ture of ethyl phenyl sulfone and the epoxy ketone **2** which was used directly in the next step.

The crude reduction product was diluted with 50 mL of benzene, cooled in an ice bath, and treated with 0.36 mL of a 1 M solution of $SnCl_4$ in benzene. After 3 min, the mixture was partitioned between ether and 2 N NaOH, and the organic layer was washed with 2 N NaOH, 1 N HCl, saturated $NaHCO_3$, and brine. After drying over $MgSO_4$, the solvent was removed under reduced pressure. The (±)-α-multistriatin was separated from the ethyl phenyl sulfone by bulb-to-bulb distillation using a Büchi Kugelrohr oven [90 °C (7 torr)], providing 980 mg (50% overall yield from the epoxy ester **10**) of material of >95% chemical and stereochemical purity by VPC (100 °C, 15% Carbowax) and 180-MHz ^1H NMR analysis. The spectral properties (IR, ^{13}C NMR, and 180-MHz ^1H NMR) corresponded to those reported for the α isomer of multistriatin.[1,8]

Acknowledgments. Financial support for this work was provided by the National Institutes of Health (grant no. CA-16616), Chevron Research Company and the Eli Lilly Company, and the National Science Foundation (departmental equipment grant no. CHE-76-05512). We would also like to thank Mr. Jerry L. Adams for preparing the aldehyde ester **8**.

Registry No.—**1**, 54815-06-4; **2**, 59014-13-0; **7**, 69291-66-3; **8**, 69291-67-4; **9**, 69291-68-5; **10**, 69291-69-6; **11**, 69291-70-9; methylenetriphenylphosphorane, 3487-44-3; α-lithioethyl phenyl sulfone, 69291-71-0; ethyl phenyl sulfone, 599-70-2.

References and Notes

(1) (a) W. E. Gore, G. T. Pearce, and R. M. Silverstein, *J. Org. Chem.*, **40**, 1705 (1975); (b) W. J. Elliott and J. Fried, *ibid.*, **41**, 2475 (1976); (c) G. T. Pearce, W. E. Gore, and R. M. Silverstein, *ibid.*, **41**, 2797 (1976); (d) K. Mori, *Tetrahedron*, **32**, 1979 (1976); (e) G. J. Cernigliaro and P. J. Kocienski, *J. Org. Chem.*, **42**, 3622 (1977).
(2) P. A. Bartlett, F. R. Green III, and E. H. Rose, *J. Am. Chem. Soc.*, **100**, 4852 (1978), and references cited therein.
(3) N. L. Allinger, *J. Am. Chem. Soc.*, **81**, 232 (1959).
(4) M. Julia and J.-M. Paris, *Tetrahedron Lett.*, 4833 (1973).
(5) Prepared in the same manner as the methyl ester reported by A. Zamojski, *Rocz. Chem.*, **40**, 451 (1966).
(6) P. A. Bartlett and J. Myerson, *J. Am. Chem. Soc.*, **100**, 3950 (1978).
(7) E. J. Corey and M. Chaykovsky, *J. Am. Chem. Soc.*, **87**, 1345 (1965).
(8) G. T. Pearce, W. T. Gore, and R. M. Silverstein, *J. Magn. Reson.*, **27**, 497 (1977).

Use of ^{15}N–^1H and ^{15}N–^{13}C Coupling Constants for the Measurement of Uracil Monoanion Tautomerism[1]

Robert L. Lipnick* and John D. Fissekis

Memorial Sloan-Kettering Cancer Center, New York, New York, 10021

Received December 5, 1978

We have measured the proton and carbon-13 couplings to nitrogen-15 (J_{H_6,N_1}, J_{H_5,N_1}, J_{H_5,N_3}, and J_{C_6,N_1}) for uracil-1,3-$^{15}N_2$, its *N*-deuteriomethyl derivatives (I–IV), and the corresponding monoanionic species (Ia ⇌ Ib, IIa, and IIIb). These parameters were found to be sensitive probes for the determination of the uracil monoanion tautomeric equilibrium (Ia ⇌ Ib) by reference to the fixed tautomer models, IIa and IIIb. Similar measurements were performed employing J_{H_5,H_6}, $\delta_{H_6} - \delta_{H_5}$, and $\delta_{C_6} - \delta_{C_5}$. The population of the N_1H tautomer (Ia), based upon the weighted average derived from four of these coupling constants, is 48.5%. If a correction is made for the effect of *N*-methyl substituents on the tautomer models, IIa and IIIb, the weighted average is 52.2%. The above population determinations are in excellent agreement with those made previously using other methods. The potential of this approach in the study of similar equilibria for oligonucleotides is discussed.

The investigation of chemical tautomerism is of considerable importance in the study of heterocyclic molecules. In many instances, the determination of the structure of such tautomeric species and their relative stabilities is of considerable biological importance. A wide range of chemical and spectroscopic methods (e.g., IR, UV, and NMR) have already been applied to this problem, with varying degrees of success. For the most part, these studies are based upon the assumption that substitute fixed tautomer parameters, which can be obtained from two or more partially methylated derivatives, are good models for the otherwise unmeasurable intensive parameters of the corresponding tautomeric species.

It is known that ^{15}N–^1H and ^{15}N–^{13}C coupling constants are sensitive to changes in hybridization of the nitrogen in question and that such variations are likely to be both large and highly specific when the parameter is measured for each tautomeric species. Recently, this approach has been applied successfully to the problem of histidine tautomerism.[2]

We have now applied this method to the quantitative measurement of uracil monoanion tautomerism. The use of this system permits a direct comparison of our results with those obtained by other methods.

Experimental Section

We have prepared uracil-1,3-$^{15}N_2$ (I) from urea-$^{15}N_2$, 99.6% ^{15}N (KOR Isotopes, Cambridge, Mass.), and propiolic acid (Aldrich) in 77% yield, according to the procedure employed by Harada and Suzuki for the synthesis of the nonlabeled material.[3a] The uracil-1,3-$^{15}N_2$ was randomly alkylated with 1 equiv of dimethyl-d_6 sulfate, 99% *d* (Aldrich), in the presence of 1 equiv of aqueous sodium hydroxide to yield a mixture of 1-methyl-d_3-uracil-1,3-$^{15}N_2$ (II), 3-methyl-d_3-uracil-1,3-$^{15}N_2$ (III), and 1,3-dimethyl-d_6-uracil-1,3-$^{15}N_2$ (IV), which was separated chromatographically. Each of the components was identified by comparison of its UV spectra in neutral and alkaline pH's with those derived from authentic samples of the corresponding nonlabeled derivatives. UV measurements were performed on a Varian Superscan 3 spectrophotometer. The experimental details of these isotopic syntheses and the separation procedures used will be reported elsewhere.[3b]

NMR measurements were performed in D_2O solution on a JEOL-PFT-100 spectrometer, operating at ambient probe temperature, 22 °C. Field stabilization was provided through internal ^2H lock on the deuterated solvent. Measurements of the monoanionic species were made at pD ≅ 12.0.[4] Under these conditions uracil and its monomethyl derivatives should exist solely as the monoanionic forms, as calculated from the known pK_a's of these molecules.[5,6] The pD adjustments were made by adding 5-μL aliquots of 10% NaOD solution from a micropipet and monitoring changes with an Ingold 6025-02 combination microelectrode and a Beckman Research pH meter. For the analysis of the proton spectra, all coupling constants were extracted directly from the average of the appropriate repeated spacings, as $J/\Delta\gamma \ll 0.1$ in all such cases, allowing a first-order treatment.[7]

Results and Discussion

We have measured the ^{15}N–^1H and ^{15}N–^{13}C coupling constants from the proton and natural abundance ^{13}C NMR spectra, respectively, for both the neutral (I–IV) and the

0022-3263/79/1944-1627$01.00/0

Table I. Proton, Nitrogen-15, and Proton, Proton Coupling Data for Uracil-*1,3*-$^{15}N_2$ and Its *N*-Deuteriomethyl Derivatives in D_2O as a Function of Ionization and Tautomerism[a]

species	J_{H_6,N_1}	J_{H_6,N_3}	J_{H_5,H_6}	J_{H_5,N_1}	J_{H_5,N_3}	$\delta_{H_6} - \delta_{H_5}$
I	3.30	0.25[b]	7.69	4.46	2.63	1.732
	(3.5)[c]		(7.8)[c]	(4.4)[c]	(2.5)[c]	
			(7.70)[d]			(1.93)[d,e]
Ia, Ib	6.54	~0	6.83	2.81	1.73	1.864
			(6.80)[d]			(1.87)[d]
II	2.48	~0	7.82	4.73	2.69	1.808
			(7.82)[d]			(2.09)[d,e]
IIa	2.32	~0	7.32	3.97	0.70	1.727
			(7.32)[d]			(1.73)[d]
III	3.36	~0	7.62	4.49	2.75	1.641
IIIb	10.46	~0	6.38	1.68	2.72	1.924
			(6.26)[d]			(1.92)[d]
IV	2.54	~0	7.80	4.82	2.96	1.739
			(7.78)[d]			(2.00)[d]
IV[f] (pD = 12.3)	2.47	~0	7.80	4.79	2.93	1.737

[a] Spectra were obtained at 500-Hz sweep width FT, 10–100 scans, 16 κ data points, nominal resolution = 0.06 Hz. Solutions were of ~3 mg of each derivative in ~0.5 mL of D_2O. Coupling constants are expressed in hertz; chemical shift differences are in parts per million. [b] The instrument was exceptionally well tuned on this occasion. [c] Data from Roberts et al.[8] [d] Data from Stolarski et al.[9] [e] The data of Stolarski et al.[9] are not directly comparable as their measurements for the neutral species were made in Me_2SO-d_6 solution. [f] Obtained as a control to illustrate the negligible effect of pH on these parameters if no ionization occurs.

Table II. Carbon-13, Nitrogen-15 Coupling Data for Uracil-*1,3*-$^{15}N_2$ and Its *N*-Deuteriomethyl Derivatives in Derivatives in D_2O as a Function of Ionization and Tautomerism[a]

species	J_{C_6,N_1}	J_{C_5,N_3}	J_{C_4,N_3}	J_{C_2,N_1}	J_{C_2,N_3}	$\delta_{C_6} - \delta_{C_5}$
I[b]	11.2	6.8	8.5	~16.9	~16.9	41.94
						(41.85)[c]
Ia, Ib	6.7	unresolved multiplet				50.08
						(50.6)[d]
II	12.8	5.5				47.14
IIa	12.8	≦1.0				44.88
						(44.8)[d]
III	11.6	4.9				40.44
IIIb	≦1.0	unresolved multiplet				57.27
						(45.5)[d,e]
IV	12.2	5.5				45.47

[a] Spectra were run at 2.5-kHz sweep width FT, 300–700 scans, 8 κ data points, nominal resolution = 0.6 Hz. Solutions were made up from ~30 mg of each derivative in ~1 mL of D_2O in a 10-mm sample tube. Coupling constants are in hertz; chemical shift differences are in parts per million. [b] The ^{13}C NMR spectra for I were obtained from a Me_2SO-d_6 solution because of the limited solubility of this species in D_2O. These spectra were obtained from 9.3 κ scans, with a 5-s pulse interval, such that the two otherwise undetected carbonyl signals C_2 and C_4 were observed. They appeared at 51.28 and 64.15 ppm, respectively, downfield from C_5. Ellis et al.[10] found C_2 and C_4 at 51.17 and 63.98 ppm downfield from C_5. In our measurement and that of Ellis et al.,[10] C_5 was 59.78 ppm downfield from internal Me_4Si. [c] From Ellis et al.[10] [d] From Stolarski et al.[9] [e] This discrepancy between our results and those of Stolarski et al.[9] is considered in the Results and Discussion Section.

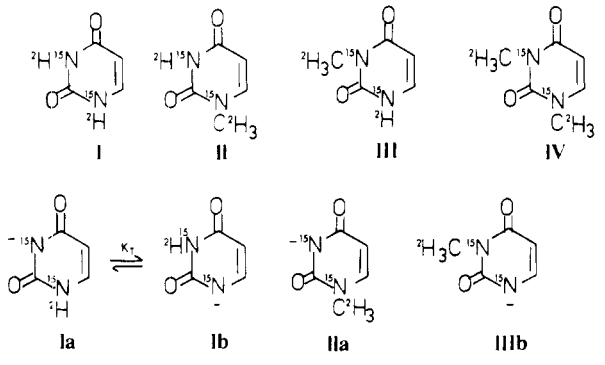
corresponding monoanionic species (Ia, Ib, IIa, and IIb) (Chart I). The results are presented in Tables I and II. Sample ^1H and ^{13}C spectra are shown in Figure 1.

We have employed the deuteriomethyl derivatives (II and III) as fixed tautomer models (IIa and IIIb) rather than the usual methyl derivatives for instrumental reasons, in order to remove unnecessary signals in the ^1H and ^{13}C (no NOE) spectra, and thereby narrow the FT spectral width to achieve greater spectral precision. For the same reason, no internal chemical shift standard has been employed, so that chemical shifts are expressed relative to the most shielded signal, which is nominally taken as $\delta = 0$. These relative assignments for both the ^1H and ^{13}C NMR spectra are in excellent agreement with literature values.[8-10]

Our values for J_{H_6,N_1}, J_{H_5,N_1}, and J_{H_5,N_3} for uracil-*1,3*-$^{15}N_2$ (I) are in agreement with the earlier measurements of Roberts, Lambert, and Roberts.[8] In addition, we have resolved a four-bond coupling, J_{H_6,N_3}, of 0.24 Hz, and have assigned the hitherto ambiguous pair J_{H_5,N_1}, J_{H_5,N_3} on the basis of their variation with respect to the N_1H and N_3H model tautomers of the corresponding methyl monoanions, IIa and IIIb.

Most ^{13}C spectra were obtained under conditions of rapid pulsing and relatively brief accumulation times. Under these conditions, only two signals, corresponding to C_5 and C_6, could be observed due both to the lack of NOE of the C_2 and C_4 carbonyls and to their respective long T_1 relaxations. However, in an overnight run with long pulse delay, the C_2 and C_4

Chart I. Neutral and Monoanionic Species of Uracil-*1,3*-$^{15}N_2$ and Its Deuteriomethyl Derivatives



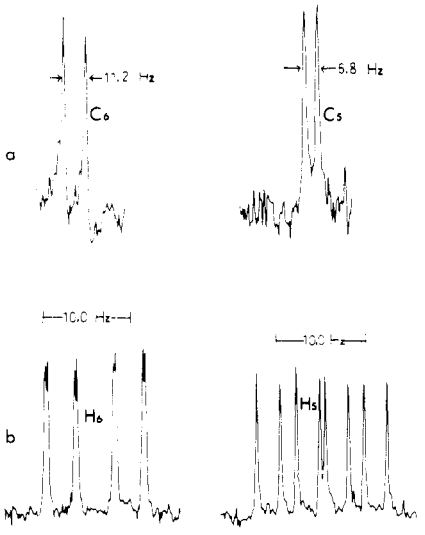


Figure 1. (a) The splittings J_{C_6,N_1} and J_{C_5,N_3} in the natural abundance ^{13}C NMR spectrum of I in Me_2SO-d_6. (b) The expanded proton NMR spectrum of I in D_2O. The small splitting in each of the four H_6 signals is due to the long-range coupling J_{H_6,N_3} of 0.25 Hz.

signals could be detected downfield at 51.28 and 64.15 ppm, in excellent agreement with the literature (Table II). The C_2 and C_4 carbonyls in the uracil-1,3-$^{15}N_2$ appear as a quartet and doublet, respectively, by virtue of their one or two one-bond couplings to the labeled nitrogens. We believe that this observed difference in multiplicity for these two signals provides the most unambiguous data yet in support of the original C_2 and C_4 carbonyl assignment.[11]

The geminal ^{15}N–C–H coupling across an sp^2 hybridized carbon atom is known to be related to the electronic structure of the C–N π system,[12] and for III J_{H_6,N_1} was found to increase 7.10 Hz on conversion from the neutral species to its N_1 monoanion (IIIb), consistent with the substantial contribution of the canonical form IIIb′ (Scheme I).

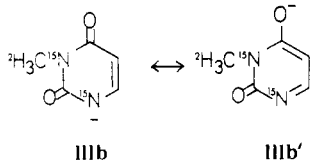
Similarly, the vicinal ^{15}N–C–C–H couplings J_{H_5,N_1} and J_{H_5,N_3} are expected to vary with electronic and stereochemical factors, and the one-bond ^{13}C–^{15}N coupling J_{C_6,N_1} should depend upon the s characters of these two atoms comprising their common σ bond.[13]

We have employed the above coupling constant variations for uracil-1,3-$^{15}N_2$ and its N-deuteriomethyl derivatives to make a new determination of the uracil monoanion tautomeric equilibrium, Ia $\rightleftharpoons$ Ib (Chart I).

The ith observed NMR coupling constant, P_i^{obsd}, for the uracil monoanion (Ia $\rightleftharpoons$ Ib) represents the time average of the intensive parameters, P_i^{Ia} and P_i^{Ib}, for the corresponding tautomeric species. Since the latter values cannot be obtained directly, the values P_i^{IIa} and P_i^{IIIb} obtained from the fixed tautomer models[14] IIa and IIIb are employed.

Thus, if X_{Ia} and X_{Ib} represent the mole fractions of tautomers Ia and Ib, respectively, $P_i^{obsd} = X_{Ia}P_i^{IIa} + X_{Ib}P_i^{IIIb}$, and since $X_{Ia} + X_{Ib} = 1$, then $X_{Ia} = (P_i^{obsd} - P_i^{IIIb})/(P_i^{IIa} - P_i^{IIIb})$. The results of this calculation ("uncorrected") are presented in Table III as %Ia or $100X_{Ia}$. The values are spread over a narrow range (47.9–49.4%), with the weighted average

Scheme I. Two Canonical Forms of the 3-Methyl-d_3-uracil-1,3-$^{15}N_2$ Monoanion



IIIb IIIb′

Table III. Populations of Uracil Monoanion Tautomers Calculated from This Work

soln	parameter	parameter change	%Ia "uncorrected"	%Ia "corrected"
1	J_{H_6,N_1}	7.26 Hz	48.2	53.2
2	J_{H_5,N_1}	2.05 Hz	49.4	56.6
3	J_{H_5,N_3}	1.96 Hz	49.1	44.4
4	J_{H_5,H_6}	0.74 Hz	47.9	51.4
5	weighted av[a] of soln 1–4		48.5	52.2
6	$\delta_{H_6} - \delta_{H_5}$	0.197 ppm	30.5	41.5
7	$\delta_{C_6} - \delta_{C_5}$	12.39 ppm	58.3	45.6

[a] The weighted averages (solution 5) were obtained from the equation (%Ia) weighted av = $\sum[(\%Ia)_{soln\ i}(\text{parameter change})_i]/\sum(\text{parameter change})_i$, where $(\%Ia)_{soln\ i}$ is the ith solution value of %Ia and $(\text{parameter change})_i$ is the parameter change in hertz or parts per million.

Table IV. Populations of Uracil Monoanion Tautomers Obtained from the Literature

parameter	%Ia	source
258 nm (UV)	43	Nakanishi et al. (1961)[6]
286 nm (UV)	51	Nakanishi et al. (1961)[6]
UV	47	Shapiro and Kang (1971)[15]
J_{H_5,H_6} (NMR)	48	Stolarski et al. (1977)[9]

Table V. Additivity of *N*-Methyl Substitution for Uracil-1,3-$^{15}N_2$ NMR Parameters[a]

parameter	Δ_{calcd}	Δ_{obsd}
J_{H_6,N_1}	−0.76 Hz	−0.76 Hz
J_{H_5,H_6}	+0.06 Hz	+0.11 Hz
J_{H_5,N_1}	+0.30 Hz	+0.36 Hz
J_{H_5,N_3}	+0.18 Hz	+0.33 Hz
$\delta_{H_6} - \delta_{H_5}$	−0.015 ppm	+0.007 ppm
J_{C_6,N_1}	+2.0 Hz	+1.0 Hz
$\delta_{C_6} - \delta_{C_5}$	+3.70 ppm	+3.53 ppm
J_{C_5,N_3}	−3.2 Hz	−1.9 Hz

[a] Strict additivity implies that Δ_{calcd} and Δ_{obsd} obtained from eq 1 and 2, respectively, are the same within experimental error for a given parameter.

(according to the magnitude of $P_i^{IIa} - P_i^{IIIb}$) in excellent agreement with literature values. (Table IV).

We have also calculated this equilibrium composition using "corrected" model intensive parameters, P_i^{Ia} and P_i^{Ib}, which are obtained with the assumption that the two electronic changes, relating to the transformations N–H → N–CH_3 and N–H → N^-, are additive within the same uracil monoanion tautomer.

For the limited case of methyl substitution alone at two different sites, the principle of additivity can be tested by comparing the calculated and observed parameter changes, Δ_{calcd} and Δ_{obsd}, respectively, for the change uracil → 1,3-dimethyluracil, based upon eq 1 and 2. The results are summarized in Table V. In general, we find good agreement, within experimental error of the two results.

$$\Delta_{calcd} = (P_i^{I} - P_i^{II}) + (P_i^{I} - P_i^{III}) \quad (1)$$

$$\Delta_{obsd} = P_i^{I} - P_i^{IV} \quad (2)$$

This self-consistency provides some justification for applying the same additivity concept to the above "corrected" model parameters employed in the measurement of uracil monoanion tautomerism. That is, in each of the N-1 and N-3 methyl derivatives of uracil, we assume that the measured

effect of the methyl substituent upon each coupling constant is invariable on going from the neutral to the monoanionic species. Thus, $P_i^{Ia} = P_i^{I} + (P_i^{IIa} - P_i^{II})$ and $P_i^{Ib} = P_i^{I} + (P_i^{IIIb} - P_i^{III})$. The results of four calculations employing this approach labeled "corrected" are summarized in Table III. In this case, a "correction" has yielded a range in values 44.4–56.6%, considerably larger than that obtained by the first approach. Interestingly, the weighted average in this case, 52.2%, is in very good agreement with that derived by the previous approach and other literature values (Table V). The lower precision in the second case suggests a fallacy in the additivity assumption implicit in the "corrected" calculations. This is not surprising in view of the known discrepancies of strongly mesomeric groups in NMR calculations employing linear free energy relationships[16] and the substantial differences in electronic character when comparing the neutral and monoanionic species of uracil. Since we have no information regarding the source and magnitude of all systematic errors inherent in the "uncorrected" values, there is no guarantee that their smaller spread or greater precision necessarily reflects a greater accuracy than that obtained from the average of the "corrected" measurements.

The parameter J_{C_6,N_1} appears to be of potential value for these measurements, but unfortunately it is small in magnitude for the N_1 monoanion (IIIb). Its actual value could not be experimentally resolved. An "uncorrected" calculation, assuming a population of 48.5% Ia obtained from the weighted average of the other coupling constant parameters, would require that $J_{C_6,N_1}^{IIIb} = 1.0$ Hz, within the range expected for a narrow unresolved carbon signal.

Our data for the monoanionic species Ia $\rightleftharpoons$ Ib, IIa, and IIIb should be directly comparable with those obtained under the same conditions by Stolarski et al.[9] for the nonenriched compounds. However, inspection of the chemical shift differences $\delta_{H_6} - \delta_{H_5}$ and $\delta_{C_6} - \delta_{C_5}$ extracted from these two sets of experiments reveals a single discrepancy with regard to the value of $(\delta_{C_6} - \delta_{C_5})^{IIIb}$ for the 3-methyluracil monoanion (IIIb, Table II). This discrepancy suggests an error in the measurement of $(\delta_{C_6} - \delta_{C_5})^{IIIb}$ for this species by Stolarski et al. Their measurements produce a $(\delta_{C_6} - \delta_{C_5})^{obsd}$ for the uracil monoanion Ia $\rightleftharpoons$ Ib which is outside the expected range $(\delta_{C_6} - \delta_{C_5})^{IIIb}$ as defined by the model fixed tautomer species IIa and IIIb. Indeed, the value of each parameter, P_i^{IIa} and P_i^{IIIb} for the species IIa and IIIb, respectively, should define the limits for the corresponding parameters, P_i^{obsd}, of the tautomeric equilibrium mixture, Ia $\rightleftharpoons$ Ib. We believe that the error in $(\delta_{C_6} - \delta_{C_5})^{IIIb}$ resides in their measurement of $\delta_{C_6}^{IIIb}$, which unlike the value for $\delta_{C_5}^{IIIb}$ does not satisfy the above minimum requirement.

Finally, we have employed the chemical shift differences $\delta_{H_6} - \delta_{H_5}$ and $\delta_{C_6} - \delta_{C_5}$ in similar "uncorrected" and "corrected" calculations. The "uncorrected" values of %Ia obtained from these chemical shift differences are qualitatively correct, but in poor agreement with our other results and those obtained previously by different methods. The corrected values (Table III) fall within the previously established range and suggest some potential for their use in future studies.

In this work, we have demonstrated that ^{15}N–^{1}H and ^{15}N–^{13}C coupling constants provide self-consistent values in the determination of tautomeric equilibria in the uracil monoanion, the results being entirely comparable with literature values obtained by other methods. While this approach lacks the detection sensitivity of UV and IR methods, it is certain to be of great value in the study of more complex systems such as oligonucleotides, in which overlapping bands may obscure any direct determinations. In such cases, selective isotopic enrichment would be of great advantage in the use of the NMR method.

Acknowledgment. We are indebted to Mr. Marvin Olsen and Dr. Brian Otter for their assistance and suggestions regarding some of the NMR experiments and to Dr. George Bosworth Brown for his continuing interest in this work.

Registry No.—I, 69381-24-4; Ia, 69381-25-5; Ib, 69381-25-5; II, 69381-26-6; IIa, 69381-27-7; III, 69381-28-8; IIIb, 69381-29-9; IV, 69381-30-2.

References and Notes

(1) This investigation was supported in part by Grant No. CA 08748 awarded by the National Cancer Institute and a Biomedical Research Grant No. 5 S07 RR 0534-04 awarded by the National Institutes of Health, DHEW, to R.L.L.
(2) F. Blomberg, W. Maurer, and H. Ruterjans, *J. Am. Chem. Soc.*, **99**, 8149 (1977).
(3) (a) K. Harada and S. Suzuki, *Tetrahedron Lett.*, 2321 (1976); (b) R. L. Lipnick and J. D. Fissekis, *J. Labelled Compd. Radiopharm.*, in press.
(4) Basicity measurements were made with the combination electrode used for H_2O and corrected according to the equation pD = pH(meter reading) + 0.40 from P. K. Glasoe and F. A. J. Long, *J. Phys. Chem.*, **64**, 188 (1960).
(5) (a) D. Shugar and J. J. Fox, *Biochim. Biophys. Acta*, **9**, 199 (1952); (b) P. A. Levene, L. W. Bass, and H. S. Simms, *J. Biol. Chem.*, **70**, 229 (1926).
(6) K. Nakanishi, N. Suzuki, and F. Yamazaki, *Bull. Chem. Soc. Jpn.*, **34**, 53 (1961).
(7) F. A. Bovey, "Nuclear Magnetic Resonance Spectroscopy", Academic Press, New York, 1969, p 97.
(8) B. W. Roberts, J. B. Lambert, and J. D. Roberts, *J. Am. Chem. Soc.*, **87**, 5439 (1965).
(9) R. Stolarski, M. Remin, and D. Shugar, *Z. Naturforsch. C*, **32**, 894 (1977).
(10) P. D. Ellis, R. B. Dunlop, A. L. Pollard, K. Seidman, and A. D. Cardin, *J. Am. Chem. Soc.*, **95**, 4398 (1973).
(11) (a) D. E. Dorman and J. D. Roberts, *Proc. Natl. Acad. Sci. U.S.A.*, **65**, 19 (1970); (b) A. J. Jones, M. W. Winkley, D. M. Grant, and R. K. Robbins, *ibid.*, **65**, 27 (1970); (c) A. J. Jones, D. M. Grant, M. W. Winkley, and R. K. Robbins, *J. Am. Chem. Soc.*, **92**, 4079 (1970).
(12) M. Witanowski and G. A. Webb, "Nitrogen NMR", Plenum Press, New York, 1973, p 294.
(13) Reference 12, p 286.
(14) (a) J. Elguero, C. Marzin, A. R. Katritzky, and P. Linda, "The Tautomerism of Heterocycles", "Advances in Heterocyclic Chemistry", Suppl. 1, Academic Press, New York, 1976, pp 14–20, 39–55; (b) L. M. Jackman and S. Sternhell, "Applications of Nuclear Magnetic Resonance Spectroscopy in Organic Chemistry", 2nd ed., Pergamon Press, New York, 1969, pp 380–384.
(15) R. Shapiro and S. Kang, *Biochim. Biophys. Acta*, **232**, 1 (1971).
(16) M. T. Tribble and J. G. Traynham, *Adv. Linear Free Energy Relat. 1972*, 143–201 (1972).