# Nitrogen-15 Magnetic Resonance Spectroscopy. VI. Pyrimidine Derivatives[1,2]

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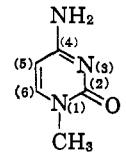
*Proton and nitrogen-15 magnetic resonance spectra of several derivatives of pyrimidine have been studied. Long-range $^{15}N$–H couplings were observed in uracil-$^{15}N_2$, 2,4-dichloropyrimidine-$^{15}N_2$, 2,4-dimethoxypyrimidine-$^{15}N_2$, 1-methyl-4-methoxy-2-pyrimidone-$^{15}N_2$, and 1-methylcytosine-$^{15}N_3$. A particularly large $^{15}N_{(1)}$–C–H coupling (12.5 c.p.s.) has been observed in 2,4-dichloropyrimidine-$^{15}N_2$. 1-Methylcytosine-$^{15}N_3$ has been shown to protonate at $N_{(3)}$ in agreement with previously reported work on 1-methylcytosine labeled only in the amino group.*

## Introduction

Our previous investigations of nitrogen-15 magnetic resonance spectroscopy[1] have prompted a study of possible applications to the structural determination of nitrogen-containing natural products. Purine and pyrimidine nucleosides were particularly attractive subjects for such an investigation, since the previously reported empirical correlations between hybridization of nitrogen and nitrogen-15 chemical shifts[1a] and coupling constants[1b] might be expected to help in the identification of tautomeric structures and sites of protonation or hydrogen bonding. The initial effort has been directed toward the cytidine system as represented by the nucleoside analog, 1-methylcytosine (I). Recently, Miles, Bradley,



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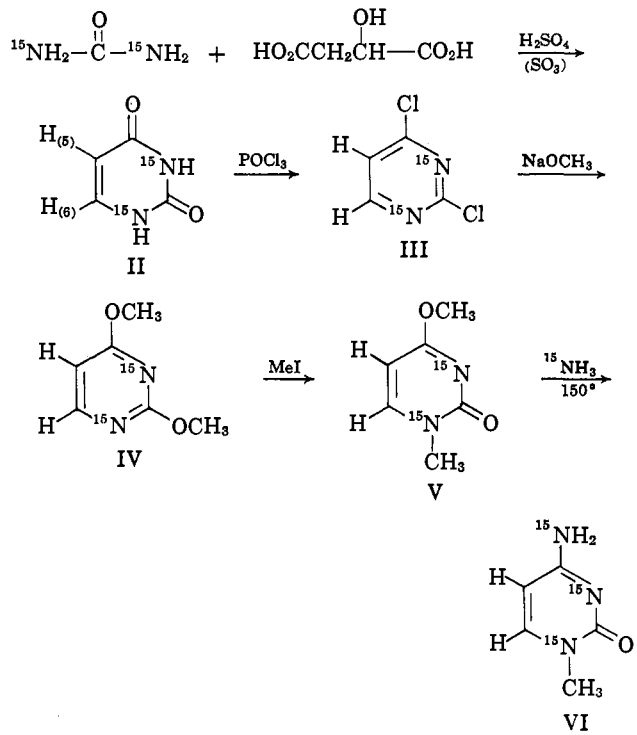
and Becker[5] reported a proton magnetic resonance study of the same system labeled with nitrogen-15 in the amino group. Our results, in agreement with theirs, further demonstrate the value of nitrogen-15 in magnetic resonance studies of the structures of nitrogen-containing tautomeric systems.

## Results and Discussion

The predominant tautomeric form of the cytosine system, although the object of several ultraviolet, infrared, and nuclear magnetic resonance studies,[5] has until recently been a subject of controversy. The use of proton magnetic resonance, as an aid to the solution of problems such as this, is often complicated by rapid proton exchange and by quadrupole broadening associated with nitrogen-14. Proton exchange in addition usually precludes the use of an aqueous medium. Direct observation of N–H resonances must therefore be obtained in nonaqueous solvents, which, of course, might well lead to conclusions which are not correct for aqueous solutions. Rapid exchange of protons in aqueous solution complicates but does not necessarily preclude the use of $^{15}N$ chemical shifts to determine the structures. In hope of using $^{15}N$ shifts for this purpose we have synthesized several $^{15}N$-labeled pyrimidine derivatives leading up to 1-methylcytosine. The solubility of some of the compounds prevented study of $^{15}N$ spectra and additional information was obtained from the proton spectra which were free of the quadrupole line broadening effects often associated with nitrogen-14-containing substances.

Our synthetic objective, 1-methylcytosine-$^{15}N_3$ (VI), was prepared by the following route. All compounds



had been reported previously and were identified by comparison with unlabeled samples. The labeled urea used as starting material was prepared[1b] from ammonium-$^{15}N$ chloride containing >95% $^{15}N$, and the ammonia used in the conversion of V to VI contained approximately 70% $^{15}N$.

The proton magnetic resonance spectrum of uracil-$^{15}N_2$ (II) was difficult to observe because of the low solubility

(1) For previous papers in this series, see: (a) J. B. Lambert, G. Binsch, and J. D. Roberts, *Proc. Natl. Acad. Sci. U. S.*, **51**, 735 (1964); (b) G. Binsch, J. B. Lambert, B. W. Roberts, and J. D. Roberts, *J. Am. Chem. Soc.*, **86**, 5564 (1964).

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(3) National Academy of Sciences–National Research Council Postdoctoral Fellow, 1963–1964.

(4) National Science Foundation Predoctoral Fellow, 1962–1965.

(5) H. T. Miles, R. B. Bradley, and E. D. Becker, *Science*, **142**, 1569 (1963), and references therein.

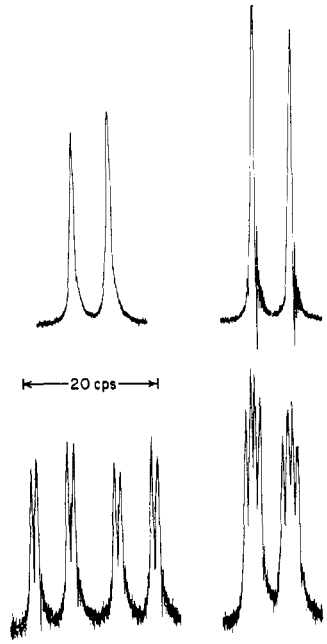
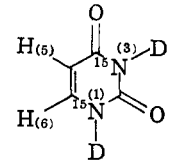



Figure 1. The 60-Mc.p.s. proton spectrum of 2,4-dichloropyrimidine-$^{14}N_2$ (top) and -$^{15}N_2$ (bottom). The splitting patterns are described in the text. The field increases from left to right.

of the material in most solvents. However, an adequate spectrum was obtained from a supersaturated solution of the compound in dimethyl sulfoxide. All resonance peaks were greatly complicated by extensive, long-range $^{15}N$–H couplings. Although this effect extends the possibility of structure correlation, it can be a nuisance when the spin–spin coupling patterns become too highly complicated. The two $^{15}N$-bonded protons gave two doublets centered at 10.78[6] and 10.96 p.p.m., each of which was perturbed by further couplings. The $^{15}N$–H coupling constants, 91 and 97 c.p.s., agree well with values expected of protons directly bonded to an $sp^2$-hybridized nitrogen atom.[7] The spectrum confirmed the diketo structure usually ascribed to uracil. The high-field $^{15}N$–H doublet, which was further split by 5.8 c.p.s., was assigned to the proton bonded to $^{15}N_{(1)}$. This same splitting appeared in the absorption of $H_{(6)}$ and corresponds closely to the coupling of *ortho* protons in a six-membered ring containing $sp^2$-hybridized atoms.[8] The low-field doublet, assigned to the proton bonded to $^{15}N_{(3)}$, was further split by a 1.8-c.p.s. coupling, but the other coupling nucleus was not identified. The protons $H_{(5)}$ and $H_{(6)}$, respectively, exhibited a broad resonance centered at 5.50 p.p.m. and a set of four doublets centered at 7.38 p.p.m. Allowance for $J_{H_{(5)}H_{(6)}}$ (determined from the spectrum of the unlabeled material to be 7.8 c.p.s.) and $J_{H_{(6)},H_{(1)}}$ (see above, 5.8 c.p.s.) in the four doublets produced by $H_{(6)}$ left a splitting of 3.5 c.p.s. which may reasonably be assigned to $J_{H_{(6)},^{15}N_{(1)}}$. In order to study the absorption of $H_{(5)}$, the spectrum of uracil-$^{15}N_2$,$d_2$, (VII), generated by exchange with deuterium oxide, was examined. The absorption of $H_{(6)}$ was reduced to the expected pair of doublets and

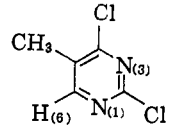


VII

that of $H_{(5)}$ to a set of four doublets. Allowance for $J_{H_{(5)}H_{(6)}}$ from the resonances from $H_{(5)}$ now left two $H_{(5)}$–$^{15}N$ splittings of 4.4 and 2.5 c.p.s. Although the coupling of $H_{(5)}$ with specific nitrogen nuclei could not be determined, it is evident that $H_{(5)}$ couples significantly with at least four and possibly five of the other magnetically active nuclei in the uracil-$^{15}N_3$ molecule.

Because of the low solubility of uracil in all solvents and the relatively low sensitivity of $^{15}N$ with our present spectrometer, the nitrogen-15 magnetic resonance spectrum of uracil-$^{15}N_2$ could not be recorded.

The proton magnetic resonance spectrum of unlabeled 2,4-dichloropyrimidine (Figure 1) in carbon disulfide consists of the two doublets expected of an AX system at 7.48 and 8.62 p.p.m. with $J_{H_{(5)},H_{(6)}} = 5.6$ c.p.s. In the corresponding spectrum of the nitrogen-15-labeled material (Figure 1), the two doublets were replaced by two sets of four doublets centered at 7.47 and 8.60 p.p.m. After allowance for the known $J_{H_{(5)},H_{(6)}}$, it was found that each proton was significantly coupled with both nitrogen-15 nuclei, the low-field proton by 12.5 and 0.9 c.p.s. and the high-field one by 1.4 and 0.8 c.p.s. Examination of the proton spectrum of 2,4-dichloro-5-methylpyrimidine (VIII, $H_{(6)}$, 8.60 p.p.m.) confirmed that the low-field resonance corresponds to $H_{(6)}$ and the high-field resonance to $H_{(5)}$. Although the



VIII

coupling constants could not be definitely assigned to specific nuclei, it is reasonable to assume that the large coupling of 12.5 c.p.s. takes place between $H_{(6)}$ and $^{15}N_{(1)}$. Large $^1H$–C–$^{15}N$ couplings have been observed previously between the aldehydic proton and nitrogen-15 in formamide-$^{15}N$ (19.0 c.p.s.)[9] and N,N-dimethylformamide-$^{15}N$ (15.6 c.p.s.).[10] However, the H–C–N bonding in amide and pyrimidine systems are electronically rather dissimilar but probably more alike than in benzalmethylamine, for which a $^1H$—C=$^{15}N$ coupling is reported[1b] as 3.9 c.p.s.[11] The large coupling here may well result from noncontact contributions, and it is worth noting in this connection that the average chemical shift of the two nitrogen-15 atoms in 2,4-dichloropyrimidine is 290 p.p.m. below external anhydrous ammonia.

The proton nuclear magnetic resonance spectrum of 2,4-dimethoxypyrimidine-$^{15}N_2$ (IV) was similar to that

(6) All chemical shifts are measured in parts per million (p.p.m.) downfield from internal TMS.

(7) For a discussion of this particular case in terms of the mechanism of coupling, see J. B. Lambert, Ph.D. Thesis, California Institute of Technology, 1965, pp. 95–96.

(8) L. M. Jackman, "Applications of Nuclear Magnetic Resonance Spectroscopy in Organic Chemistry," Pergamon Press Inc., New York, N. Y., 1959, p. 85.

(9) B. Sunners, L. H. Piette, and W. G. Schneider, *Can. J. Chem.*, 38, 681 (1960).

(10) A. J. R. Bourn and E. W. Randall, *J. Mol. Spectry.*, 13, 29 (1964).

(11) $^{15}N$–C–H coupling through an $sp^3$-hybridized carbon atom is characteristically small and usually does not exceed 1.5 c.p.s.[1b]

of the 2,4-dichloro compound (III) discussed above. The coupling constants were found to be $J_{H_{(6)},^{15}N_{(1)}}$ = 12.2 c.p.s., $J_{H_{(6)},^{15}N_{(3)}}$ = 0.6 c.p.s., $J_{H_{(5)},H_{(6)}}$ = 5.9 c.p.s., and $J_{H_{(5)},^{15}N_{(1)}}$, $J_{H_{(5)},^{15}N_{(3)}}$ = 1.4, 0.6 c.p.s. (indeterminate). The nitrogen-15 spectrum was not studied.

The proton spectrum of 1-methyl-4-methoxy-2-pyrimidone-$^{15}N_2$ (V) in acetic acid exhibited a doublet at 3.56 p.p.m. assigned to the $^{15}N$–$CH_3$ group ($J_{^{15}N_{(1)}CH_3}$ = 1.2 c.p.s.), a singlet at 3.92 p.p.m. assigned to the O-methyl group, a pair of doublets centered at 6.09 p.p.m. assigned to $H_{(5)}$ ($J_{H_{(5)},H_{(6)}}$ = 7.3 c.p.s., $J_{H_{(5)},^{15}N_{(1)}, \text{ or } ^{15}N_{(3)}}$ = 3.9 c.p.s., indeterminate), and a pair of doublets centered at 7.96 p.p.m. assigned to $H_{(6)}$ ($J_{H_{(6)},H_{(6)}}$ = 7.3 c.p.s., $J_{H_{(6)},^{15}N_{(1)}}$ = 1.8 c.p.s.). The assignments are based on the assumption that the relative positions of $H_{(5)}$ and $H_{(6)}$ are the same as found in uracil, and must be regarded as tentative. The nitrogen-15 spectrum consisted of a sharp peak 225 p.p.m. below external anhydrous ammonia-$^{15}N$ and a much broader resonance at 148 p.p.m. If the assumption is made that long-range coupling will be more extensive for $^{15}N_{(1)}$ than for $^{15}N_{(3)}$, the high-field absorption may be assigned to $^{15}N_{(1)}$ and that at low field to $^{15}N_{(3)}$.

Finally, the site of protonation of 1-methylcytosine-$^{15}N_3$ was studied. The proton spectrum of the free base, saturated in dimethyl sulfoxide, was rather obscure because of extensive couplings. The spectrum consisted of two multiplets centered at 5.76 and 7.86 p.p.m. These peaks have previously been assigned to $H_{(5)}$ and $H_{(6)}$, respectively.[5] The low-field multiplet appeared to be a pair of doublets with couplings of 8.1 and 2.9 c.p.s. However, the presence of a multitude of broader, less intense peaks prevented any meaningful analysis. The high-field peak was even more difficult to interpret. Although five very broad peaks could be discerned, no recognizable pattern could be picked out. The expected doublet for the amino protons either overlapped with the absorption of the ring protons or was so complicated by long-range coupling as to be indistinguishable from noise.

The proton spectrum of the hydrochloride of 1-methylcytosine-$^{15}N_3$ in dimethyl sulfoxide was much more susceptible to analysis. The amino hydrogens appeared as two doublets. Although one-half of one doublet overlapped the absorption of $H_{(1)}$, the peaks arising from the 30% of nitrogen-14 present served to position each doublet. The $^{15}N$–H coupling constant of the observable doublet was 94.2 c.p.s. It was of interest that the observed doublet was further split by a 4.4-c.p.s. coupling while the observed half of the other doublet was not noticeably split. It thus appears that one amino proton preferentially couples with another nucleus. Since the published spectrum[5] of 1-methylcytosine hydrochloride labeled only in the amino group does not show this effect, the other coupling nucleus must be one of the ring $^{15}N$'s, probably $^{15}N_{(3)}$. The absorption of $H_{(5)}$ was again very complex and could not be analyzed. The absorption of $H_{(6)}$ appeared as a broad doublet ($J$ = 5.7 c.p.s.) which overlapped one-half of one amino proton doublet. The acidic proton could not be observed because of rapid exchange.

The proton spectrum of the labeled hydrochloride in liquid sulfur dioxide was observed at low temperature in order to slow down exchange of the acidic proton. Below −30°, a clean doublet, absent from the spectrum taken in dimethyl sulfoxide at room temperature, was present at 12.52 p.p.m. The 94.0-c.p.s. $^{15}N$–H coupling constant strongly supports attachment of the acidic proton to a trigonally hybridized nitrogen-15 nucleus.[1b] The results are thus compatible with protonation at $^{15}N_{(3)}$. The remainder of the spectrum was similar to that taken in dimethyl sulfoxide solution at room temperature except for slight changes in chemical shifts. When the sulfur dioxide solution was raised to room temperature, the doublet arising from the acidic proton disappeared. Lowering the temperature to −58° caused no significant change in the spectrum taken at −30°.

Because of the limited solubility of 1-methylcytosine and the hydrochloride in aprotic solvents, the nitrogen-15 spectra of these substances were not recorded.

## Experimental Section

Proton spectra were determined on the Varian A-60 spectrometer. Nitrogen-15 spectra were obtained on a Varian Model V-4300B spectrometer operated at 6.08 Mc.p.s. and 14,100 gauss.[1a] Low-temperature spectra were obtained on the Varian A-60 spectrometer fitted with a low-temperature probe.[12]

*Urea-$^{15}N_2$* was prepared according to the procedure of Leitch and Davidson[13] from ammonium-$^{15}N$ chloride (96.0% $^{15}N$; Merck Sharp and Dohme of Canada) and diphenyl carbonate.

*Uracil-$^{15}N_2$* was prepared by the condensation of urea-$^{15}N_2$ with malic acid in fuming sulfuric acid according to the procedure of Davidson and Baudisch.[14]

*2,4-Dichloropyrimidine-$^{15}N_2$* was prepared from uracil-$^{15}N_2$ and phosphorus oxychloride by the procedure of Hilbert and Johnson.[15]

*2,4-Dimethoxypyrimidine-$^{15}N_2$* was prepared by treatment of 2,4-dichloropyrimidine-$^{15}N_2$ with methanolic sodium methoxide according to the procedure of Hilbert and Johnson.[16]

*1-Methyl-4-methoxy-2-pyrimidone-$^{15}N_2$* was prepared by rearrangement of 2,4-dimethoxypyrimidine-$^{15}N_2$ with methyl iodide according to the procedure of Hilbert and Johnson.[16]

*1-Methylcytosine-$^{15}N_3$* was prepared from 1-methyl-4-methoxy-2-pyrimidone-$^{15}N_2$ and ammonia-$^{15}N$ (generated from ammonium-$^{15}N$ chloride containing approximately 70% $^{15}N$) according to the procedure of Miles, Bradley, and Becker.[5]

*The hydrochloride of 1-methylcytosine-$^{15}N_3$* was prepared by dissolving the free base in excess aqueous hydrochloric acid and evaporating the solution to dryness.

(12) We wish to thank Dr. S. L. Manatt of the Jet Propulsion Laboratory, Pasadena, Calif., for kindly extending us the use of this instrument.
(13) L. C. Leitch and W. M. Davidson, *Sci. Agr.*, **29**, 189 (1949).
(14) D. Davidson and O. Baudisch, *J. Am. Chem. Soc.*, **48**, 2379 (1926).
(15) G. E. Hilbert and T. B. Johnson, *ibid.*, **52**, 1152 (1930).
(16) G. E. Hilbert and T. B. Johnson, *ibid.*, **52**, 2001 (1930).