ton,[11] 1.79 at 80° for the unsubstituted compound, and the observation of a large positive ρ value of +3.77.[12]

(11) L. J. Steffa and E. R. Thornton, *J. Amer. Chem. Soc.*, **89**, 6149 (1967).
(12) W. H. Saunders, Jr., D. G. Bushman, and A. F. Cockerill, *ibid.*, **90**, 1775 (1968).

Peter J. Smith,* S. K. Tsui
Departments of Chemistry and Chemical Engineering
University of Saskatchewan
Saskatoon, Saskatchewan, S7N 0W0, Canada
Received April 16, 1973

Tautomerism of the Nucleoside Antibiotic Formycin, as Studied by Carbon-13 Nuclear Magnetic Resonance[1]

Sir:

Tautomerism of the nucleic acid bases has been well studied because of the obvious biological significance. In this communication I wish to report the carbon-13 spectra of several nucleoside antibiotics and related compounds and present evidence for prototropic tautomerization. The carbon-13 spectra of a wide variety of nucleosides have been previously reported.[2] In general all the carbon resonances have exhibited reasonably narrow spectral lines. This is in sharp contrast to the ^{13}C spectra of formycin A (7-amino-3-β-D-ribofuranosyl-1*H*-pyrazolo[4,3-*d*]pyrimidine) and formycin B (1,6-dihydro-3-β-D-ribofuranosyl-7*H*-pyrazolo[4,3-*d*]pyrimidin-7-one) shown in Figure 1.[3] Formycin A[4,5] and 8-azaadenosine[6,7] (7-amino-3-β-D-ribofuranosyl-*v*-triazolo[4,5-*d*]pyrimidine) are cytotoxic adenosine analogs while formycin B is a cytotoxic inosine analog. The chemical shifts and line assignments are given in Table I. The assignments are based upon partial decoupling experiments, the previous assignments for the naturally occurring nucleosides,[2] and a comparison of the chemical shifts in the series $\Delta\delta$(adenosine–inosine) and $\Delta\delta$(formycin A–formycin B). The unusual broadening is a function of sample temperature and solvent composition, but it is not particularly sensitive to concentration. In Figure 1 the narrow downfield line is the C2 carbon. The line widths of the other heterocyclic base carbons were 15–30 Hz at 25°. As the temperature was raised the line widths narrowed, and at 90° both the base and the sugar carbons had line widths $\leq$1.5 Hz. The

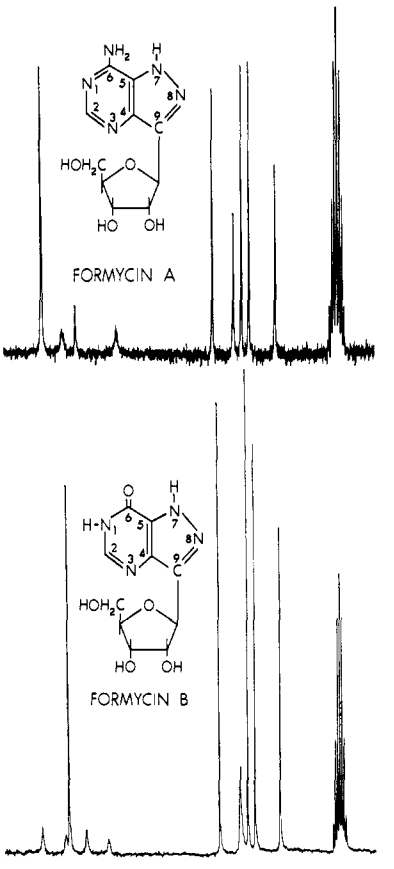

Figure 1.

samples were prepared quickly to minimize the absorption of water. In a separate experiment it was observed that the small amount of water present was due to water of hydration of the formycin samples. The relatively high melting point of DMSO-d_6 prevented us from lowering the temperature to observe the spectra in the "slow exchange" limit. However, the line broadening of base carbons does demonstrate that the formycin molecules are involved in a dynamic equilibrium. Scalar relaxation of the carbons resulting from ^{13}C–^{14}N or ^{13}C–^{1}H coupling can be ruled out as the dominant relaxation process because the line width was relatively independent of concentration in the range where the viscosity was very dependent upon nucleoside concentration. The hydrogen bonding properties of the solvent and the large uncertainties in extracting kinetic and thermodynamic parameters from a detailed analysis of the line shape (when the spectra are only recorded above the coalescence temperature) vitiate the extraction of these parameters from the present experiments.

The ^{13}C spectra of a series of indoles,[8] indazoles, and pyrazoles[9] were also recorded to see if the line broadening would be evidenced in other compounds. The details of this study will be presented separately, but for indole, indazole, and five derivatives of indazole the ^{13}C spectra did not exhibit any significant line broadening under the present experimental conditions. On the other hand, pyrazole and 3-methyl-

(1) Portions of this work were presented at the 13th ENC, May 1972.
(2) (a) A. J. Jones, D. M. Grant, M. W. Winkley, and R. K. Robins, *J. Phys. Chem.*, **74**, 2684 (1970); (b) A. J. Jones, M. W. Winkley, D. M. Grant, and R. K. Robins, *Proc. Nat. Acad. Sci. U. S.*, **65**, 27 (1970); (c) D. E. Dorman and J. D. Roberts, *ibid.*, **65**, 19 (1970); (d) A. J. Jones, D. M. Grant, M. W. Winkley, and R. K. Robins, *J. Amer. Chem. Soc.*, **92**, 4079 (1970); (e) H. H. Mantsch and I. C. P. Smith, *Biochem. Biophys. Res. Commun.*, **46**, 808 (1972); (f) A. R. Tarpley, Jr., and J. H. Goldstein, *J. Amer. Chem. Soc.*, **93**, 3573 (1971).
(3) Dr. L. F. Johnson of Varian Associates has also recorded the spectrum of formycin B and observed similar line broadenings. Dr. D. Grant and coworkers have informed me that they have also observed the line broadenings in formycin A and formycin B.
(4) G. Koyama, K. Maeda, and H. Umezawa, *Tetrahedron Lett.*, **6**, 597 (1966).
(5) D. C. Ward, A. Cerami, E. Reich, G. Acs, and L. Altwerger, *J. Biol. Chem.*, **244**, 3243 (1969).
(6) J. A. Montgomery and H. J. Thomas, *J. Med. Chem.*, **15**, 305 (1972).
(7) J. A. Montgomery, H. J. Thomas, and S. J. Clayton, *J. Heterocycl. Chem.*, 7, 215 (1970).
(8) R. G. Parker and J. D. Roberts, *J. Org. Chem.*, **35**, 996 (1970).
(9) R. F. M. White and H. Williams, *Phys. Methods Heterocycl. Chem.*, **4**, 121 (1971), and references therein.

Table I. ^{13}C Chemical Shifts[a] and Assignments

	C2	C4	C5	C6	C8	C9	C1′	C2′	C3′	C4′	C5′
Formycin A	151.6	138.7	123.0	151.6		143.7	78.3	75.5	72.7	86.3	62.8
Adenosine	152.5	149.1	119.4	156.2	140.1		88.1	73.6	70.8	86.0	61.8
8-Azaadenosine	156.1	148.8	124.1	156.7			89.7	72.9	70.7	86.2	61.8
Formycin B	143.3	136.7	128.0	153.6		144.7	77.7	74.9	72.1	85.7	62.5
Inosine[c]	148.2	146.0	124.5	156.7	138.9		87.9	74.3	70.4	85.8	61.4
4-Mercapto-1*H*-pyrazolo-[3,4-*d*]pyrimidine[b]	146.3 (C6)	148.8 (C8)	116.8 (C9)	179.4 (C4)	C7 = 136.4 (C3)						
6-Azauridine	148.7	156.8	136.6				89.7	72.6	70.6	84.9	62.3
Uridine	151.2	163.8	102.2	141.6			88.3	74.0	70.3	85.2	61.3
6-Azathymine	149.7	157.5	142.7		CH_3 = 15.9						
Thymine	151.6	165.0	107.8	137.8	CH_3 = 11.9						
Thymidine	150.5	163.8	109.5	136.2	CH_3 = 12.3		83.9	39.5	70.5	87.3	61.4

[a] Values given are in ppm from TMS. DMSO-d_6 was used as solvent and as an internal reference, and the values were changed to a TMS scale using δ(TMS–DMSO-d_6) = 39.5 ppm. The formycin A and formycin B were a gift from Dr. H. Umezawa. The 8-azaadenosine was a gift from Dr. J. A. Montgomery. [b] The assignments in parentheses correspond to the IUPAC numbering system. In the table the chemical shifts are listed under the corresponding adenine carbons for comparison. [c] These values are from ref 2a and were converted to a TMS scale using δ(TMS–benzene) = 127.6 ppm.

pyrazole showed broadened resonances that narrowed with increasing temperature. The lines narrowed with the addition of H_2O or D_2O and became very narrow with the addition of small amounts of acid or base. These data are consistent with the observation that the broadening results from prototropic tautomerization. With these compounds a careful study of the concentration and solvent dependence of the line widths must be completed to determine the effect of self association[9] and interaction with the solvent.[9,10] The only other compound studied that exhibited the pronounced line broadening was 4-mercapto-1*H*-pyrazolo[3,4-*d*]pyrimidine. This compound is a better model for the formycin ring system than the indazoles and like the formycins it has two sites where tautomeric equilibria may be important.

The ^{13}C spectra of 8-azaadenosine, 6-azathymine, and 6-azauridine all exhibited narrow resonances. The chemical shifts of these compounds (Table I) further illustrate the large perturbations of the electronic structure of the ring system when a nitrogen atom is introduced, as has been noted in other compounds.[11] This perturbation of the electronic structure of the nucleosides may have a significant effect on the presence of the rare tautomeric forms of the nucleosides. Theoretical calculations (G. P. Ceasar and J. Greene, personal communication) have shown that the change in the ring structure in going from adenine to formycin A can have significant effects on the relative stabilities of the amino and imino tautomers.[12] The present experiments provide convincing evidence that prototropic tautomerization is an important consideration in the nucleoside antibiotics formycin A and formycin B.

Acknowledgments. The author wishes to acknowledge Dr. Thomas C. Farrar of JEOL, Inc., and Dr. George Gray of Varian Associates for the use of their carbon-13 spectrometers and for their kind hospitality during my visits. This research was partially supported by the National Science Foundation (GU-1154) and the American Cancer Society (IN-18M).

Thomas R. Krugh
Department of Chemistry, University of Rochester
Rochester, New York 14627
Received June 29, 1972

(10) M. L. Roumestant, P. Viallefont, J. Elguero, and R. Jacquier, *Tetrahedron Lett.*, 6, 495 (1969).
(11) R. J. Pugmire, M. J. Robins, D. M. Grant, and R. K. Robins, *J. Amer. Chem. Soc.*, 93, 1887 (1971).
(12) B. Pullman and A. Pullman, *Advan. Heterocycl. Chem.*, 13, 77 (1971).

Base and Acid Catalyzed Protonation of the Acrylate Radical Dianion at the β Position. Spectral and Kinetic Evidence

Sir:

We report the results of a spectral and kinetic study of the reaction of the hydrated electron, e_{aq}^-, with acrylic acid and acrylate ion and of the subsequent protonation reactions of these radical anions. Data presented below show that in neutral aqueous solution the acrylate radical dianion undergoes protonation on the carboxyl group. In alkaline solution, the dianion radical undergoes protonation at the β carbon. *The latter protonation reaction is catalyzed by hydroxide ions.* The radical anions of acrylamide and methacrylamide were found[1] to undergo protonation at the carboxamide group in weakly acidic solution and at the β-carbon atom in alkaline solution. However, the latter protonation reaction was *not* catalyzed by OH^- ions.

Details of the pulse radiolytic experimental conditions used are described elsewhere.[2] Acrylic acid (Matheson Coleman and Bell or Eastman) was purified by recrystallizing from the melt, vacuum distilled under nitrogen, and recrystallized from the melt a second time. $G(e_{aq}^-)$ = 2.8 was used to derive extinction coefficients.

The specific rates of reaction[3] of e_{aq}^- with acrylic acid and acrylate ion are respectively (2.2 ± 0.1) ×

(1) E. Hayon, N. N. Lichtin, and V. Madhavan, Abstracts of Papers for the 21st Annual Meeting of the Radiation Research Society, St. Louis, Mo., May 1973, p 83.
(2) M. Simic, P. Neta, and E. Hayon, *J. Phys. Chem.*, 73, 3794 (1969); J. P. Keene, E. D. Black, and E. Hayon, *Rev. Sci. Instrum.*, 40, 1199 (1969).
(3) For an earlier pulse radiolysis study of aqueous acrylic acid, see K. W. Chambers, E. Collinson, F. S. Dainton, W. A. Seddon, and F. Wilkinson, *Trans. Faraday Soc.*, 63, 1699 (1967).