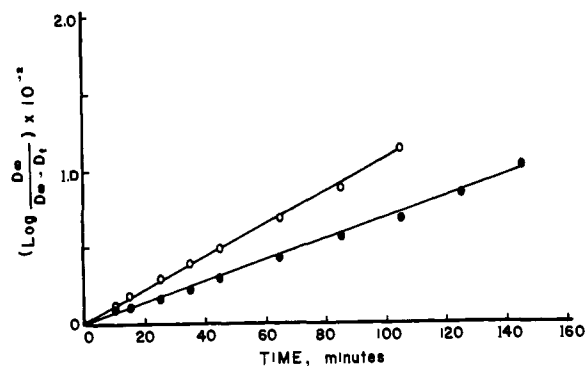
**Synthesis of Photohydrates.** Structures of photohydrates **2** and **3**, assigned on the basis of similarity to the known photohydrates of pyrimidines, in particular the UV and NMR spectral data, have been confirmed by synthesis via the corresponding bromohydrins **6** and **7** (Scheme II). The mechanism of formation of bromohydrins of pyrimidines has been well established.<sup>28</sup> Reduction of halohydrins with zinc/acetic acid has recently been used for the preparation of various photohydrates of pyrimidines<sup>29</sup> and since it here involves reduction at C(5), the configuration at C(6) remains unchanged. The synthetic products were identical with the corresponding photoproducts, **2** and **3**, and permit the assignment of absolute configuration at C(6) for both compounds.

**Reversal or Dehydration Reactions of ho<sup>6</sup>hdUrd (**2**, **3**) and c-dUrd (**4**, **5**).** As mentioned previously all four photoproducts could be converted to parent compound by heating or by treatment with acid or base at ambient temperature. Acid-catalyzed dehydration was found to follow first-order kinetics with respect to the concentrations of each photoproduct. As shown in Figure 5 a plot of  $\log (C_0/C_t)$ , i.e.,  $\log [D_\infty/(D_\infty - D_t)]$ , vs.  $t$  in each case gave a straight line after an initial faster rate. The slopes are  $1.22 \times 10^{-2}$ ,  $1.32 \times 10^{-2}$ ,  $8.15 \times 10^{-3}$ , and  $6.76 \times 10^{-3} \text{ min}^{-1}$ , respectively, for compounds **2**, **3**, **4**, and **5**. Earlier the  $k$  value for the corresponding riboside, ho<sup>6</sup>hUrd, was found to be  $1.15 \times 10^{-2} \text{ min}^{-1}$  in 1 N HCl,<sup>30</sup> in good agreement with the present findings. The slower rates



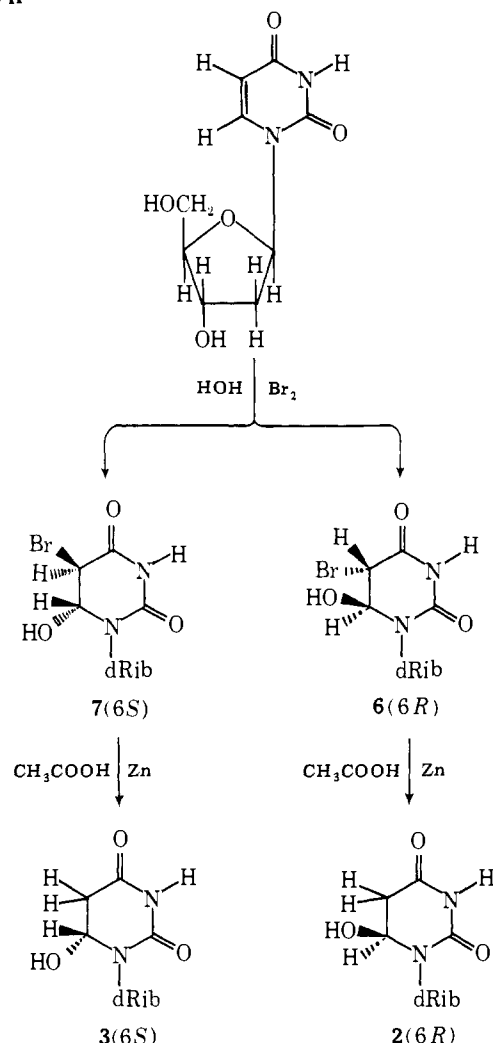
**Figure 4.** (A) CD spectra of 6*S* isomers: (–)-ho<sup>6</sup>hdUrd, c-dUrd, and (–)-Br<sup>5</sup>ho<sup>6</sup>hUrd and (B) of 6*R* isomers: (+)-ho<sup>6</sup>hdUrd, c-dUrd, and (+)-Br<sup>5</sup>ho<sup>6</sup>hUrd.

observed for **4** and **5** may be accounted for in terms of weaker basicity of the ether oxygen in comparison to the hydroxy group of **2** and **3**. The rates of base-catalyzed dehydration on the other hand were ~30-fold greater for the cyclic photoadducts than the normal photohydrates. The greater reactivity of the cyclic photoadducts to basic β-elimination may be attributed to relief of ring strain. A major segment of this reaction was found to follow either first-order or second-order kinetics. It is known that alkaline reversal of pyrimidine hydrates can follow either kinetic order depending on the concentration of the base (solution pH) and the nature of the hydrates.<sup>30</sup> Therefore a more thorough study is desirable for more explicit



**Figure 5.** Acid-catalyzed (1 N HCl) dehydration of photohydrates (upper curve) and photocycloadducts (lower curve) analyzed at 22 °C by the appearance of 262-nm absorbancy maximum at time intervals.

#### Scheme II



kinetic understanding. However, the results clearly show that the UV spectrum of dUrd is regenerated when each of the four photoproducts is treated with acid or base with rates comparable to those observed for similar pyrimidine photohydrates.

In conclusion, we have presented evidence for the formation of two isomeric cyclonucleosides along with two hydrates as photoproducts of dUrd in aqueous solution. Apparently this newly recognized minor process competes with addition of water which takes place in a stereochemical random fashion<sup>7</sup> as in the case of Urd<sup>8</sup> producing **2** and **3** in equal proportions. The unequal yields of **4** and **5** reflect the influence of steric

factors on the intramolecular cycloaddition.<sup>26</sup> Failure to detect the corresponding cyclonucleosides in the NMR study of Urd<sup>8</sup> is understandable in that a crude mixture was used to obtain a poorly resolved 100-MHz spectrum. However, while this manuscript was being revised, observation of cyclonucleosides on irradiation of Urd in methanolic solution was reported.<sup>31</sup> Both the photocycloaddition and photohydration processes require an intermediate with a relatively positive center at C(6). The possible significance of such a zwitterionic intermediate in a biochemical environment was pointed out as early as 1958,<sup>2b</sup> but has received little attention. The present finding serves as an important example that nucleophiles other than water may be added to this intermediate in a biological milieu. Such an occurrence could result in cross-linkages or adducts between nucleic acids and other biomolecules, and be of greater biological consequence than the well-known photohydration reaction.

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## The Fischer Indole Synthesis and Pinacol Rearrangement in the Mass Spectrometer

G. L. Glish and R. G. Cooks\*

Contribution from the Department of Chemistry, Purdue University, West Lafayette, Indiana 47907. Received April 13, 1978

**Abstract:** The gas-phase acid-catalyzed elimination of NH<sub>3</sub> from protonated phenylhydrazones is shown to be analogous in detail to a solution reaction, the Fischer indole synthesis. This is demonstrated using new mass spectrometric procedures based on mass-analyzed ion kinetic energy (MIKE) measurements in conjunction with isotope labeling. Specifically, (a) the product of the rearrangement sequence is shown to be identical with the protonated indole while (b) selection of compounds labeled to various extents from a single mixture—a uniquely facile procedure in MIKES—gives the expected label incorporation patterns in the ammonia elimination products. Formation of 3H-indole from suitable substituted ketones represents a variant on the Fischer synthesis which is also observed in the gas phase. The present methodology for characterizing gas-phase analogues of solution reactions should be generally applicable. This is further demonstrated by applying it to the pinacol rearrangement, where correspondence between the isolated and condensed phase chemistry is again observed.

Studies on ionic chemistry in the gas phase have borrowed heavily from both the concepts and the mechanistic probes of condensed-phase physical organic chemistry. This is evident in studies on gas-phase substituent effects,<sup>1</sup> steric effects,<sup>2</sup> ortho effects,<sup>3</sup> anchimeric assistance,<sup>4</sup> and tautomerism,<sup>5</sup> and in the gas-phase analogues of nucleophilic substitution,<sup>6</sup>

electrophilic aromatic substitution,<sup>7</sup> transesterification,<sup>8</sup> and the Beckmann rearrangement.<sup>9</sup> Mechanistic probes used in the gas phase have included kinetic isotope effects<sup>10</sup> and orbital symmetry studies<sup>11</sup> among other traditional methods. It is noteworthy, however, that with rare exceptions, product analysis in the gas phase has been limited to mass measurement