Nuclear Magnetic Resonance Studies of Methyl Derivatives of Cytosine
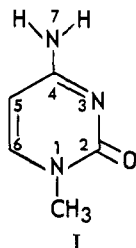
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Proton nuclear magnetic resonance spectra are reported for 1-methylcytosine, its hydrochloride and hydroiodide, and several dimethylcytosine salts. Unequivocal assignments of n.m.r. lines have been made by use of ^{15}N substitution and spin decoupling. Hindered rotation of the amino group has been found in the hydrohalide salts, and in the methylamino derivative this gives rise to geometric isomers. Thermodynamic data for interconversion of the isomers were determined from the n.m.r. spectra, and estimates of the rates of rotation of the amino and methylamino groups are given. Chemical shifts and spin coupling constants are tabulated and discussed, particularly with respect to dependence on salt formation, on the nature of the anion, on the nature of the solvent (SO_2 and dimethyl sulfoxide), and on temperature.

Proton nuclear magnetic resonance (n.m.r.) has been applied in several instances to the structural investigation of cytosine and its nucleosides,[1-6] the work being stimulated in part by the relevance of these substances to nucleic acid structure and function. We present here the results of a detailed n.m.r. study of 1-methylcytosine and a number of its derivatives. In a preliminary report of this work[7] we have shown that the n.m.r. results proved that the only tautomeric form of 1-methylcytosine observed (in dimethyl sulfoxide and in sulfur dioxide solutions) is I, and that in

I

the presence of acid the molecule is protonated at N_3.[8]

In this paper further details of the experiments leading to this conclusion are given, and in addition data are presented on the hindered rotation of the amino group in the protonated molecules, on the presence of stereoisomers in 4-NCH_3 derivatives, on the magnitudes of H-H and ^{15}N-H coupling constants, on the effect of the nature of the anion on the chemical shift of the NH protons, and on the effects of protonation and methyl substitution on the chemical shifts.

Experimental Section

Spectra were obtained with a Varian A-60 spectrometer and 12-in. magnet system, using both a V-6030 room temperature probe (sample temperature 23 ± 1°) and a V-6031 variable temperature probe. The V-6040 variable temperature controller was calibrated to ±2° by using the known chemical shifts of methanol and ethylene glycol.[9] Spectra were scanned routinely at 1 c.p.s./sec. and in certain cases portions of the spectra were scanned at 0.2 c.p.s./sec. The spectrometer frequency calibration was found to be slightly temperature dependent and was calibrated at each temperature by means of audio side bands. Frequencies of sharp peaks are accurate to ±1 c.p.s. (*ca.* 0.02 p.p.m.). Spectral resolution was usually about 0.3–0.4 c.p.s. For the determination of isomer ratios in compound X (see later discussion) integrals were obtained at 1 c.p.s./sec. The values reported are the average of five determinations.

Spin-decoupling measurements were conducted with a Varian HR-60 spectrometer equipped with a Space Avionics single side-band proton–proton decoupler and a V-4331 A variable temperature probe. The sample temperature was measured to ±0.2° with a copper-constantan thermocouple. Spectra were scanned at approximately 0.5 c.p.s./sec. Audio signals used for proton decoupling were measured to ±1 c.p.s.

The hydrochlorides were prepared by passing dry hydrogen chloride into methanolic solutions of the free bases, precipitating the salts, and recrystallizing twice from methanol.

(1) C. D. Jardetzky and O. Jardetzky, *J. Am. Chem. Soc.*, **82**, 222 (1960).
(2) J. P. Kokko, J. H. Goldstein, and L. Mandell, *ibid.*, **83**, 2909 (1961).
(3) L. Gatlin and J. C. Davis, Jr., *ibid.*, **84**, 4464 (1962).
(4) H. T. Miles, *ibid.*, **85**, 1007 (1963).
(5) A. R. Katritzky and A. J. Waring, *J. Chem. Soc.*, 3046 (1963).
(6) O. Jardetzky, P. Pappas, and N. G. Wade, *J. Am. Chem. Soc.*, **85**, 1657 (1963).
(7) H. T. Miles, R. B. Bradley, and E. D. Becker, *Science*, **142**, 1569 (1963).
(8) We have assigned the number 7 to the exocyclic nitrogen in order to avoid unnecessarily cumbersome nomenclature for the methylated derivatives. This convention also permits a clear and succinct designation of the protons attached to this nitrogen, and when necessary, of the isotopic species.
(9) Varian Associates' calibration curve supplied with V-6040 controller.

Table I. Chemical Shifts and Spin–Spin Coupling Constant for Cytosine Derivatives in SO₂ at −60°

Compound	No.	Chemical shifts, p.p.m.							Coupling constants, c.p.s.						
		1-CH₃	3-CH₃	7-CH₃	5-H	6-H	3-H	7-H	$J_{^{15}N-H}$	J_{5H-6H}	J_{3H-6H}	J_{6H-7H}	$J_{^{15}N-CH_2}$	$J_{^{15}N-5H}$	J_{7H-CH_2}
1-Methylcytosine hydrochloride[a]	III	3.55			6.28	7.82	11.44	7.45 7.83		7	2.5				
1-Methylcytosine-7-¹⁵N hydrochloride[b]	IV	3.55			6.27	7.82	11.42	7.42 7.82	93.8 96.8	7.5	2.5			0.7	
1-Methylcytosine hydroiodide	V	3.58			6.33	7.87	11.02	7.28 7.45		7.5	2.1				
1,3-Dimethylcytosine hydrochloride[c]	VII	3.58	3.65		6.42	7.80		7.25 8.00		7.5					
1,3-Dimethylcytosine hydroiodide	VIII	3.58	3.67		6.37	7.82		7.00 7.82		7.5					
1,7-Dimethylcytosine hydrochloride[d]	X A	3.54		3.23	6.38	7.70	11.09	8.65		7.8	2.6				
	X B	3.52		3.18	6.52	7.90	11.54	8.03		7.8	2.3	0.7			5.0
1,7-Dimethylcytosine-7-¹⁵N hydrochloride[e]	XI A	3.54		3.25	6.35	7.68	11.00	8.60		7.5					5
	XI B	3.52		3.18	6.25	7.88	11.50	8.03	93.5	7.5	2			1.5	5
1,7-Dimethylcytosine hydroiodide	XII	3.58		3.33	6.35	7.77	11.50	7.50		8.0	2.6				
		3.58		3.25	6.35	7.97	10.92	7.23		8					
1:1 Mixture of III and V		3.58			6.32	7.85	11.22	7.35 7.65							

[a] 1-Methylcytosine was prepared by the method of E. H. Flynn, J. W. Hinnan, E. L. Caron, and D. O. Woolf, Jr., J. Am. Chem. Soc., 75, 5867 (1953); cf. footnote c. The hydrochloride and hydroiodide salts of the bases were prepared as indicated in the Experimental Section. [b] Reference 7. [c] G. E. Hilbert, J. Am. Chem. Soc., 56, 190 (1934). [d] G. W. Kenner, C. B. Reese, and A. R. Todd, J. Chem. Soc., 855 (1955). [e] See Experimental Section.

The hydroiodides were prepared by dissolving the free base in aqueous hydroiodic acid, evaporating to dryness under reduced pressure, and recrystallizing the salt several times from methanol.

1,7-Dimethylcytosine-7-¹⁵N (IX) was prepared by distilling 250 mg. of methylamine-¹⁵N (>95% ¹⁵N; Merck of Canada, Ltd.) at 10⁻⁵ mm. into a tube containing 5 ml. of methanol and an equivalent amount (1.08 g.) of 1-methyl-4-methoxypyrimidone-2. The tube was sealed and heated at 100° for 8 hr. The product (900 mg.) was recrystallized from methanol to m.p. 181–182°, undepressed by mixture with an authentic sample of the ¹⁴N compound. This preparation incidentally demonstrates that no excess of amine is required in the reaction.

Solutions in dimethyl sulfoxide-d₆ (DMSO) (New England Nuclear, 99% deuterated, <0.05% water) were prepared with precautions to avoid absorption of water from the atmosphere, transferred to capped n.m.r. samples tubes, and were not degassed. Sulfur dioxide solutions were prepared by distilling SO₂ (Matheson) in vacuo into chilled sample tubes containing weighed amounts of the compounds and a drop of tetramethylsilane, and sealing the tubes under vacuum. The spectrum of DMSO showed only a weak quintet at 2.55 p.p.m. due to the undeuterated material and a weak, sharp peak at ca. 3.38 p.p.m. due to water, while the spectrum of SO₂ showed no n.m.r. lines. Sample concentration ranged from 0.3 to 1 M.

No difficulties were encountered in solubility or sample preparation except for 1,7-dimethylcytosine hydrochloride. Although this compound dissolves when DMSO is first added, crystals form after a few minutes, followed by separation of most of the solute. It is possible that these crystals are composed of the less soluble of the two geometrical isomers of the cation. In order to obtain the solution spectra reported, it was usually necessary to heat the sample tube to redissolve the crystals which had separated and cool it to room temperature before measurement. Solution of this compound in SO₂ presented no difficulties.

Spectra of hydroiodides were found to change slowly with time. The reported spectra were measured on fresh solutions.

Chemical shifts are reported in p.p.m. relative to tetramethylsilane as an internal reference.

Spectra and Assignment of N.m.r. Lines

Spectra of selected cytosine derivatives are shown in Figures 1–3. Table I summarizes the chemical shift and spin-coupling results (from first-order analysis, which is entirely adequate) for all compounds studied in liquid SO₂ at −60° and Table II summarizes the results for those compounds studied in DMSO at room temperature (23°). A brief description of salient features of the spectra and the justification of the peak assignments given in the tables follows.

1-Methylcytosine and 1-Methylcytosine-7-¹⁵N. Figures 1a and b show the low-field portions of the spectra of these compounds in DMSO. The doublets at 5.67 and 7.62 p.p.m. are clearly due to 5-H and 6-H, coupled by about 7 c.p.s. (confirmed by proton spin decoupling). The literature assignment[1] of the higher-field doublet to 5-H and the lower-field doublet to 6-H was made on the basis of methyl substitution at the 5-position in the related compound uracil. This assignment is entirely reasonable, but firmer evidence is provided by the observation of 1-N, 6-H coupling in cytosine hydrochloride.[10] The data reported here for 5-methyldeoxycytidine (see below) also confirm this assignment.

The sharp line at 3.23 p.p.m. (Table II) is unquestionably assigned to the 1-methyl protons.

The broad line at 7.00 p.p.m., with an area appropriate to two protons, is split into a 90-c.p.s. doublet by ¹⁵N substitution in the amino group, thus unequivocally establishing the chemical shift of the amino

(10) J. P. Kokko, Ph.D. Dissertation, Emory University, 1964.
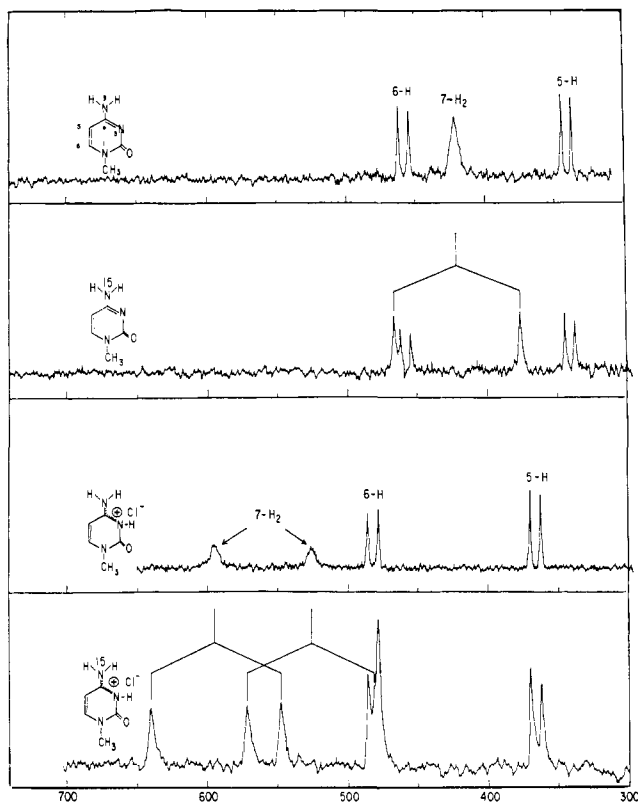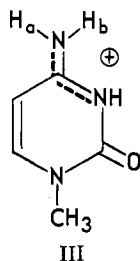
Figure 1. Spectra of 1-methylcytosine and its hydrochloride in DMSO at 23°. In this and the following figures the abscissa is in c.p.s. with respect to internal tetramethylsilane (designations a–d in order from top).

Figure 2. Spectra of 1-methylcytosine hydrochloride and hydro-iodide in SO_2 at $-60°$ (designations a–c in order from top).

protons and proving the amino form to be the correct tautomeric structure.

1-Methylcytosine Hydrochloride and 1-Methylcyto-sine-7-¹⁵N Hydrochloride. The low-field portions of the spectra of these compounds in DMSO at 23° are shown in Figures 1c and d, and in SO_2 at $-60°$ in Figures 2b and c. The features due to 5-H and 6-H are readily assigned. The NH protons give rise to three broad peaks in the ¹⁴N compound in SO_2 at $-60°$ (Figure 2b), each with an area due to one proton. The nonequivalence of the two amino protons results from hindered rotation about the C_4N_7 bond[5,7] owing to its partial double bond character.

$$H_a\diagdown N\diagup H_b$$

III

Temperature variation data and further discussion of the rotational barrier will be presented in a later section. In the ¹⁵N derivative (Figure 2c) the peak at 11.43 p.p.m. is unchanged, but those at 7.42 and 7.82 p.p.m. are each split into doublets of 92.6 and 91.4 c.p.s., respectively, thus permitting their unambiguous assignment to the amino protons. Assignment of the two peaks individually to H_a and H_b is less certain and is deferred to a later section. In DMSO solution only the two lines due to the amino protons (split by ¹⁵N)
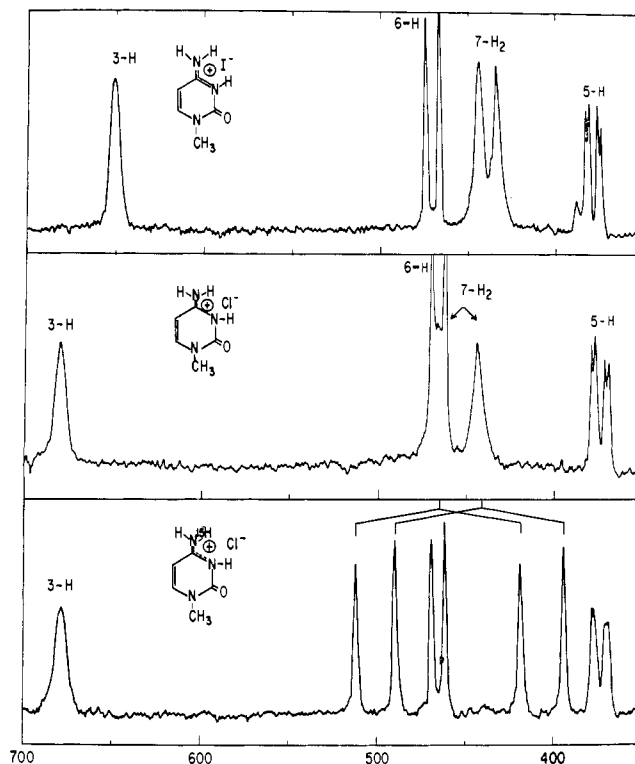
are found, the line due to 3-H being too broad for observation.

In the ¹⁴N compound in SO_2 at $-60°$ (Figure 2b) the line due to 5-H at 6.28 p.p.m. is split as expected by the 7-c.p.s. coupling to 6-H and is further split by 2.5 c.p.s. This latter splitting arises from coupling to the proton responsible for the peak at 11.43 p.p.m., as shown by proton spin decoupling results. Irradiation of the 11.43-p.p.m. peak with a strong decoupling field causes collapse of the 2.5 c.p.s. splitting, while not affecting the 7-c.p.s. 5-H and 6-H coupling. Optimum decoupling is observed at a side-band frequency of 311 c.p.s., compared with a chemical shift difference of 309 c.p.s. The ¹⁵N results permit assignment of the 11.43-p.p.m. peak to 3-H, rather than to one of the amino protons. As pointed out in our earlier note,[7] the magnitude of this coupling, 2.5 c.p.s., eliminates the possibility of protonation at the oxygen, rather than at N_3.

The doublet assigned to 5-H (6.18 p.p.m. in DMSO) is split further by 0.7 c.p.s. in the ¹⁵N analog but not the ¹⁴N compound. The splitting is evidently due to coupling to ¹⁵N and further supports the assignment of the higher-field doublet to 5-H rather than 6-H.

1-Methylcytosine Hydroiodide. As shown in Figure 2a and Table I, chemical shifts for carbon-bound protons in this molecule are quite close to, but not identical with, those of the analogous hydrochloride, and NH chemical shifts are altered substantially. The marked effect of the anion is consistent with the suggestion by Jardetzky, *et al.*,[6] that cytidine hydrochloride exists as ion pairs in appropriate solvents, such as DMSO. Further effects of the anion are noted later.

Even without ¹⁵N substitution the peaks in the hy-droiodide may be assigned readily by analogy to the

Table II. Chemical Shifts and Spin–Spin Coupling Constants for Cytosine Derivatives in DMSO-d_6 at 23°

| Compound | No. | Chemical shifts, p.p.m. | | | | | | | Coupling constants, c.p.s. | | | | |
		1-CH₃	3-CH₃	7-CH₃	5-H	6-H	3-H	7-H	J^{15}_{N-7H}	J_{5H-6H}	$J^{15}_{N-CH_3}$	J^{15}_{N-5H}	J_{7H-CH_3}
1-Methylcytosine[a]	I	3.23			5.67	7.62		7.00		7			
1-Methylcytosine-7-¹⁵N	II	3.23			5.67	7.63		7.02	90	7			
1-Methylcytosine hydrochloride	III	3.35			6.18	8.12	8.85	10.00		8			
1-Methylcytosine-7-¹⁵N hydrochloride	IV	3.35			6.18	8.10	8.84	9.95	92.6 91.4	7		0.7	
1,3-Dimethylcytosine	VI	3.20	3.18		5.70	7.02		7.35		7			
1,3-Dimethylcytosine hydroiodide	VIII	3.40	3.43		6.13	8.05		9.34		8			
1,7-Dimethylcytosine-7-¹⁵N	IX	3.27		2.80	5.75	7.62		7.50	94	7	1.2		4.8
1,7-Dimethylcytosine hydrochloride	X	3.40		3.05	6.40	8.32		9.54		7.7			
		3.37		3.02	6.35	8.02		11.02		7.7			5.0
1,7-Dimethylcytosine-7-¹⁵N hydrochloride	XI	3.42			6.40	8.34				7.5			
		3.38		3.02	6.33	8.02		11.00	94	7.7	1.5		5.0
1,7-Dimethylcytosine hydroiodide	XII	3.37		2.97	6.03	7.97	10.89	9.87		7.5			5.0
		3.37		2.97	6.33	8.25		8.39		7.5			
1,7-Dimethylcytosine-7-¹⁵N hydroiodide	XIII	3.40		3.01	6.10	8.02		9.94	94	7.5			
		3.40		3.01	6.38	8.30				7.5			
Deoxycytidine	XIV				5.80	7.85		7.32		7.5			
Deoxycytidine hydrochloride	XV				6.25	8.30	8.82	9.94		7.5			
5-Methyldeoxycytidine[b]	XVI					7.68	6.67	7.80					
5-Iododeoxycytidine[b]	XVII					8.31	6.91	7.37					

[a] Sources of compounds given in Table I. [b] Obtained from Calbiochem, Los Angeles, Calif.

hydrochloride. The only potentially ambiguous point involves the correlation of the amino peaks at 7.28 and 7.45 p.p.m. in the hydroiodide with those at 7.45 and 7.83 p.p.m. in the hydrochloride, since the lower-field peak in one case does not necessarily correspond to the lower-field peak in the other. To establish the correspondence and provide further support for the hypothesis of ion-pair formation we obtained the spectrum of an equimolar mixture of the hydrochloride and the hydroiodide in SO₂. With the expected rapid anion exchange we should then observe peaks half-way between those of either species alone. The peaks for 1-methyl, 5-H, 6-H, and 3-H were all at precisely the expected frequencies (Table I). The observed peaks of the amino group were at 7.35 and 7.65 p.p.m. On the basis of the correlation of low-field peaks in the hydrochloride and hydroiodide, the expected values are 7.36 and 7.64 p.p.m., whereas the alternative correlation would predict peaks at 7.45 and 7.55 p.p.m. Clearly the first alternative is correct.

1,3-Dimethylcytosine. Except for the 1- and 3-methyl lines, which are almost coincident, there is no difficulty in making the assignments given in Table II. The double bond at C₄N₇ should permit existence of stereoisomers in which the NH proton is *cis* or *trans* to the 3-position. The observation of only one NH line at room temperature indicates rapid rotation of the NH or predominance of one isomer. (See further discussion of rotational barriers below.) We were unable to obtain spectra of this compound in SO₂ at low temperature because of its low solubility, possibly the result of SO₂ adduct formation.

1,3-Dimethylcytosine Hydrochloride and Hydroiodide. The spectra in SO₂ at −60° are shown in Figure 4. The assignment of peaks to 5-H and 6-H is straight-forward, but there is some ambiguity in assignment of the 1-methyl and 3-methyl lines. The assignment in Tables I and II is made so that the peak assigned to 1-

methyl is consistent with the 1-methyl frequencies of the other salts. The NH₂ line positions again show a marked dependence on anion. We assume that the lower-field NH peak in the hydrochloride corresponds to the lower-field line in the hydroiodide, as was found for the compounds discussed in the last section. As would be expected from our previous assignment of the 2.5-c.p.s. splitting of the 5-H proton to a 3-H–5-H coupling, this splitting is not observed when the 3-H is replaced with a 3-CH₃.

1,7-Dimethylcytosine-7-¹⁵N. The assignments in Table II are readily made. The 7-methyl line is split into a 4.8-c.p.s. doublet by coupling with 7-H (confirmed by an equivalent splitting of the 7-H peaks into quartets) and is further split by about 1.2 c.p.s., presumably because of coupling of the ¹⁵N to the methyl protons. The 1.2-c.p.s. ¹⁵N–CH₃ coupling is in accord with the relatively small coupling constants observed with other N–CH₃ compounds.[11]

1,7-Dimethylcytosine Hydrochloride and Hydroiodide and 1,7-Dimethylcytosine-7-¹⁵N Hydrochloride. The spectra of these compounds (Figure 3) display considerably more lines than those of the previous molecules. The spectral complexity is due to the presence of stereoisomers in which the 7-CH₃ is either *cis* or *trans* to N₃.[5] The 6-H lines are clearly separated into two 7.5-c.p.s. doublets at about 7.70 and 7.90 p.p.m., the latter being more intense for the hydrochloride and less intense for the hydroiodide. A similar situation occurs in the 5-H region, except that the chemical-shift difference between the isomers is smaller, thereby causing overlapping peaks. This region is further complicated by an additional 2.5-c.p.s. splitting. Spin-decoupling measurements show that this splitting is due to coupling between 5-H and 3-H. The 3-H

(11) (a) E. Grunwald, A. Loewenstein, and S. Meiboom, *J. Chem. Phys.*, **27**, 641 (1957); (b) I. D. Kuntz, Jr., P. von R. Schleyer, and A. Allerhand, *ibid.*, **35**, 1533 (1961).
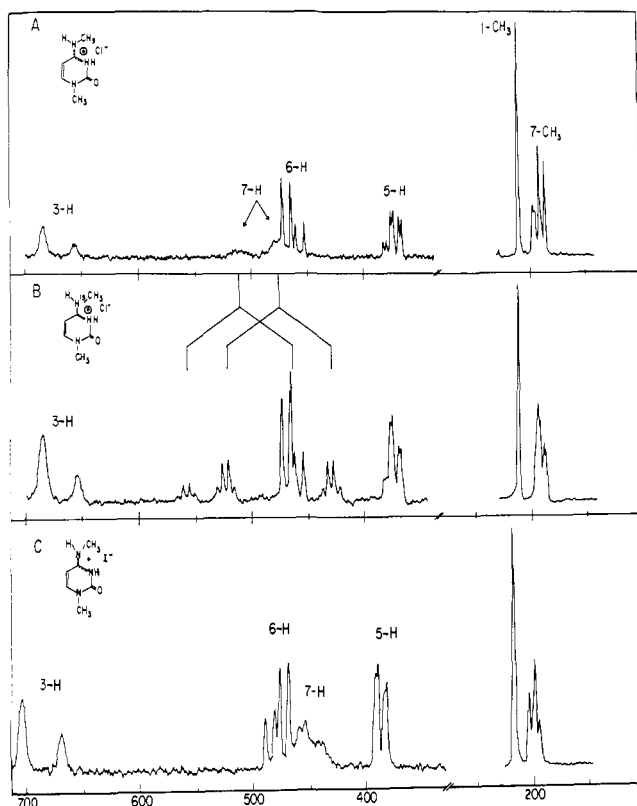
Figure 3. Spectra of 1,7-dimethylcytosine hydrochloride and hydroiodide in SO₂ at −60°.

Figure 4. Spectra of 1,3-dimethylcytosine hydrochloride and hydroiodide in SO₂.

proton gives rise to the peaks at 10.9 to 11.5 p.p.m. As in the case of 1-methylcytosine hydrochloride, substitution of ^{15}N at the 7-position permits unequivocal distinction between the lines due to 7-H and 3-H, the former at 8.03 and 8.60 p.p.m. being split by 93.5 c.p.s. as a result of coupling to ^{15}N. From the spectrum of the ^{15}N derivative it is clear also that 7-H couples to the 7-CH₃ protons with $J = 5.0$ c.p.s. In the ^{14}N analog the 7-CH₃ lines are readily identified by the 5.0-c.p.s. splitting and the coupling to 7-H is confirmed by spin decoupling. The more abundant geometric isomer is responsible for the 5-c.p.s. doublet at 3.18 p.p.m. and the less abundant for the 5-c.p.s. doublet at 3.23 p.p.m. In the ^{15}N analog the 7-CH₃ region shows not only the 5-c.p.s. splitting due to coupling of the CH₃ to 7-H but also an additional 1.5-c.p.s. splitting, which must be due to coupling of the CH₃ to $^{15}N_7$. The four lines thus produced for the more abundant isomer spread over a wide enough frequency range to obscure partially the lines of the less abundant isomer. The 1-CH₃ line at 3.52 p.p.m. is also doubled by the stereoisomerism; the less abundant isomer gives rise to a shoulder at 3.54 p.p.m., which is not visible in Figure 3A but is quite pronounced in a slower scan.

The spectrum of the hydroiodide in SO₂ (Figure 3C) is quite similar in terms of chemical shifts to that of the hydrochloride, except for differences in 3-H and 7-H.[12] In DMSO the 3-H line is seen; this is the only

(12) The fact that two of the three exchangeable protons in III and V and in VII and VIII had shown approximately equal changes in chemical shift upon change of anion, while the third had shown almost no change, suggested the possibility that the anion effect might be used as a basis for assigning the H_a and H_b proton signals in the cis and trans isomers. The more complex anion effect in the case of X and XII, however (Table I), indicates that this effect does not at present provide a reliable basis for such an assignment.
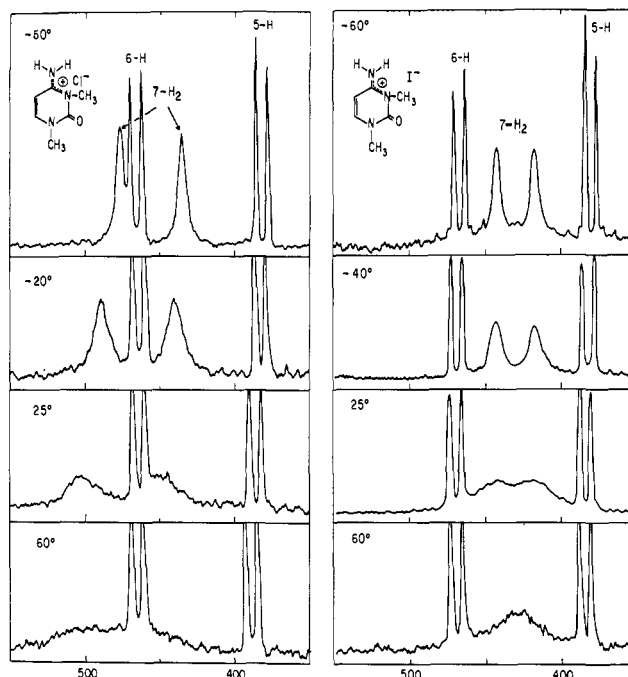
molecule studied where this line is not broadened beyond recognition at room temperature in DMSO.

Deoxycytidine, Deoxycytidine Hydrochloride, 5-Methyldeoxycytidine, and 5-Iododeoxycytidine. The spectra of these compounds in DMSO are included in Table II for comparison. It should be noted that the spectra of the cytosine protons in these nucleosides are quite similar to those of the 1-methyl derivatives.

The spectra of the 5-substituted compounds (XVI and XVII) provide further confirmation of the 5-H and 6-H assignments given previously. Both XVI and XVII have two peaks for the NH₂ protons at 23°, which coalesce at increased temperature. Evidently there is a barrier to internal rotation in these compounds considerably higher than that in I, and space-filling molecular models[13] suggest considerable steric hindrance. This phenomenon is under further investigation. (The possible occurrence of an imino form[3,4] has been considered, but the chemical shift of 6-H in XVI agrees well with that of I, not that of the imino model compound VI. In addition, the detailed temperature dependence is in accord with hindered rotation of the NH₂ group and seems to be inconsistent with an imino structure.)

Temperature Effects

Temperature variation has a profound effect on many features of the spectra of the substituted cytosine salts. Figures 4–6 illustrate the salient points: in general, with increasing temperature the line due to 3-H broadens, the lines due to 7-H broaden and then coalesce to a single peak with concomitant frequency shifts, and with X in DMSO solution the lines assigned to 5-H and 6-H in *cis* and *trans* isomers broaden and coalesce. The behavior of the n.m.r. lines of X is un-

(13) Improved Corey–Pauling space filling atomic models with Koltun connectors were supplied by the American Society of Biological Chemists.
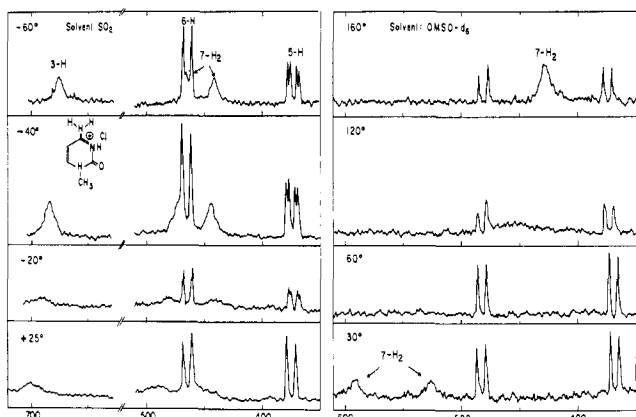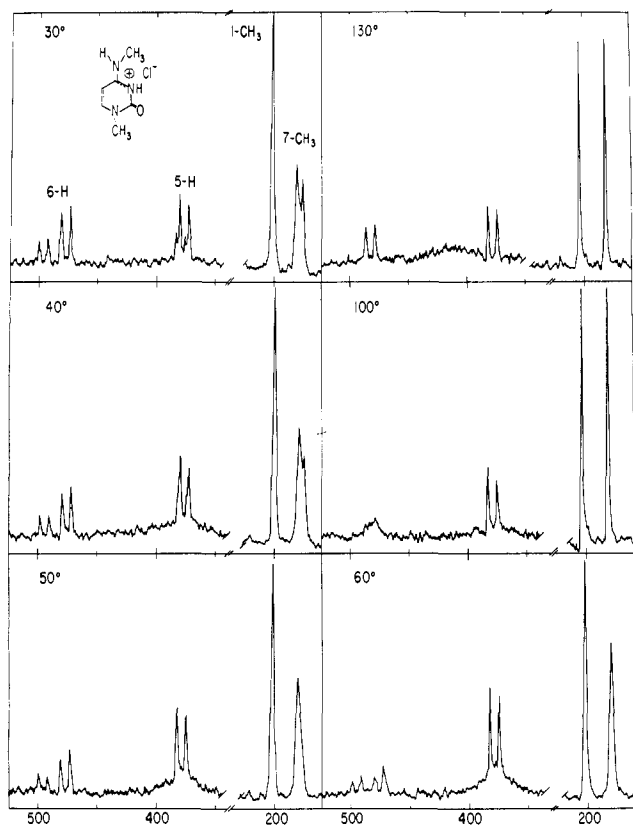
Figure 5. Spectra of 1-methylcytosine hydrochloride.

Figure 6. Spectra of 1,7-dimethylcytosine hydrochloride in DMSO.

Figure 7. Spectra of 1,7-dimethylcytosine hydrochloride in SO_2.

ambiguously ascribed to internal rotation of the 4-$NHCH_3$ group. By analogy coalescence of the NH_2 lines in III, VII, and VIII is probably also due to increase in rotational frequency of the 4-NH_2 group, but the situation is complicated here, since exchange of protons by ionization could accomplish the same type of averaging.

In principle these data may be interpreted in terms of exchange rates and the activation energy for internal rotation. Because of limitations in the data and possible variation of resonance frequencies with temperature, we have felt it inadvisable to attempt a detailed study of these rate processes, but it is possible to make some *rough* estimates of rates from our data.[14]

(14) For slow rate processes (relative to the differences in chemical shifts involved) a broadening of resonance lines is observed; for more
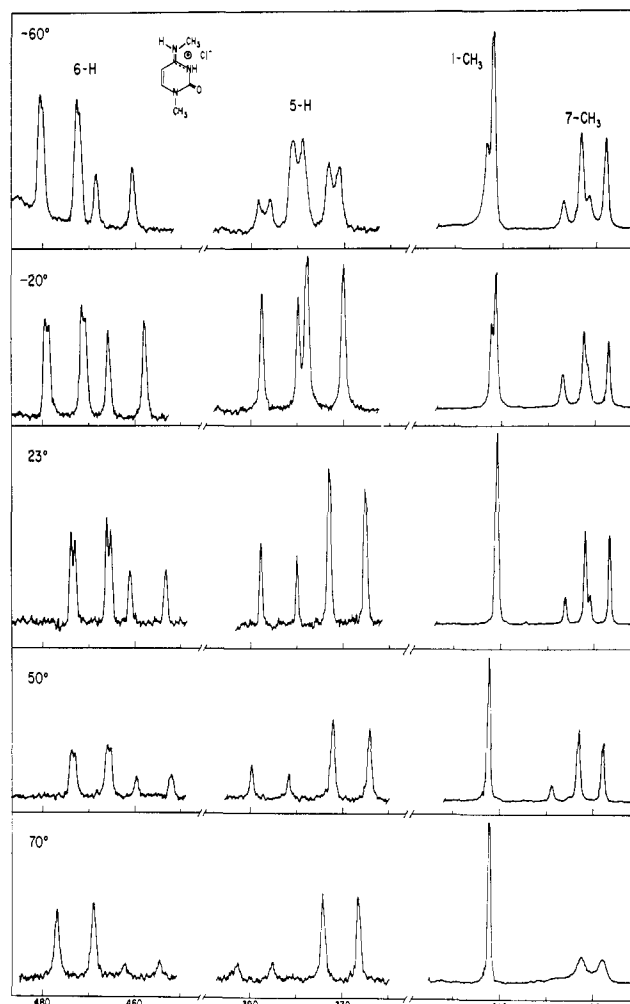
For example, for the 1,3-dimethyl derivatives VII and VIII (see Figure 4), the mean lifetime of the NH_2 group in a given conformation is about 8 msec. at 40° in liquid SO_2, and the activation energy is about 4–5 kcal./mole. There is little difference between the chloride and iodide salts. For the 1,7-dimethyl derivative X in DMSO, coalescence of the 6-H lines at *ca.* 100° gives an approximate mean lifetime of 12 msec. while the broadening of the lines at 60° indicates a lifetime for the less abundant isomer of about 100 msec. On the other hand, for X in SO_2 solution (Figure 7) the broadening of the 5-H lines indicates a lifetime of about 500 msec. at 60° for the less abundant isomer. Thus, both the site of substitution and the nature of the solvent play major roles in the internal rotation process.

Figures 6 and 7 show that 7-H begins exchanging at an appreciable rate above 50–60°, as shown by its effect on the 5-c.p.s. splitting of the 7-CH_3 lines.

Interconversion of Geometrical Isomers

While the amount of information on rates and activation energies is quite limited, it is possible to obtain from the spectra of X some useful thermodynamic

rapid rates the separate lines coalesce. For a detailed discussion see J. A. Pople, W. G. Schneider, and H. J. Bernstein, "High Resolution Nuclear Magnetic Resonance," McGraw-Hill Book Co., Inc., New York, N. Y., 1959, Chapter 10.

data on the interconversion of the two geometric isomers. Figure 7 shows typical spectra of X in SO_2. If we let A and B represent, respectively, the less abundant and more abundant isomers, then the equilibrium constant (assuming equal activity coefficients) is given by $K = (B)/(A)$ for the reaction $A \rightleftarrows B$. Over the range 0–60° the peaks due to 5-H and 6-H for the two isomers are sufficiently well separated from each other and from other peaks to permit accurate area measurements and hence determination of the concentration ratio (B)/(A). Figure 8 shows a plot of log K vs. $1/T$. The circles denote the average values of K obtained from separate area ratio measurements of the 5-H and 6-H peaks, while the extremes of the vertical bars give the separate values for 5-H and 6-H. From this plot the following values (in kcal./mole) are obtained for the reaction is written above: $\Delta H = +1.3$, $\Delta F_{298} = 0.6$, and $(T\Delta S)_{298} = +1.9$. Thus the reaction may be said to be entropically driven, with an entropy change, $\Delta S = +6.5$ e.u., that is rather large for a cis–trans isomerization of this sort. The entropy change can probably be accounted for largely by changes in the organization of solvent molecules around the cationic species.

It would be desirable to have similar data for X in DMSO, but there is too much overlap of peaks, and the signal/noise ratio is low. For the hydroiodide XII, Figure 3 shows that the abundance of the two isomers is reversed, but again strong overlap of peaks prevents the extraction of quantitative information.

The question now arises as to just which geometric isomer is the more abundant. We believe that this question can be answered by analysis of some of the spin-coupling data in the next section.

Spin–Spin Coupling

In addition to the common coupling constants already mentioned, such as $J_{5H,6H}$ and $J_{(HNCH_3)}$, there are several spin–spin couplings of considerable interest. One of the most important is that responsible for the 0.7-c.p.s. splitting of the 6-H peaks of isomer XB, as shown in Figure 7. This splitting cannot result from coupling to 3-H, since that proton is exchanging too rapidly above 0°, as indicated by loss of the 2.5-c.p.s. splitting in the 5-H peaks. The splitting must then arise from long-range (five-bond) coupling to the amino hydrogen, 7-H. The data in Figure 7 support this conclusion, for the 0.7-c.p.s. splitting disappears in the same temperature range as that due to the coupling of 7-H to 7-CH₃. Spin decoupling results, while not definitive, provide further support for the assignment of this coupling.

Five-bond couplings of this sort, involving conjugated systems, are generally thought to be larger when the coupled protons are connected by an all trans bond arrangement,[15] and in the somewhat related

$$
\begin{array}{c}
H_2C=N \diagdown \diagup H(1) \\
N \\
(4)H \diagdown \bigcirc \diagup NO_2 \\
(3)H \diagdown \diagup H(2) \\
NO_2
\end{array}
$$
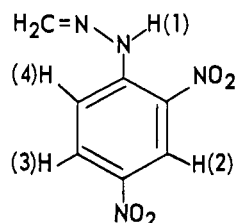
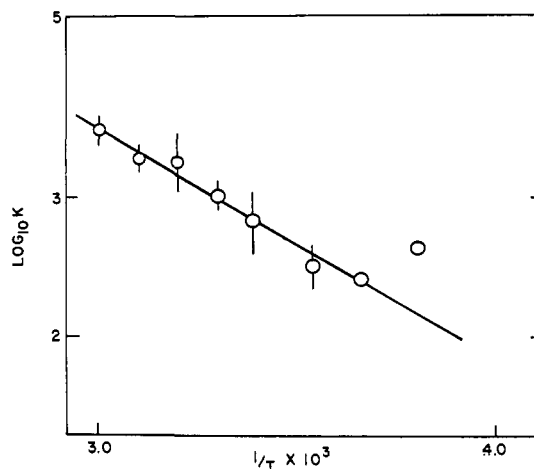(15) C. N. Banwell and N. Sheppard, *Discussions Faraday Soc.*, **34**, 115 (1962).

Figure 8. Equilibrium constant for interconversion of geometrical isomers of 1,7-dimethylcytosine hydrochloride in SO_2 as a function of temperature (see text for exact definition of K).

molecule, Karabatsos, et al.,[16] found that $J_{13} = 0.7$ c.p.s., while $J_{12} \leq 0.1$ c.p.s. Hence we believe that the isomer of X showing the 0.7-c.p.s. splitting (XB) is

$$
\begin{array}{c}
a \\
H_3C \diagdown \diagup H \\
N \quad \oplus \\
\diagdown NH \\
\diagdown \diagup O \\
N \\
CH_3
\end{array}
$$
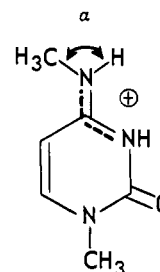
While steric effects are probably unimportant for this particular molecule, a careful study of the spectra of other derivatives with bulky substituents on both the amino group and on 3-N might provide more conclusive evidence for the assignment.

It may be noted from Figure 7 that at low temperature the splitting of 6-H due to this long-range coupling tends to disappear. This low-temperature effect may be due partially to a small decrease in $J_{6H,7H}$ resulting from slight alterations in the position (e.g., in the angle α formed by H–N–CH₃) of the amino protons, but is largely accounted for by line broadening. The increased width at low temperature could result from additional unresolved splitting due to $J_{3H,6H}$, from coupling of 6-H to 1-N, and quadrupolar broadening, or from over-all line broadening due to viscosity effects.

In the unsubstituted cytosine hydrochloride (III) one might also expect such a five-bond coupling between 6-H and one of the amino protons. Figure 9 shows that in the ¹⁵N analog IV, the 6-H lines are somewhat broadened but do not disclose a resolvable splitting. One pair of the amino proton lines shows a splitting of 0.6 c.p.s., while the other is broadened appreciably. A possible explanation of these data is that 0.6 c.p.s. represents the geminal amino coupling, which is present in both the H_a and H_b lines but is obscured in H_b because of the trans 6-H, 7-H coupling. This explanation implies then that the identification of

(16) G. J. Karabatsos, B. L. Shapiro, F. M. Vane, J. S. Fleming, and J. S. Ratka, *J. Am. Chem. Soc.*, **85**, 2784 (1963).
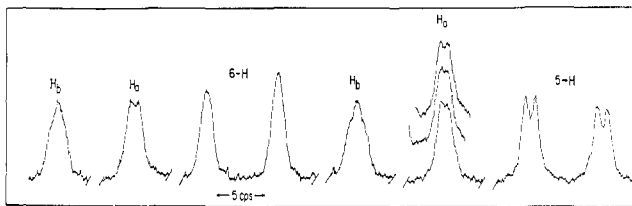
Figure 9. Spectrum of 1-methylcytosine-7-^{15}N hydrochloride in SO$_2$ at 23°.

the amino lines in Figure 9 as H$_a$ and H$_b$ is consistent with the labeling in formula III.

Attention should also be called to the values of the ^{15}N–H coupling constants in Tables I and II. In IV, for example, the two amino hydrogens are evidently differently hybridized, as indicated by the 2–3-c.p.s. difference in J. There is also a solvent effect of these J's, the values decreasing in DMSO. Here too there is apparently some rehybridization as a result of solvent effects. (It should be noted that in aniline-^{15}N, $J_{(^{15}N-H)}$ *increases* on hydrogen bond formation, whereas in the cytosine case there is a decrease in going to the hydrogen-bonding solvent DMSO.[17] See further discussion of hydrogen bonding in the next section.)

Discussion of Chemical Shifts

A few points shown in Tables I and II merit comment. Comparison of each of the free bases with its protonated form shows that 5-H and 6-H shift downfield by about 0.5 p.p.m. on protonation. This shift is in accord with some delocalization of charge through the pseudo-aromatic ring. There is considerable consistency among all the free bases and among all the salts in the values of δ_{5H} and δ_{6H} except for the free base 1,3-

(17) E. D. Becker, unpublished work.

dimethylcytosine, where $\delta_{6H} \approx 7.0$ p.p.m. rather than *ca.* 7.6, as in the other bases. This must be associated with the change in bonding leading to the exocyclic double bond and probable change in aromatic character of the ring.

The amino hydrogens are, of course, affected markedly in chemical shift by protonation, as shown in Table II. The average downfield shift is about 1.5 p.p.m. The chemical shifts of these protons are also dependent upon solvent, as indicated in the tables and as depicted in Figure 5. Comparison of the room temperature spectra in the two solvents shows that the amino protons are shifted downfield in DMSO by about 1.5 p.p.m. relative to their position in SO$_2$. That this results from hydrogen bonding in DMSO is shown by the temperature effects of Figure 5. At 160° the coalesced peak is over 2 p.p.m. at higher field than the average of the peak positions at 30°. This is the direction one expects a hydrogen-bonded proton to shift as the temperature is increased and hydrogen bonds are broken. In SO$_2$, on the other hand, there is only a small change in δ_{NH_2} with temperature and it is in the direction opposite to that expected for hydrogen bonding. In XII, the only molecule for which the 3-H peak is discernible in DMSO, there is very little difference between its chemical shift in DMSO and SO$_2$. If this result is general for this class of compounds, it would lead to the rather surprising conclusion that the 3-H does not hydrogen bond significantly to the solvent.

Finally, it may be noted from Tables I and II that in X and XI δ_{6H} is more dependent upon the geometrical isomerism than δ_{5H}, even though 5-H is much closer to the methylamino group. This finding may be understood in terms of the effect of solvent redistribution postulated to explain the large entropy difference between the two isomers.

The Dissociation Energy of the Tetrasulfide Linkage

I. Kende,[1] T. L. Pickering, and A. V. Tobolsky

The thermal decomposition of dimethyl tetrasulfide has been studied using a kinetic technique. It is suggested that the decomposition occurs as follows: Me–S$_4$–Me $\xrightarrow{2k_d f}$ MeS$_x$· + MeS$_{4-x}$·. The radical fragments were scavenged by a stable free radical and the first-order rate constant was found to be given by $2k_d f = 9.55 \times 10^{17} \exp(-36,600/RT)$. It is suggested that the experimental activation energy may be equated to the dissociation energy of the sulfur–sulfur bond in methyl tetrasulfide.

Introduction

The question of the strength of sulfur–sulfur bonds in various systems has been of great interest for many

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years. For example, the value of the dissociation energy for S$_2$ has been the subject of much controversy[2] and only recently has a reliable value become available.[3-5] The only other reliably known values for the dissociation energy of sulfur–sulfur bonds are those determined in various disulfide compounds. These values are of the order of 65 to 75 kcal./mole for simple aliphatic disulfides.

In contrast to the dissociation energy of disulfides, which is about 70 kcal./mole, we propose that the dis-

(2) (a) T. Cottrell, "The Strengths of Chemical Bonds," Butterworth & Co., Ltd., London, 1958; (b) A. G. Gaydon, "Dissociation Energies and Spectra of Diatomic Molecules," 2nd Ed., Chapman and Hall, Ltd., London, 1953.
(3) J. Berkowitz and J. B. Marquart, *J. Chem. Phys.*, 36, 275 (1963).
(4) L. Brewer, *ibid.*, 31, 1143 (1959).
(5) R. Colin, P. Goldfinger, and M. Juenehomme, *Nature*, 187, 408 (1960).