Carbon-13 Nuclear Magnetic Resonance of 5-Substituted Uracils[1]

Paul D. Ellis,* R. Bruce Dunlap,* Andrew L. Pollard, Kurt Seidman, and Alan D. Cardin[2]

Contribution from the Department of Chemistry, University of South Carolina, Columbia, South Carolina 29208. *Received June 16, 1972*

Abstract: ^{13}C chemical shifts and J_{C_6H} coupling constants are reported for 17 5-substituted uracils. Overall, the chemical shifts at C-5 and C-6 of the 5-substituted uracils exhibit no obvious correlation with substituent electronegativity. Instead, when the 5-substituted uracils are considered as trisubstituted ethylenes, the chemical shift data are shown to be rationalized in terms of the ability of the C-5 substituent to behave as a mesomeric acceptor or donor. It is also demonstrated that the correlation of the chemical shifts at C-6 can be used to identify two categories of 5-substituted uracils whose parent deoxynucleotide derivatives are inhibitors of the enzyme, thymidylate synthetase. It is suggested that ^{13}C nmr spectroscopy is a potentially useful tool for predicting the effectiveness of certain modified substrates as enzymatic inhibitors.

Uracil and certain of its 5-substituted analogs are involved in a number of biochemically significant roles (*i.e.*, constituents of RNA and DNA) in all living systems. The 5-substituted uracils and their nucleoside and nucleotide counterparts function either as substrates, products, or inhibitors of certain enzymes which catalyze the synthesis, degradation, or interconversion of pyrimidine compounds.[3] Certain naturally occurring uracil derivatives are known to participate in intracellular mechanisms regulating pyrimidine metabolism, while a number of synthetic 5-substituted uracils and their derivatives are of value as chemotherapeutic agents.[3,4]

The investigation of the 5-substituted uracils reported herein is motivated by our basic interest in the mechanistic aspects of enzymes such as thymidylate synthetase, thymidine kinase, and dihydrouracil dehydrogenase, which interact with the uracils or their nucleoside or nucleotide derivatives. For example, three deoxynucleotide derivatives of the 5-substituted uracils, namely deoxyuridine 5′-monophosphate (dUMP), thymidine 5′-monophosphate, and 5-fluorodeoxyuridine 5′-monophosphate, interact with thymidylate synthetase in the role of substrate, product, and inhibitor, respectively.[5] Often important insight relative to the mechanism of action of an enzyme can be gathered by examining the factors which are responsible for the mode and degree to which substrates or their analogs bind to the catalyst. In many cases, the nature and position of substituents on a substrate molecule are correlated with both the extent to which such compounds interact with enzymes and their ability to function as substrates or inhibitors in an enzymatic reaction.[6] Acknowledgment of the factors operative in substrate binding is also of great value in the design of affinity labeling reagents and inhibitors of possible chemotherapeutic utility.

In the present study, we have utilized ^{13}C nmr spectroscopy to characterize 17 5-substituted uracils with respect to their chemical shifts and J_{C_6H} coupling constants. Tarpley and Goldstein[7] have used a similar technique to study uracil and the halouracils (see Discussion), and other groups have applied this method to determine and assign the ^{13}C nmr spectra of the naturally occurring nucleosides and nucleotides.[8] The variations in chemical shifts at C-5 and C-6 as a function of the substituent show a relatively poor correlation with respect to substituent electronegativity, and instead are rationalized in terms of the ability of the substituent to behave as a mesomeric donor or acceptor. Finally, we speculate on the utility of ^{13}C nmr studies of 5-substituted uracils in predicting the effectiveness of the deoxynucleotide analogs as inhibitors of the thymidylate synthetase reaction.

Experimental Section

Materials. Uracil and its 5-nitro, thio, amino, fluoro, methyl, hydroxymethyl, and hydroxy derivatives were purchased from Sigma Chemical Co. (St. Louis, Mo. 63178). Uracil-5-carboxylic acid was purchased from Aldrich Chemical Co. (Cedarknolls, N. J. 07927), and 5-trifluoromethyluracil was a gift from Dr. Daniel V. Santi, Department of Chemistry, University of California, Santa Barbara, Calif. 93106. These compounds did not need further purification and were used as obtained.

5-Formyluracil was prepared by the oxidation of 5-hydroxymethyluracil by potassium persulfate in the presence of a catalytic amount of silver nitrate as described by Brossmer and Ziegler.[9] The methyl ester of uracil-5-carboxylic acid was synthesized by refluxing the corresponding acid chloride in absolute methanol.[10] 5-Cyanouracil was prepared by the sequence of reactions described by Prystas and Sorm,[11] and 5-methoxyuracil was prepared by the method outlined by Chesterfield.[12]

Proton nmr and thin layer chromatography were used to characterize the purity of the compounds employed in this research. Two thin layer systems were used here: (A) a tertiary mixture of *tert*-

(1) Presented in part at the 162nd National Meeting of the American Chemical Society, Washington, D. C., Sept 1971.

(2) National Science Foundation Undergraduate Research Participant, 1971.

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(12) J. H. Chesterfield, J. F. W. McOmie, and M. S. Tute, *J. Chem. Soc.*, 4590 (1960).

Table I. Carbon-13 Chemical Shifts and J_{C_6H} for the 5-Substituted Uracils[a]

Substituent	δ_{C_2}[b]	δ_{C_4}[b]	δ_{C_5}[b]	δ_{C_6}[b]	δ_{C_2}[c]	δ_{C_4}[c]	δ_{C_5}[c]	δ_{C_6}[c]	J_{C_6H}
F[d]	−109.48	−117.81	−99.25	−85.64	1.47	5.95	−39.47	15.99	182.0
OCH_3[e]	−110.19	−120.31	−95.40	−82.11	0.76	3.45	−35.62	19.52	180
OH	−109.20	−120.59	−91.04	−79.92	1.75	3.17	−31.26	21.71	178
NO_2	−109.88	−115.71	−85.56	−107.78	1.07	8.05	−25.78	−6.15	186
NH_2	−109.00	−20.90	−75.88	−81.12	1.95	2.86	−21.34	25.75	178
CH_2OH[f]	−110.47	−122.89	−72.00	−97.30	0.48	0.84	−12.22	4.33	178
CHO[g]	−109.76	−121.78	−69.62	−108.49	1.19	1.98	−9.84	−6.86	178
CH_3[d]	−110.67	−124.08	−66.96	−96.91	0.28	−0.32	−7.18	4.72	177.8
S)−2	−110.35	−121.38	−65.93	−107.10	0.60	2.38	−6.15	−5.47	183
Cl	−109.57	−119.25	−65.52	−99.10	1.38	4.51	−5.74	2.53	184.0
CO_2CH_3[h]	−109.88	−119.40	−62.48	−108.88	1.07	4.36	−2.70	−7.25	180
CF_3[i]	−109.88	−119.20	−61.36	−102.94	1.07	4.56	−1.58	−1.31	182.5
CO_2H[j]	−109.60	−122.49	−69.61	−109.40	1.35	1.27	−0.93	−7.77	185
H[d]	−110.95	−123.76	−59.78	−101.63	0.00	0.00	0.00	0.00	180.5
Br[d]	−109.77	−119.39	−53.98	−101.56	1.18	4.37	5.80	0.07	184.7
CN[k]	−109.36	−120.39	−46.49	−111.26	1.59	3.37	13.09	−9.63	185
I[d]	−110.25	−120.84	−27.14	−106.42	0.70	2.92	32.64	−4.79	184.5

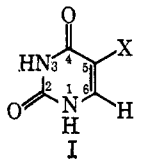
[a] All chemical shifts and coupling constants are reported in parts per million and hertz, respectively. [b] Chemical shifts of the uracil carbons with respect to internal DMSO. The ^{13}C chemical shift of DMSO is −40.5 ppm with respect to TMS. [c] Chemical shifts of the *i*th carbon in the 5-substituted uracil with respect to the *i*th carbon in uracil. [d] The chemical shifts of the H, CH_3, F, Cl, Br, and I compounds have been previously reported by Tarpley and Goldstein.[7] Their values for the chemical shifts agree with ours for the H, CH_3, and F compounds within experimental error. The values reported for Cl, Br, and I are theirs after converting to a DMSO chemical shift scale. Likewise, the values of J_{C_6H} for these compounds are those of Tarpley and Goldstein. [e] The chemical shift of the methyl carbon is −17.71 ppm with respect to DMSO, and J_{CH} for the methyl is 147 Hz. [f] The chemical shift of the CH_2 carbon is −15.19 ppm with respect to DMSO, and J_{CH} for the methylene is 145 Hz. [g] The chemical shift of the COH carbon is −145.66 ppm with respect to DMSO, and J_{CH} is 180 Hz. [h] The chemical shifts of the carbonyl and methyl carbons are −122.57 and −10.99 ppm, respectively, with respect to DMSO. J_{CH} for the methyl carbon is 147.5 Hz. [i] The chemical shift of the CF_3 carbon is −82.31 ppm with respect to DMSO, and J_{CF} is 270 Hz. [j] The chemical shift of the carbonyl carbon is −124.47 ppm with respect to DMSO. [k] The chemical shift of the nitrile carbon is −73.98 ppm with respect to DMSO.

butyl alcohol, methyl ethyl ketone, ammonium hydroxide, and water in the ratios 40:30:10:20, respectively, and (B) a mixture of benzene, pyridine, and acetic acid in the ratios of 16:4:1, respectively. All the compounds used gave a single spot on Brinkmann Instruments (Westbury, N. Y. 11590) 0.1-mm cellulose MN 300 UV_{254} prepared thin layer plates. The compounds synthesized herein gave the following R_f values: 5-formyluracil (A) 0.78, (B) 0.36; 5-cyanouracil (A) 0.66, (B) 0.24; 5-methoxyuracil (A) 0.28, (B) 0.48; methyl ester of uracil-5-carboxylic acid (A) 0.32, (B) 0.21.

Nmr Measurements. The ^{13}C nmr spectra were determined on a Varian XL-100-15 nmr spectrometer operating in the Fourier transform mode. All the samples were dissolved in DMSO-d_6 with approximately 2% DMSO added to each sample. The concentration of the substituted uracils was 0.1 *M* for chemical shift determinations and 0.2 *M* or higher for evaluations of J_{C_6H}. The DMSO-d_6 furnished the internal lock while the DMSO was used for a chemical shift reference. The precision in the reported chemical shifts and coupling constants is 0.05 ppm and 1 Hz, respectively. The DMSO-d_6 (99.5%) was obtained from Diaprep and it was used without further purification.

Results

The ^{13}C chemical shifts and J_{C_6H} coupling constants of 17 5-substituted uracils (see structure I for numbering system) are presented in Table I. The order of presentation in Table I is that of increased shielding of carbon 5. The chemical shifts are reported in two ways: (1) in ppm with respect to internal DMSO, and (2) the chemical shift of carbon *i* in the 5-substituted compound is reported with respect to the analogous carbon in uracil. The latter method of reporting chemical shifts will prove very useful in subsequent discussions concerning substituents effects.



I

Discussion

Overall Trends. The ^{13}C chemical shifts presented in Table I have the expected order of sensitivity to the presence of a C-5 substituent, namely, C-2 < C-4 < C-6 < C-5. The observed ranges of ^{13}C chemical shifts for the systems studied are 2, 6, 72, and 34 ppm, for C-2, C-4, C-5, and C-6, respectively. Examination of the coupled and completely ^1H noise decoupled ^{13}C spectra of the various uracils in conjunction with previous work leads to a completely unambiguous assignment of the various ^{13}C resonances.[8]

Tarpley and Goldstein[7] were able to successfully correlate the ^{13}C chemical shifts in a *limited* number of 5-substituted uracils with substituent electronegativity. It is of interest to see if this overall correlation holds for the 12 additional substituents reported here. A plot of substituent electronegativity, E_x, *vs.* the ^{13}C chemical shifts of carbons 5 and 6 is presented in Figure 1. The E_x values are those of Dailey and Schoolery.[13] It is clear from Figure 1 that the linear relationship between ^{13}C chemical shifts and E_x that Tarpley and Goldstein obtained no longer holds for a more complete list of substituents. In fact, *there is no obvious relationship between the ^{13}C chemical shifts of C-5 and C-6 in the 5-substituted uracils and the substituent electronegativity* parameters of Dailey and Schoolery. In view of these results, we must look elsewhere in an attempt to rationalize the observed patterns of chemical shifts.

It is convenient to consider the 5-substituted uracils as either a set of trisubstituted ethylenes or monosubstituted benzenes. This is not an unreasonable premise for discussion since the trends in the ^{13}C chemical shifts reported here are not unlike those that have

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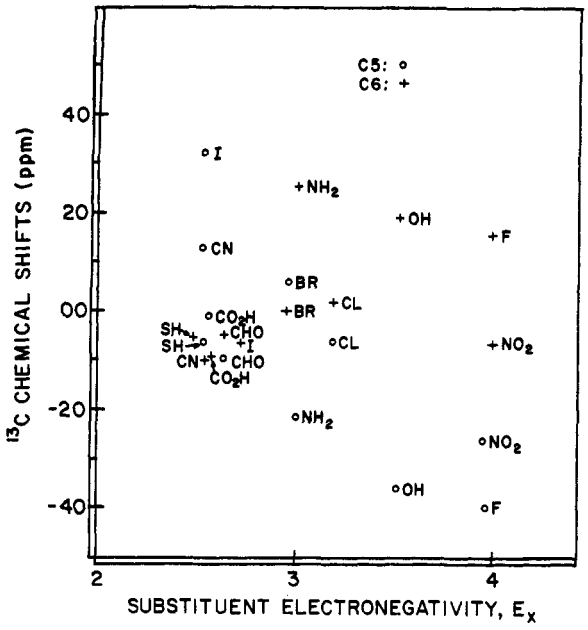

Figure 1. A plot of substituent electronegativity, E_x, *vs.* the ^{13}C chemical shifts of C-5 (○) and C-6 (+) in 5-substituted uracils. The chemical shifts are with respect to the analogous carbon in uracil.

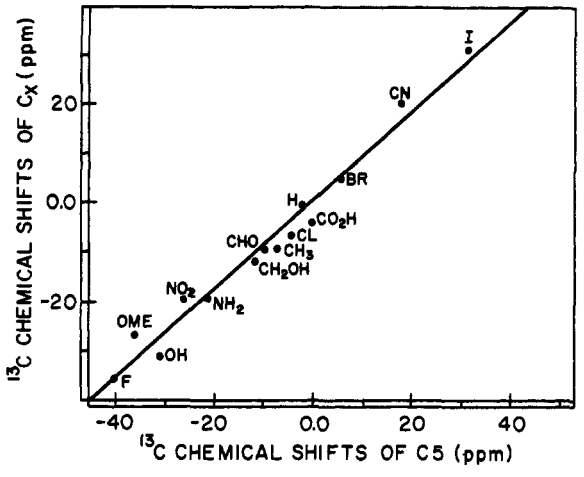

Figure 2. A plot of the ^{13}C chemical shifts of C-5 in 5-substituted uracils *vs.* the ^{13}C chemical shifts of the substituted carbon in an analogous series of monosubstituted benzenes.

been reported earlier for substituted benzenes[14,15] and vinyl compounds.[16-18] The overall trends in the ^{13}C chemical shifts of monosubstituted benzenes and simple vinyl compounds can be rationalized in terms of *mesomeric* arguments that reflect the competition between the carbon bearing the substituent, C_x, to form a double bond with the atom (or group) X, or C_β and the possible ionic character in these bonds. This argument is represented schematically in II and III.

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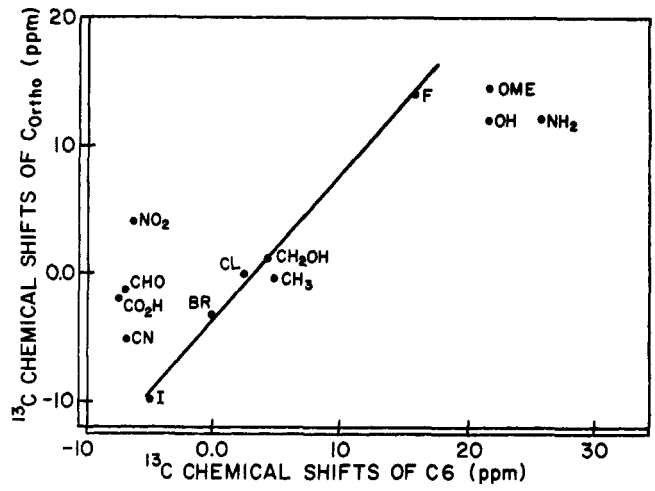
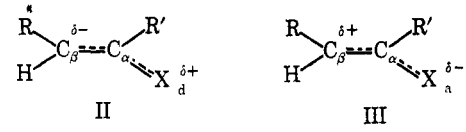


Figure 3. A plot of the ^{13}C chemical shifts of C-6 in 5-substituted uracils *vs.* the ^{13}C chemical shifts of the ortho carbon in an analogous series of monosubstituted benzenes.

Here, X_a and X_d represent mesomeric acceptor and donor groups, respectively.



II III

It is important to ascertain if there is a close linear relationship between the ^{13}C chemical shifts of C-5 and C-6 in the uracils with those for C_x and C_o in the monosubstituted benzenes. If such a relationship exists, one could, therefore, imply that whatever mechanisms are operational in determining the ^{13}C chemical shifts in monosubstituted benzenes are also present to about the same extent in the 5-substituted uracils. Furthermore, if there is a good correlation between the above mentioned chemical shifts, then we can apply the mesomeric arguments depicted in structures II and III to rationalize the observed trends in the ^{13}C chemical shifts in the 5-substituted uracils. Plots of the ^{13}C chemical shift of C-5 *vs.* C_x and C-6 *vs.* C_o are presented in Figures 2 and 3, respectively. Note the excellent linear relationship between the ^{13}C chemical shifts of C_5 and C_x. However, the analogous plot for C-6 and C_o has considerably more scatter to it. This increased scatter undoubtedly represents a limited breakdown in the assumption that structures II and III can completely represent the electronic changes in the substituted uracils or substituted benzenes.

The data presented in Figure 3 indicate that carbon-6 in a substituted uracil is significantly different than the ortho carbon in a substituted benzene with regard to the ability of the substituent to alter the electron distribution and hence the ^{13}C chemical shift of this carbon. The methods of Savitsky, Ellis, Namikawa, and Maciel[17] can be employed to describe the factors which contribute to the ^{13}C chemical shifts of C-5 and C-6 in the substituted uracils. However, before we examine these chemical shifts in detail, let us consider a brief summary of the model used by Savitsky, *et al.*, to describe the ^{13}C chemical shifts in the vinyl systems.

Savitsky and coworkers employed the Karplus and Pople[19] formulation of ^{13}C chemical shifts in an attempt to rationalize the patterns of ^{13}C chemical shifts in unsymmetrically substituted ethylenes in terms of the mesomeric arguments depicted by structures II and III. Briefly, the Karplus–Pople treatment of chemical shifts can be described by eq 1. In this expression,

$$\sigma^{A} = \sigma_d^{AA} + \sigma_p^{AA} + \sum_{B \neq A} \sigma^{AB} + \sigma^{A,ring} \quad (1)$$

σ_d^{AA} is the local diamagnetic term, σ_p^{AA} the local paramagnetic term, σ^{AB} is the contribution from currents on other atoms to the chemical shift of atom A, and $\sigma^{A,ring}$ is the contribution to the shielding of A from "ring currents." By local, we mean atom A's contribution to its own shielding. It is well known that the local paramagnetic term, σ_p^{AA}, dominates the ^{13}C chemical shifts.[19-21] The local paramagnetic term, σ_p^{AA}, in the Karplus–Pople treatment is given by eq 2

$$\sigma_p^{AA} = -[e^2\hbar^2/2m^2c^2\Delta E]\langle r^{-3}\rangle_{2p} \sum_B Q_{AB} \quad (2)$$

where

$$Q_{AB} = \frac{4}{3}\delta_{AB}(P_{x_Ax_B} + P_{y_Ay_B} + P_{z_Az_B}) - \frac{2}{3}(P_{y_Ay_B}P_{z_Az_B} + P_{z_Az_B}P_{x_Ax_B} + P_{x_Ax_B}P_{y_Ay_B}) + \frac{2}{3}(P_{y_Az_B}P_{z_Ay_B} + P_{z_Ax_B}P_{x_Az_B} + P_{x_Ay_B}P_{y_Ax_B}) \quad (3)$$

and

$$\langle r^{-3}\rangle_{2p} = (1/24a_0^3)(3.25 - 0.35q_A)^3 \quad (4)$$

In these equations, ΔE represents a "mean electronic excitation" energy, $\langle r^{-3}\rangle_{2p}$ is the mean inverse cube of the distance from the nucleus for an electron in a 2p orbital, q_A is the net charge on carbon A, and the $P_{\mu\nu}$ are elements of the first-order density matrix in the neglect of overlap approximation. The Karplus–Pople treatment of chemical shifts has severe limitations in its applicability.[21,22] However, for a limited series of analogous compounds where the ΔE parameter can be considered a constant, useful information can be obtained in terms of variations in the $\langle r^{-3}\rangle_{2p}$ term and the elements of the density matrix.

Using eq 2–4, and a set of π-molecular orbitals with an *explicit* dependence in terms of parameters that reflects a sort of competition between C_β and atom X (or group) to form π bonds to carbon C_α, while also showing the ionic characters in these bonds, Savitsky and coworkers[17] derived the following expressions for σ_p^{AA}

$$\sigma_p^{AA} = -130[0.677 - 0.323f(I-1)\{2 + (4/9) \times [(I+1)(1-I)f]^{1/2}\} \quad \text{for donors} \quad (5)$$

$$\sigma_p^{AA} = -130[1.323 - 0.323f(I+1)]\{2 + (4/9) \times [(I+1)(1-I)f]^{1/2}\} \quad \text{for acceptors} \quad (6)$$

Here, the parameter I determines the extent of bond ionicity, and f is a measure of the relative importance of C_β-to-C_α π bonding in comparison to the C_α-to-X π bonding.

Carbon-6 in Substituted Uracils. Inherent in Figure 3 is the ambiguity of correlating relative substituent effects of ^{13}C chemical shifts in terms of substituent electronegativity alone. The line drawn in Figure 3 goes through the halogens. When X is CH_2OH, CH_3, OMe, and H, the ^{13}C chemical shifts of carbon-6 fall on or very near the line through the halogens. Whereas, when X is OH, NH_2, CN, CO_2H, and NO_2, their ^{13}C chemical shifts depart dramatically from the line. Fluctuations in bonding and charge density within the σ framework could be responsible for the observed trends in the ^{13}C chemical shifts of carbon-6. If this was the case, these changes in bonding should be reflected in the carbon–hydrogen coupling constant, J_{CH}. Examination of Table I yields no obvious relationship between J_{CH} and the chemical shifts of carbon 6. However, these departures from the line can be correlated with the ability of the substituent to be either a mesomeric donor or acceptor. When X is a mesomeric donor group, there are positive deviations from the line and when X is an acceptor group there are negative deviations from the line.

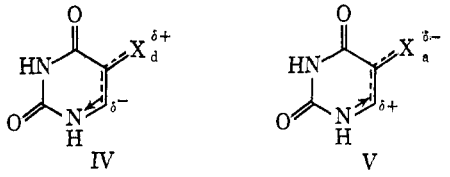
When X is F, OMe, OH, and NH_2, one could consider the I values to be approximately the same for the latter four substituents. That is, it is assumed that these substituents can polarize the C–X σ bond to about the same extent. Within the scope of this model, the deviations from the halogen line can arise because of different f values for the substituents. From eq 5, it can be seen that for a given I value, $I \leq 1$, as f *decreases* the paramagnetic term becomes less negative or the carbon-6 more shielded. For the model to be consistent with this data it requires that $f_{NH_2} < f_{OH} < f_{OMe} < f_F$. Therefore, the model is consistent with the following order for mesomeric donor ability for the above substituents, $NH_2 > OH > OMe > F$. This order is in accord with previous concepts on the mesomeric donor ability of these substituents.

Likewise, the same arguments hold for the X acceptor groups. When X is NO_2, CHO, CO_2H, CN, or I, the I values for these substituents can be considered reasonably constant. From eq 6 and ref 17, as f *increases* for a given I, σ_p^{AA} becomes more shielded. For the model to be consistent with the data $f_{NO_2} < f_{CHO} < f_{CO_2H} < f_{CN} < f_I$. That is, carbon-6 is more deshielded with respect to the halogen line in the following order: $NO_2 > CHO > CO_2H > CN > I$. Therefore, the model implies that the order of mesomeric acceptor ability of the substituents is $NO_2 > CHO > CO_2H > CN > I$. Again, this order is the generally accepted order of the ability of these substituents to act as mesomeric acceptor groups.

In the preceding discussion, we have established a reasonable correlation between the mesomeric donor or acceptor ability of the substituent and deviations from the line drawn in Figure 3. Herein also lies a possible explanation for the difference between the

(19) M. Karplus and J. A. Pople, *J. Chem. Phys.*, **38**, 2803 (1963).
(20) J. A. Pople, *ibid.*, **37**, 53 (1962).
(21) P. D. Ellis, G. E. Maciel, and J. W. McIver, Jr., *J. Amer. Chem. Soc.*, **94**, 4069 (1972).
(22) P. D. Ellis, Ph.D. Thesis, University of California at Davis, 1970.

ortho carbon in substituted benzenes and carbon 6 in the corresponding uracils, that is, mesomeric delocalization. In substituted benzenes any charge that builds up on the ortho carbon can be delocalized further to the para carbon. However, in the substituted uracils the efficiency of the delocalization is considerably reduced with respect to benzene derivatives. This may be shown schematically in the following structures. The arrows in structures IV and V indicate possible pathways for charge delocalization,



IV V

for mesomeric donor and acceptor groups, respectively. The pathway indicated in IV would seem to be not very efficient, since it requires delocalization of electrons to an already electron rich center. Therefore, C-6 in uracil derivatives (for donor X groups) would be *more* shielded than the ortho carbon in the substituted benzenes. Likewise, the pathway indicated by V may not be as efficient as the delocalization in the substituted benzenes, and hence carbon-6 would be less shielded (for X acceptor groups) than the ortho carbon in substituted benzenes. These trends with C-6 and C_o are not unlike those that were observed by Maciel[14] for vinyl carbons and C_o. Therefore, these trends enforce our initial assumption that uracil derivatives can be considered as trisubstituted ethylenes.

The remaining substituents that cannot be conveniently thought of as mesomeric donors or acceptors can be discussed in terms of their ability to polarize structures II and III. For the substituents where X is given by H, CH_3, and CH_2OH, there is little effect on the chemical shift of C-6. For Br and Cl there appears to be a cancellation of effects, that is, a small increase in I and a similar increment in f. This follows from the more detailed discussion of eq 5 and 6 given by Savitsky, *et al.*[17]

C-5 in Substituted Uracils. The reliability of the predictions of the trends in the ^{13}C chemical shifts for C-5 is somewhat lower than that of C-6, presumably because of the large changes in bonding that occur at C-5. These large changes in bonding, in all probability, will invalidate the assumption of a constant ΔE. In comparison, C-6 is relatively "isolated" from the substituent, whereas C-5 interacts with the substituent not only *via* the partial π bond formed between C-5 and X but also with the σ bond to X. This situation does not allow an easy separation of the mesomeric effects, *i.e.*, I constant and subsequent changes in f or *vice versa*. Furthermore, the neighbor anisotropy effects of the substituent are larger at C-5 than at C-6. These problems preclude a meaningfully detailed analysis of the trends exhibited in Figure 2. However, the qualitative discussion of the data in terms of possible fluctuations in I and f seems in order.

It appears from Figure 2 and Table I that for the substituents F, OMe, OH, NO_2, and NH_2 the C-5 becomes more shielded in the same order. Evidently, for these substituents, the inductive σ withdrawal of these substituents dominates the observed trends as opposed to the mesomeric donor or acceptor ability of the substituent. For the substituents CH_2OH, CH_3, Cl, CHO, CO_2H, H, and Br there is an apparent cancellation of inductive effects and mesomeric effects. The increased shielding of C-5 for X equal to Br, CN, and I may be due to several factors, none of which are very obvious. It is common to attribute these shieldings to a neighbor anisotropy effect of these substituents. However, the range of chemical shifts observed in going from Br to I is approximately 27 ppm. This chemical shift difference is too large to be attributed to the neighbor anisotropy effect. It is well known[18,23] that the magnitude of this effect is independent of the nucleus involved. The range of proton chemical shifts is only approximately 10 ppm and, therefore, the magnitude of this neighboring group anisotropy effect cannot exceed this range. The effect of iodine as a substituent on ^{13}C chemical shifts is just not understood.

Correlation with Enzymatic Behavior. We sought to determine whether or not the ^{13}C chemical shift data and our arguments for rationalizing this information could be extended to aid in understanding and evaluating the electronic component involved in the interaction of the uracils (or their corresponding nucleoside or nucleotide derivatives) with enzymatic systems. A convenient enzymatic case in point is that of thymidylate synthetase. 5-Fluoro-,[4] 5-trifluoromethyl-,[24] and 5-formyldeoxyuridine monophosphate[25] are potent inhibitors of thymidylate synthetase ($K_i \cong 10^{-8}$ M) while 5-hydroxymethyldeoxyuridine monophosphate[26] and thymidylate itself exhibit smaller inhibition constants ($K_i \cong 10^{-5}$ M) which are characteristic of "so-called" product inhibitors. Finally, 5-HOdUMP[27] and 5-NH_2dUMP[28] are strong inhibitors of cellular growth whose main site of action appears to be that of thymidylate synthetase.

An analysis of the nmr data presented herein, especially the correlation depicted in Figure 3, reveals that the inhibitors of thymidylate synthetase fall into two categories represented by: (A) 5-fluoro-, 5-hydroxy-, and 5-aminouracil, and (B) 5-formyl-, 5-trifluoromethyl-, 5-hydroxymethyl-, and 5-methyluracil (thymine). On close scrutiny, the C-5 substituents of the uracils in category A produce large, but similar chemical shift displacements when compared with uracil (C-4, 2.86–5.95; C-5, −21.34 to −39.47; and C-6, 15.99–25.75) and feature C-5 heteroatom bonding systems. In comparison, the C-5 substituents of the uracils in category B are joined to C-5 by carbon–carbon linkages and elicit smaller chemical shift displacements from uracil (C-4, −0.32 to 4.56; C-5, −1.58 to −12.22; and C-6, −6.86 to 4.72). In other words, the ^{13}C data indicate that the uracils in category A share certain similar properties with one another which are distinctly different from those of uracil and thymine,

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particularly with regard to the electronic conditions inferred from the ^{13}C chemical shifts of C-5 and C-6. However, the uracils grouped in category B display ^{13}C chemical shifts not unlike uracil itself. Thus, if extended to the deoxynucleotide level, the analysis of the ^{13}C data of the substituted uracils provides an electronic basis for differentiating among the inhibitors of thymidylate synthetase and is employed here to tentatively identify at least two mechanisms by which 5-substituted dUMP derivatives inhibit this enzyme.

A current view of the initial steps in the reaction mechanism of thymidylate synthetase envisages the binding of dUMP to the active site of the enzyme followed by the attack of an enzyme-bound nucleophile, most probably a sulfhydryl group in the thiolate anion form, at the C-6 position of the substrate.[29-31] If the behavior of the C-5 substituents of the uracils in category A is rationalized by considering –F, $-NH_2$, and –OH as mesomeric donors, the latter substituents funnel electrons into the uracil ring system, causing a substantial increase in the π-electron charge density at C-6. Thus, we speculate that the inhibitory properties of the dUMP compounds derived from the uracils in category A result in part because the inhibitor satisfies the rather stringent structural requirements for substrate binding, but more importantly because the C-5 substituent is a mesomeric donor and causes an increase in π-electron density at C-6 which is not conducive to successful attack by the nucleophilic sulfhydryl group of the enzyme.

The deoxynucleotide analogs of the uracils in category B include thymidylate and share structural and electronic properties with each other. However, 5-FdUMP[25] and 5-F_3CdUMP[24] are 1000-fold better inhibitors of thymidylate synthetase than are thymidylate and 5-$HOCH_2$dUMP. It is interesting to note that the C-6 chemical shifts of 5-formyluracil and 5-trifluoromethyluracil are both negative with respect to the C-6 of uracil itself. These data could be interpreted to indicate that C-6 of the corresponding irreversible inhibitors has a lower π-electron density than that of C-6 in dUMP; such a condition would make the C-6 of the inactivators more susceptible to nucleophilic attack than the corresponding carbon in dUMP.

Thus, this ^{13}C nmr investigation of 5-substituted uracils has suggested a means of categorizing deoxynucleotide inhibitors of thymidylate synthetase with regard to the electronic effects induced by their C-5 substituents. This technique is of potential value in predicting the effectiveness of substituted uracils as inhibitors of thymidylate synthetase before the more laborious procedure of testing on the deoxynucleotide level. We are presently extending such observations to include other enzymes which interact with pyrimidine bases, nucleosides, or nucleotides, and ^{13}C nmr studies of the interaction of ^{13}C enriched substrates and inhibitors with these enzymes are now in progress.

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