Experimental Section

Preparation of *exo*-2-Chloro-*endo*-2-methylnorbornane. This compound was prepared by the method of Toivonen et al.[9]

Preparation of Camphene Hydrochloride. Camphene hydrochloride was prepared from camphene and dry HCl and from methylcamphenilol or camphene hydrate by reported procedure.[8] All of the three samples gave identical rate of solvolysis.

Preparation of the Various α-Methyl-Substituted 1-Chloro-1-methylcyclopentanes 8-11. 2,2-Dimethylcyclopentanone was prepared by the procedure of Bartlett.[19] The other cyclopentanones were obtained commercially or as a donation of K. Greenlee (ChemSampCo). The tertiary alcohols were obtained by the addition of MeMgI to the ketone. The alcohols were converted to the chlorides by our earlier reported procedure.[8]

Preparation of *exo*- and *endo*-2,3,3-Trimethyl-2-norbornyl *p*-Nitrobenzoates (4a,b). The endo alcohol (mp 144-145 °C) was obtained by the addition of MeMgI to camphenilone. The *exo*-alcohol was obtained by converting the *endo*-alcohol into the chloride by using HCl. The crude chloride mixture was hydrolyzed to give the crude *exo*-alcohol, which was purified by recrystallization, mp 105.5-106 °C. The alcohols were converted into their *p*-nitrobenzoates by using an earlier reported procedure.[10]

Kinetics Measurements. The rates of ethanolysis of the chlorides and the solvolysis of *p*-nitrobenzoates in 80% aqueous acetone were measured by the titrimetric procedure described earlier.[10]

Registry No. 1, 465-30-5; 7, 19138-54-6; 8, 6196-85-6; 9, 94944-56-6; 10, 94944-57-7; 11, 91138-79-3; 1-methylcyclopentyl *p*-nitrobenzoate, 19013-42-4; 2-methyl-*exo*-norbornyl *p*-nitrobenzoate, 22467-58-9; 2-methyl-*endo*-norbornyl *p*-nitrobenzoate, 13351-30-9; 2,3,3-trimethyl-*exo*-norbornyl *p*-nitrobenzoate, 13421-46-0; 2,3,3-trimethyl-*endo*-norbornyl *p*-nitrobenzoate, 13389-76-9; 2,3,3-trimethyl-*endo*-norbornan-2-ol, 13429-57-7; 2,3,3-trimethyl-*exo*-norbornan-2-ol, 13429-40-8; MeMgI, 917-64-6; camphenilone, 13211-15-9; HCl, 7647-01-0; *tert*-butyl chloride, 507-20-0.

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^{17}O NMR Studies on 5-Substituted Uracils

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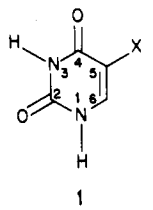
The ^{17}O NMR chemical shifts of eight ^{17}O-enriched 5-substituted uracils have been measured at 95 °C in ^{17}O-depleted water. The chemical shift range from the methoxy to the nitro compound for oxygen 2 is 40 ppm; the range for oxygen 4 for the same compounds is 20 ppm. The ^{17}O data for oxygen 2 gives a good correlation with Hammett and DSP treatments. A plot of the ^{17}O chemical shifts with the data for oxygen 2 and with the ^{17}O data for para-substituted anisoles gives a good correlation. Data for oxygen 4, an ortho-type position, does not correlate as well with the Hammett relationship.

Adenine–thymine base pair specificity of certain small molecules that intercalate with DNA has been attributed to hydrogen bonding between hydroxyl functions on the intercalator and the 2-carbonyl of thymine.[1] ^{17}O NMR spectroscopy is an excellent potential method to examine directly such interactions. As a first step in such investigations it is important to understand the effects of structural changes on the ^{17}O chemical shift of the carbonyl groups of the bases. The investigation reported here examines one aspect of structural changes on these pyrimidine bases carbonyl resonances: the effect of varying substituents at the 5-position of uracil (1). Several 5-



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substituted uracils and their nucleotide analogues have found use as chemotherapeutic agents[2] and, consequently, an understanding of the influence of substituents on the carbonyl oxygen chemical shifts, and the resulting inferred electronic changes, should add to the understanding of how these agents function.

Previous NMR studies on 5-substituted uracils have included proton,[3] nitrogen-15,[4] and carbon-13[5] investigations. Fiat and co-workers[6] reported the initial oxygen-17 study on uracil and thymine including assignment of the chemical shifts of their two carbonyl oxygen resonances. By carrying out this study on eight 5-substituted uracils, an evaluation of electronic properties of all nuclei in these pyrimidine systems by NMR will be completed. The determination of the oxygen chemical shifts in this system will also provide evidence of the value of using ^{17}O NMR to study tautomeric systems.

Experimental Section

The ^{17}O spectra were recorded on a JEOL GX-270 Spectrometer equipped with a 10-mm broad-band probe operated at 36.5 MHz. The instrument settings were 30-KHz spectral width, 1 K data points, 90° pulse angle (28-μs pulse width), 200-μs acquisition delay, and 16.9-ms acquisition time. The spectra were recorded

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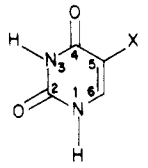
0022-3263/85/1950-0829$01.50/0

Table I. ^{17}O Chemical Shifts (ppm) of Enriched 5-Substituted Uracils[a]



X	δ_{O-2}	$\nu_{1/2(O-2)}$[b]	δ_{O-4}	$\nu_{1/2(O-4)}$[b]
OCH_3	220	195	296	140
CH_3	224	142	301	161
H	232	122	301	134
F	230	200	294	118
Cl	234	154	306	182
Br	235	182	311	190
CF_3	247	323	312	202
NO_2	261	182	317	219

[a] Measured at 95 °C in ^{17}O-depleted water and referenced to internal water at 95 °C. [b] In Hz.

without proton decoupling and with sample spinning. The signal-to-noise ratio was improved by applying a 50-Hz exponential broadening factor to the FID prior to Fourier transformation. The data point resolution was improved to ±0.2 ppm by zero filling to 8 K data points. Chemical shifts are reported relative to internal water at 95 °C. The error in the chemical shift is estimated to be ±1 ppm. The spectra of 5-substituted uracils (0.05 M solutions of enriched samples) were measured in ^{16}O normalized water (Merck) at 95 °C due to solubility limitations and to provide narrower line widths. Generally, 10^5–10^6 scans for the samples were accumulated.

All the compounds included in this study are commercially available (Sigma), except 5-methoxyuracil, which was prepared by the published procedure.[7] The ^{17}O enrichment of the uracils was carried out by heating solutions of 0.1–0.2 mmol of compound, 100 μL of $H_2{}^{17}O$ (45%, Stohler), and 250 μL of concentrated hydrochloric acid in 2.25 mL of deionized water for 8–11 days.[6] Although no quantitative measurements of exchange rates or percent enrichment were attempted, it was generally observed that uracil derivatives containing electron-donating substituents underwent ^{17}O enrichment (O-2 and O-4) slower than the parent compound and the derivatives containing electron-withdrawing substituents. In addition, the ^{17}O enrichment of O-4 was consistently faster than that of O-2 for all the compounds resulting in the area of the signal for O-4 being greater by about a factor of 2 than that for O-2. The enriched uracil derivatives were isolated by cooling the solutions of the exchanged compounds to room temperature, decanting off the solvent from the precipitated compounds, rinsing several times with acetonitrile, and drying by a stream of nitrogen. The identity of the exchanged compounds was confirmed by ^1H NMR and melting point.

Results and Discussion

The data for the ^{17}O chemical shifts for the ^{17}O-enriched 5-substituted uracils in ^{17}O-depleted water at 95 °C are given in Table I. Puzo and co-workers[8] noted, by mass spectroscopy, that position 4 of uracil and thymine was preferentially labeled relative to position 2 in an exchange reaction. Fiat and co-workers made the assignments of the two resonances of uracil and thymine based upon their relative enrichment on exchange reaction using ^{17}O-enriched water and noted the consistency of the assignments with the chemical shift of urea and amides.[6] Results from selective enrichment by synthesis of both the O-4 and O-2 in uridine and related analogues are also in accord with these assignments.[9] The assignment is in agreement with

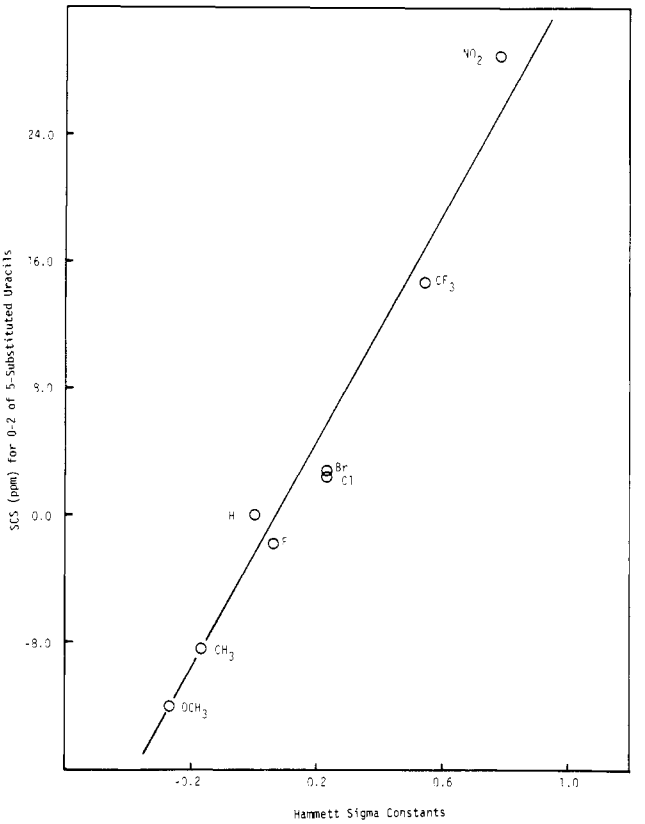


Figure 1. Correlation of O-2 chemical shifts (ppm) for 5-substituted uracils with Hammett σ constants.

Table II. Correlations with ^{17}O Chemical Shift Data for 5-Substituted Uracils[a]

correlation		slope	intercept	r[b]	n[c]
σ	δ_{O-2}	36.5	−3.06	0.982	8
δ_{O-2}	$\delta_{anisoles}$	0.643	−0.764	0.979	8
σ	δ_{O-4}	19.1	1.83	0.960	7[d]

correlation		ρ_I	$\rho_R{}^0$	f(SD/rms)[e]	r[b]	n[c]
DSP	δ_{O-2}	29.9	48.2	0.14	0.992	8

[a] Positive SCS values are downfield from the parent compound. [b] Correlation coefficient. [c] Number of data points. [d] The data for the 5-F compound was excluded. [e] See ref 16.

calculated electron densities for the two oxygen atoms.[10] We also note the preferential enrichment of the oxygen at position 4 for all the 5-substituted uracils when allowed to exchange with ^{17}O-enriched water. The chemical shift values reported here for both resonances of uracil and thymine are approximately 7 ppm downfield from those previously reported,[6] presumably a result of the 45 °C temperature difference between the measurements made in the two laboratories. Similar temperature effects on ^{17}O chemical shifts for other amides[11] have been noted.

It can be seen from Table I that both oxygen 2 and oxygen 4 experience significant shifts as a function of substituents. The range of chemical shifts from nitro to methoxy at oxygen 2 is approximately 40 ppm and the range at oxygen 4 is approximately 20 ppm. These are substantial substituent-induced chemical shifts on the two oxygen signals, particularly since it is well established that uracil and thymine exist in their keto forms both in the solid state and in solution.[10] The data reported here are

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(8) Puzo, G.; Schram, K. K.; McCloskey, J. *Nucleic Acids Res.* **1977**, *4*, 2075.
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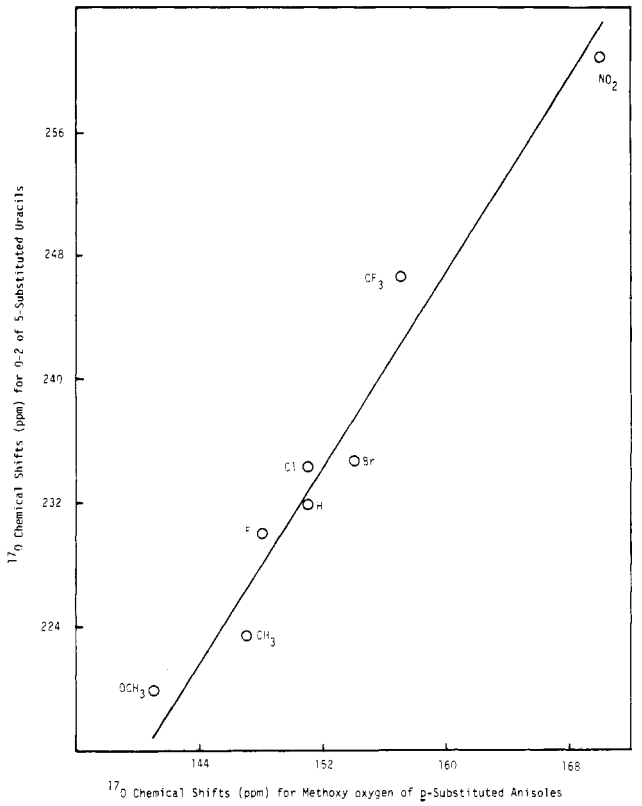

Figure 2. Correlation of O-2 chemical shifts (ppm) for 5-substituted uracils with the methoxy oxygen of para-substituted anisoles.

consistent with the keto structure since the ^{17}O signals fall in the amide carbonyl region, not the aromatic hydroxy one.

The data for oxygen 2, which is in a para-type relationship to the 5-substituents, gives a good correlation with Hammett σ constants (Figure 1, Table II). The treatment of the oxygen 2 data by the DSP method[12] gave a good correlation using σ_{R^0} values (Table II). The regression coefficients show a large dependency upon the π-delocalization term. Both these correlations indicate a significant degree of orbital overlap and interaction between the 5-substituents and the 2-oxygen. To test this point further, we have plotted the ^{17}O data for para-substituted anisoles[13] vs. the data for the 2-oxygen of the uracils (Figure 2). As can be seen, a good correlation between the two sets of data is found. This result supports the contention that considerable interaction between the 5-substituent and the 2-oxygen atom exists and compares with substituent effects noted for the benzene ring system. This is in contrast to the conclusion, drawn from ^{13}C studies,[5] that the 5-substituted uracils can be considered as trisubstituted ethylenes. The slope of the line (0.64) for the anisole and uracil data indicates that the uracil oxygen is more sensitive to substituents than the anisole oxygen. This result further indicates that the 5-substituted uracil structure should be viewed as more than an isolated trisubstituted ethylene.

The anisole ^{17}O chemical shift data gave a good correlation with the calculated π-electron density and π-bond order on oxygen,[13] suggesting a dependency of the chemical shift on the charge density bond order matrix term of the Karplus–Pople[14] expression. The only reported electron densities for the 5-substituted uracils, calculated by the SCF method,[15] show so little change with substituents that correlations with ^{17}O results were not attempted. However, the fact that a good correlation between the uracil and anisole ^{17}O data exists indicates that the O-2 chemical shift of the uracils depends to a significant extent upon the electron density on the oxygen atom. Such a conclusion is consistent with one made from hydrogen bonding studies that the ^{17}O chemical shifts of uridine were dependent, in part, upon π-electron densities.[9]

It can be seen from the data in Table I that oxygen 4 is not as sensitive to substituent effects as oxygen 2. This result is consistent with the fact that the O-4 CO bond is not as polar as the one involving O-2. The chemical shift data for oxygen 4, which is in an ortho-type relationship to the 5-substituents, do not give as good a correlation with Hammett σ constants[16] as noted for oxygen 2 (Table II). Nevertheless, qualitatively the same type of general trend noted for oxygen 2 data is observed. The results from the 5-fluoro compound give the largest deviation from the Hammett line. Previous studies have concluded that two water molecules are associated with oxygen 4 by H bonding.[5,9] H bonding by water to the highly electronegative fluorine atom, altering the association with oxygen 4, may account for the larger than expected substituent induced shift noted for 5-fluorouracil. Unfortunately, no ^{17}O data exist for ortho-substituted anisoles for comparison with oxygen 4 in the uracils. The most detailed study of ortho-substituent effects on ^{17}O chemical shifts is for benzaldehydes and acetophenones,[17] where steric inhibition of resonance is considered responsible for the major effects observed. In the uracil system one would expect ortho-type effects to be less dramatic than noted for the carbonyl systems previously studied.[15] Detailed analysis of the effects of substituents on oxygen 4 must await study of more appropriate model systems. It is apparent, however, that 5-substituents have a substantial effect on oxygen 4 of uracil and that these effects are qualitatively described by the Hammett approach.

The results from these ^{17}O studies indicate that previous conclusions regarding the electronic structure of uracil must be revised since the ^{17}O results show that there is considerable interaction between the 5-substituents and both the 2- and 4-oxygen atoms.

Acknowledgment. This work was supported by NSF (PCM 83-09575). W.D.W. is the recipient of an American Cancer Society Faculty Research Award.

Registry No. 1 (X = OCH_3), 6623-81-0; 1 (X = CH_3), 65-71-4; 1 (X = H), 66-22-8; 1 (X = F), 51-21-8; 1 (X = Cl), 1820-81-1; 1 (X = Br), 51-20-7; 1 (X = CF_3), 54-20-6; 1 (X = NO_2), 611-08-5; ^{17}O, 13968-48-4.

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