The spectra of $NiCl_2$ dissolved in melts of NaCl and LiCl do not fit the pattern for tetrahedral complexes. These results and the experimental method will be described in a later paper.

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RECEIVED FEBRUARY 19, 1963

THE TAUTOMERIC STRUCTURE OF DEOXYCYTIDINE

Sir:

The existence of cytidine as the amino tautomer in both monomeric and helical polynucleotide structures has been demonstrated in previous infrared studies on D_2O solutions.[1,2] This conclusion constituted evidence against a recently proposed DNA model[3] involving bonding to the N_7 rather than the N_1 positions of the purines.[4,5]

A recent paper[6] extended earlier n.m.r. investigations.[7,8] of the nucleosides to some deoxynucleosides in dimethyl sulfoxide solution and reported that deoxycytidine, in contrast to cytidine, exists in the imino form in this solvent as well as in D_2O. The subsequent discussions[6] of N_7 G–C bonding in the alternative DNA structure and of mechanisms of DNA–RNA interaction were based upon the existence of the imino tautomer.

We have observed the n.m.r. spectrum of purified deoxycytidine in dimethyl sulfoxide (Fig. 1A) and found that it is quite different from the one assigned to this compound by Gatlin and Davis and that, in fact, it strongly supports the amino structure. There are no NH peaks on the low field side of the H_6 doublet but instead a peak at −44 c.p.s. (relative to the aromatic peak of toluene) on the high field side of the doublet. This peak has an area indicating two protons and is assigned to the NH_2 group. The assignment of nucleoside n.m.r. peaks has been discussed in several papers.[6-8]

The spectrum of deoxycytidine hydrochloride (Fig. 1B), on the other hand, is similar in all significant features to that reported recently for deoxycytidine.[6] The difference between the spectra in Fig. 1A and 1B (and presumably between 1A and the spectrum reported by Gatlin and Davis as that of deoxycytidine) must be attributed to protonation rather than tautomerism.[9,9a]

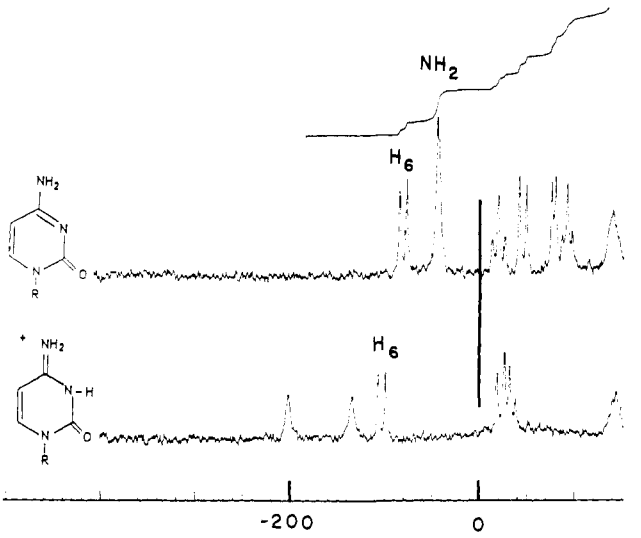

Fig. 1A (top).—60-Mc. n.m.r. spectrum of deoxycytidine in dimethyl sulfoxide (integration curve is above spectrum). Field increases from left to right. Aromatic toluene reference peak is indicated by vertical bar. Darker index marks are 200 c.p.s. apart.

Fig. 1B (lower).—60-Mc. n.m.r. spectrum of deoxycytidine hydrochloride in dimethyl sulfoxide; indexing as in Fig. 1A.

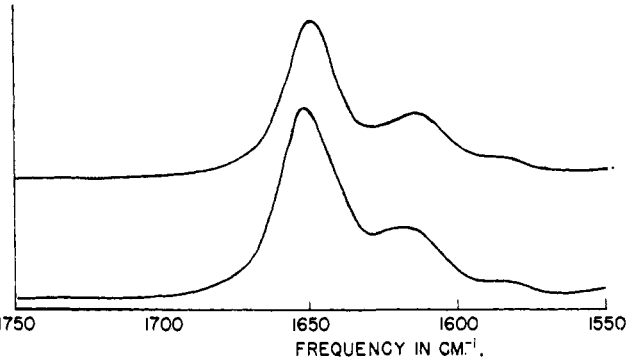

Fig. 2.—Infrared spectra of cytidine (upper curve) and deoxycytidine (lower curve) in D_2O solution, pH 7, 25-μ path length. Ordinate is absorbance on arbitrary scale.

The infrared spectrum of deoxycytidine in D_2O solution (Fig. 2) has a strong carbonyl band at 1651 cm.$^{-1}$ and a weaker ring vibration at 1618 cm.$^{-1}$ We have shown[2] that a well defined amino model compound has a strong band at 1649 cm.$^{-1}$ and a weaker one at 1625 cm.$^{-1}$, while an imino model has bands at 1671, 1657 and 1579 cm.$^{-1}$, with none near 1620 cm.$^{-1}$, thus supporting the amino and ruling out the imino form of cytidine and of deoxycytidine.

We conclude that the evidence reported here, together with that presented in ref. 2, clearly establishes the amino structure of deoxycytidine in D_2O and in dimethyl sulfoxide. The report of the imino form[6] is incorrect and can therefore provide no support for an N_7 bonding structure for DNA or for information transfer processes based upon such a structure.

The deoxycytidine hydrochloride was obtained from the California Corporation. The free nucleoside was prepared by neutralizing the hydrochloride with an equivalent of sodium hydroxide, evaporating the water, and extracting repeatedly with methanol in order to separate the nucleoside from the sodium chloride. The deoxycytidine was recrystallized five times from methanol–ether to a m.p. of 213–216° (Kofler, uncor.).[10]

(1) H. T. Miles, *Biochim. Biophys. Acta*, **35**, 274 (1959).
(2) H. T. Miles, *Proc. Natl. Acad. Sci.*, **47**, 791 (1961).
(3) R. Langridge and A. Rich, *Acta Cryst.*, **13**, 1052 (1960).
(4) K. Hoogsteen, *ibid.*, **12**, 822 (1959).
(5) L. Pauling, "The Nature of the Chemical Bond," 3rd ed., Cornell University Press, Ithaca, N. Y., 1960, p. 504.
(6) L. Gatlin and J. C. Davis, Jr., *J. Am. Chem. Soc.*, **84**, 4464 (1962).
(7) C. D. Jardetzky and O. Jardetzky, *ibid.*, **82**, 222 (1960).
(8) J. P. Kokko, J. H. Goldstein and L. Mandell, *ibid.*, **83**, 2909 (1961).

(9) The distinction between tautomerism and protonation in cytidine is made clear in ref. 2, which includes infrared evidence that cytidine is protonated at the N_3 position (the ring nitrogen not occupied by the sugar). Protonation at N_3 was supported previously by C. A. Dekker, using evidence from ultraviolet spectroscopy (*Ann. Rev. Biochem.*, **29**, 453 (1960)). Evidence that deoxycytidine and its hydrochloride have completely different structures in dimethyl sulfoxide as well as in D_2O is provided also by the infrared spectrum in this solvent. The hydrochloride has strong bands at 1720 and 1678 cm.$^{-1}$, while the free nucleoside has a strong band at 1651 cm.$^{-1}$ with a shoulder at ~1625 cm.$^{-1}$. The spectra and the deductions from them are closely analogous to those presented for the solvent D_2O (ref. 2).

(9a) The two peaks on the low field side of the H_6 doublet in Fig. 1B are presumably the external NH protons, but judgment on this and other points in the spectrum of the hydrochloride is reserved, pending further study. This reservation in no way affects the main point of the paper, which is concerned with the free nucleoside.

(10) O. Schindler, *Helv. Chim. Acta*, **32**, 979 (1949), reported m.p. 212–214° for deoxycytidine.

The n.m.r. spectra were measured in dimethyl sulfoxide solution (~0.8 *M* in solute) with a Varian model A60 spectrometer, using benzene as an external reference.

The infrared spectra were measured with a Beckman IR-7 spectrometer in D_2O solution, 25-μ path length, solvent compensated as described in earlier papers.[1,2,11]

The author is indebted to Mr. Joe Frazier and Mr. Robert Bradley for invaluable assistance in measuring the infrared and n.m.r. spectra, respectively.

(11) H. T. Miles, *Biochim. Biophys. Acta*, **30**, 324 (1958).

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A HYDROXYLATION OF ANISOLE BY HYDROGEN PEROXIDE REQUIRING CATALYTIC AMOUNTS OF FERRIC ION AND CATECHOL[1,2]

Sir:

During an investigation of possible models for metal containing enzymes which catalyze oxidation–reduction reactions, we have found that hydrogen peroxide hydroxylates anisole in an aqueous system in the presence of catalytic amounts of ferric ion and catechol. Typical reactant concentrations are given under Fig. 1. The

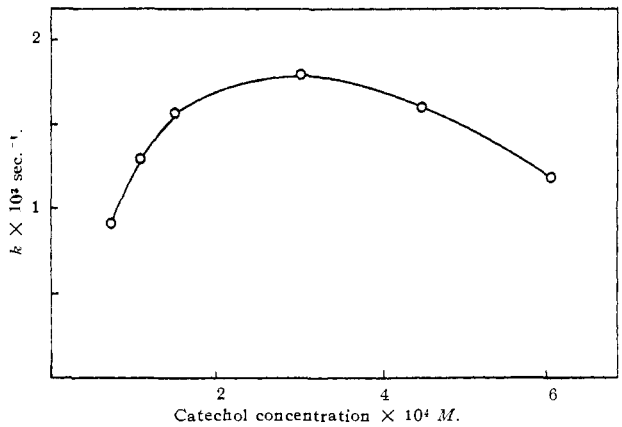


Fig. 1.—The effect of catechol concentration on the rate of reaction of hydrogen peroxide. Initial reactant concentrations: acetate buffer, 0.005 *M*, pH 4.3; $NaClO_4$, 0.15 *M*; $Fe(ClO_4)_3$, 3.94×10^{-5} *M*; anisole, approximately 0.01 *M*; hydrogen peroxide, 1.75×10^{-3} *M*; temperature, 25.0°.

hydrogen peroxide (as measured polarographically) reacts according to first-order kinetics and the reaction continues until all the hydrogen peroxide is used up. If either the ferric ion or catechol is omitted, no reaction of hydrogen peroxide occurs. If anisole alone is omitted, approximately 20 to 30% of the hydrogen peroxide reacts and then the reaction stops. The reaction will proceed if either the buffer or sodium perchlorate (necessary for the polarographic assay) is omitted.

The rate of reaction of hydrogen peroxide is directly proportional to the ferric ion concentration at constant catechol concentration. The data in Fig. 1 indicate that at low concentrations of catechol the rate increases with increasing catechol but at high concentrations the rate falls off, the maximum being around 3×10^{-4} *M* catechol.

The hydroxylated products observed in this and some other hydroxylation reactions are shown in Table I.

(1) Presented in part at the 142nd National Meeting of the American Chemical Society, Atlantic City, N. J., September, 1962, Abstracts of Papers, Division of Organic Chemistry, p. 37Q.

(2) This research was supported by a grant from the Division of General Medical Sciences of the National Institutes of Health (RG9585).

TABLE I

PRODUCTS FROM THE HYDROXYLATION OF ANISOLE BY HYDROGEN PEROXIDE[a]

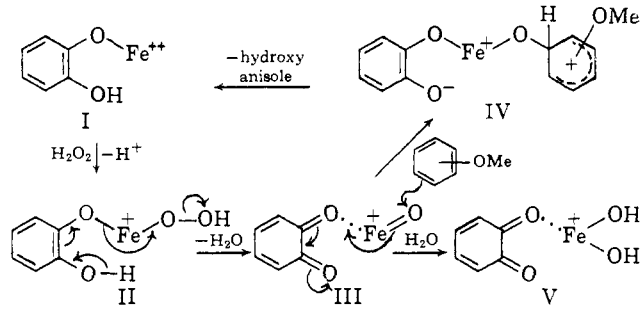
Conditions	% Yield of phenols[b]	Phenol isomer distribution % ortho	meta	para
Ferric–catechol system[c]	55	64	3	33
Ferric–hydroquinone system[d]	58	65	<5	35
Fenton reaction[e]	20	86	0	14
Udenfriend system[f]	5	88	0	12

[a] Analyzed by gas chromatography. [b] Yield based on initial amount of hydrogen peroxide. [c] Acetate buffer, 0.005 *M*, pH 4.3; $NaClO_4$, 0.15 *M*; anisole, approximately 0.01 *M*; catechol, 15×10^{-5} *M*; $Fe(ClO_4)_3$, 7.9×10^{-5} *M*; H_2O_2, 1.75×10^{-3} *M*. [d] Same as ref. *c* but with hydroquinone, 15×10^{-5} *M*, instead of catechol. [e] Acetate buffer, $NaClO_4$, anisole, and H_2O_2 same as ref. *c* and in addition $Fe(ClO_4)_2$, 2.01×10^{-3} *M*. [f] Acetate buffer, $NaClO_4$, and anisole same as ref. *c* and in addition: $Fe(ClO_4)_3$, 2.17×10^{-3} *M*; ethylenediaminetetraacetic acid, 8.0×10^{-3} *M*; L(+) ascorbic acid, 1.01×10^{-2} *M*; H_2O_2, 1.79×10^{-2} *M*.

Hydroquinone gives a reaction similar to that catalyzed by catechol but both these systems give an isomer distribution of products which is different from that observed in the Fenton reaction[3] or in a system involving ascorbic acid, originally studied by Udenfriend and coworkers[4] and more recently by others.[5] The Fenton reaction, in which stoichiometric amounts of Fe^{++} and H_2O_2 are used, apparently involves the hydroxyl radical as the hydroxylating agent,[3] and the similarity of products in the Udenfriend system indicates that the same hydroxylating species is involved.[6] Presumably a different hydroxylating agent is involved in the catechol and hydroquinone systems since higher yields of products are obtained and the isomer distribution is different. Also, ascorbic acid is present in stoichiometric amounts in the Udenfriend system, whereas the catechol and hydroquinone systems are catalytic; at least seven to ten molecules of methoxyphenol are formed for each initial molecule of catechol or hydroquinone.

We have not been able to rationalize the kinetics of the reaction in terms of a free radical chain mechanism. The products indicate that a free hydroxyl radical is not the hydroxylating species and suggest that the hydroxylating species is electrophilic. A mechanism consistent with our data is shown in Chart 1.

CHART 1



The kinetic dependence on catechol concentration suggests that a complex such as I is involved; at high catechol concentrations more than one molecule can complex with ferric ion and these complexes must be inactive since the rate decreases. The kinetic effect of hydrogen peroxide concentration is consistent with the formation of a complex such as II. A possible hy-

(3) For a recent review with references see: G. H. Williams, "Homolytic Aromatic Substitution," Pergamon Press, New York, N. Y., 1960, p. 110 ff.

(4) S. Udenfriend, C. T. Clark, J. Axelrod and B. B. Brodie, *J. Biol. Chem.*, **208**, 731 (1954).

(5) (a) R. O. C. Norman and G. K. Radda, *Proc. Chem. Soc.*, 138 (1962); (b) R. R. Grinstead, *J. Am. Chem. Soc.*, **82**, 3472 (1960); (c) R. Breslow and L. N. Lukens, *J. Biol. Chem.*, **235**, 292 (1960).

(6) This conclusion and similar results have been reported previously by Norman and Radda, ref. 5a.