0.62 g. (64%) of 2,3-diphenyllactic acid, while 0.12 g. of benzoic acid [1 mmole, corresponding to 0.5 mmole (12%) of desoxybenzoin] was obtained by ether extraction of the mother liquor.

Oxidation of Methyl Diphenylacetate by its Hydroperoxide.—A mixture of methyl diphenylacetate and methyl diphenylhydroperoxyacetate (5 mmoles of each) in pyridine (50 ml.) was treated with Triton B (10 mmoles) under nitrogen and left at room temperature for 2 hr. The peroxide-free mixture was worked up according to the general procedure. From the neutral fraction benzophenone (0.43 g., 2.4 mmoles) was isolated by means of Girard reagent. The non-ketonic residue was chromatographed to give methyl diphenylacetate (0.60 g., 2.7 mmoles; eluted with petroleum ether) and methyl benzilate (0.31 g., 1.3 mmoles; eluted with a 1:1 benzene–petroleum ether mixture). The carbonate fraction afforded upon acidification benzilic acid (0.75 g., 3.3 mmoles).

Oxidation of Methyl α-Phenylbutyrate.[14]—The ester (10 mmoles), autoxidized at −18°, absorbed 4.2 mmoles of oxygen during 3.5 hr. The neutral fraction contained 26% of the hydroperoxyester, as determined by titration. Oxidation at 0° afforded phenylethylglycolic acid, isolated from the carbonate extract in 15% yield, m.p. 130–131.5°; recorded[15] 131.5°. The neutral fraction contained 13% of hydroperoxyester.

Oxidation of Desoxybenzoin. (a) **By Oxygen.**—A solution of desoxybenzoin (1.96 g., 10 mmoles) in pyridine (20 ml.) absorbed at room temperature 14.2 mmoles of oxygen in 2 hr., the Triton added amounting to 22 mmoles. A 67% yield of benzoic acid was obtained.

(b) **By 9-Hydroperoxy-9-phenylfluorene.**[2a]—A solution of desoxybenzoin (0.65 g., 3.3 mmoles) in pyridine (5 ml.), kept under nitrogen at 0°, was treated with Triton B (10 mmoles), and a solution of the hydroperoxide (10 mmoles) in pyridine (15 ml.) was added to the mixture during 5 min. After standing at room temperature for 4 hr., the peroxide-free mixture was worked up according to the general procedure. Extraction with ether of the acidified carbonate fraction afforded 0.71 g. of recrystallized benzoic acid [5.8 mmoles, corresponding to 2.9 mmoles of desoxybenzoin (88%)]. The neutral fraction gave upon recrystallization from benzene–heptane 2.39 g. (94%) of 9-phenylfluorenol.[2a]

Acknowledgment.—The authors are indebted to Mr. Abraham Deshe for his capable assistance in carrying out the oxidation experiments.

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[CONTRIBUTION FROM THE DEPARTMENT OF PHARMACOLOGY, HARVARD MEDICAL SCHOOL, BOSTON 15, MASS.]

Proton Magnetic Resonance Studies of Purine and Pyrimidine Derivatives. IX. The Protonation of Pyrimidines in Acid Solution

BY OLEG JARDETZKY, PETER PAPPAS AND NORMA G. WADE

RECEIVED SEPTEMBER 25, 1962

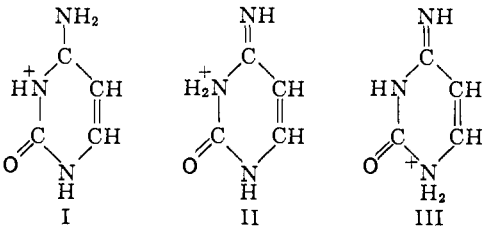
Proton magnetic spectra of the nitrogen-bound protons of a series of pyrimidines in acid solution have been observed. The area under the peak assigned to the amino group corresponds to two protons even in the most acid solutions, and separate peaks are found for protons attached to ring nitrogens. The observations support the view that the order of basicity in pyrimidines is N ring > N amino > oxygen.

Introduction

In an earlier communication[1] the suggestion has been made that the protons added to aminopurines and aminopyrimidines in acid solutions are attached to ring nitrogens, rather than to the amino groups. This inference was drawn from the relative magnitude of the chemical shifts in the nuclear magnetic resonance (n.m.r.) spectra of the pyrimidine and imidazole protons on carbon atoms adjacent to the ring nitrogens. It was therefore subject to some uncertainty, despite the fact that it was consistent with similar suggestions based on other evidence.[2-5] Less equivocal evidence for the site of protonation is provided by the n.m.r. spectra of the nitrogen-bound protons. Such spectra are not observable in aqueous solutions because of the rapid exchange of the protons with the solvent. However, in nearly anhydrous trifluoroacetic acid (TFA) the exchange can be slowed down sufficiently to allow the observation of some of the peaks. The data obtained support the view that the order of basicity in these compounds is N ring > N amino > oxygen.

Results and Discussion

A typical pyrimidine spectrum, that of cytosine in TFA, is shown in Fig. 1. In addition to the two doublets arising from the C_5- and C_4-protons at +0.45 and −0.72 p.p.m. with respect to benzene (as an external reference standard), there are three peaks at approximately −1.1, −2.9 and −3.4 p.p.m., respectively, with the solvent peak appearing at −3.9 p.p.m. The relative areas of the five peaks are approximately 1:1:2:1:1 (the width of the peaks at low fields precludes a very accurate integration, but differences in area equivalent to a single hydrogen would nevertheless have been detected). The spectrum is thus clearly inconsistent with tautomeric structures: 1, containing a tetrahedral ammonium group and a single proton in one of the other three possible positions (1, 2 or 3) (expected spectrum: two peaks, with a ratio of areas 3:1); 2, containing one proton in each of the positions 1, 2, 3 and 6 (expected spectrum: four peaks, ratio of areas 1:1:1:1), or 3, containing an amino group and two protons in one of the positions (1, 2 or 3) (expected spectrum: two peaks, ratio of areas 1:1). The broadening and the selective shift of the C_4-proton strongly suggests the protonation of N_3. If the added proton were located on the oxygen, its exchange would not affect the line width of the C_4-proton from which it is separated by five bonds, just as the exchange of the amino hydrogens does not affect the line width of the C_5-proton. The fact that three separate absorption lines can be observed also makes extremely unlikely (although does not rigorously rule out) the possibility that one or more of the protons are predominantly located on the oxygen atom: a separate resonance attributable to a hydroxyl proton is generally not observed in acid solution. Thus, the spectrum is consistent with one of the following three among the sixteen possible tautomeric structures (neglecting dipolar and nitrile forms)



I II III

The magnitude of the observed shifts allows us to favor structure I for the following reason: Taken by itself

(1) C. D. Jardetzky and O. Jardetzky, *J. Am. Chem. Soc.*, **82**, 222 (1960).
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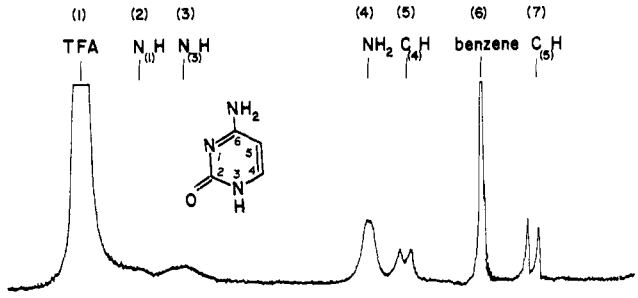

Fig. 1.—60 Mc. spectrum of cytosine in TFA; assignment of peaks: (1), solvent; (2), $N_{(1)}H$; (3), $N_{(3)}H$; (4), N-amino H; (5), $C_{(4)}H$; (6) benzene marker; (7), $C_{(5)}H$.

the shift of the $-NH_2$ group is consistent with either an aromatic amine or a quaternary ammonium grouping.[6] However, in the case of an aromatic amine the two –NH peaks would be found, as they are, at comparatively low fields, both as a result of the ring currents and as a result of the localization of charge on the ring. Furthermore, the two NH shifts would be of comparable magnitude. On the other hand, in the case of a tetrahedral ammonium configuration, the –NH peaks of the ring would appear at higher fields, since the hybridization of the tetrahedral nitrogens would not allow either ring currents or a distribution of the charge throughout the ring. In structures II and III the resonance peaks of the amino and ring protons would therefore be separated by a larger shift. The conclusion favoring structure I is in agreement with that reached by Miles[5] on the basis of infrared measurements.

Data on other pyrimidine derivatives, summarized in Table I, allow the same interpretation. It is of interest

TABLE I

CHEMICAL SHIFTS OF THE –NH PROTONS OF DIFFERENT PYRIMIDINES IN TFA (IN P.P.M.)

Pyrimidine	Ring N–H	Amino NH_2
Uracil	−2.1 to −2.3	...
Thymine	−2.1 to −2.3	...
Cytosine	−2.9 to −3.4	−1.1
Isocytosine	−2.6	−1.05
5-Fluorouracil	−2.5	...
5-Aminouracil	−2.5	−1.0
2-Amino-4-methyl-pyrimidine	...	−0.9
Thymidine	−2.3	...
Cytidine	−2.6	−1.1
Barbital	−2.3	...
Talbutal	−2.4	...
Methabarbital	−2.5	...
Butabarbital	−2.5	...

that the broadening of the C_4-proton resonance is observed in all cases and that the shifts of the peaks observed in TFA fall into the same range as one would expect for the case of a preferential protonation of the ring nitrogens.

(6) O. Jardetzky and C. D. Jardetzky, *J. Biol. Chem.*, **233**, 383 (1958).

The width of the observed broad peaks is critically dependent on the amount of water contained in the TFA and they have been detected only in preparations of the acid in which the shift of the solvent peak was −3.9 p.p.m. or lower with respect to benzene. Addition of a sufficient amount of water to shift the solvent resonance to higher fields by ~0.5 p.p.m. completely obliterates all NH peaks and narrows the C_4H resonance. The latter observation indicates that the disappearance of the peaks is not simply the result of a decreased line separation between the solvent and the –NH resonances (the lines merge if the exchange rate exceeds $2\pi\Delta\nu$, where $\Delta\nu$ is the line separation), but involves an actual increase in the exchange rate with an increased water content. Unlike the case of pyridine[7] in TFA, no splitting arising from a coupling to the nitrogen has been observed. Taking 50 c.p.s. as a rough measure of the N–H coupling constant, this indicates that the exchange rates for the NH protons in TFA solution lie within the limits 60–300 sec.$^{-1}$, the narrowest observable line being ~10 c.p.s. The possibility that the width of these lines is determined by quadrupolar relaxation of the N^{14} nucleus rather than the exchange rate is unlikely since no narrowing results from the decoupling of the N^{14} nucleus in a double resonance experiment.

Similar experiments have been performed on a series of purine derivatives. With the exception of adenine and 6-dichloroacetamido purine, which show very broad peaks ($\Delta\nu_{1/2} \simeq 30$ c.p.s.) in the region of −2 p.p.m., no additional peaks have been found, and in these cases the peaks were too broad to allow any estimate of area. The case of 8-azaguanine, whose spectrum in TFA consists of three relatively sharp lines in the benzene region, will be discussed at a later date.[8]

Experimental

The pyrimidines were purchased from Sigma Chemical Company. If the compound was sufficiently soluble, the final concentration of pyrimidines and purines dissolved in trifluoroacetic acid was 0.2 *M*. The TFA was a reagent grade product of Eastman Organic Chemicals Division, and was used without further purification. All measurements were made on a Varian 4300B 60 Mc. high resolution spectrometer, as described previously.[1]

(7) I. C. Smith and W. G. Schneider, *Can. J. Chem.*, **39**, 1158 (1961).

(8) NOTE ADDED IN PROOF.—In a recent paper Gatlin and Davis [*J. Am. Chem. Soc.*, **84**, 4464 (1962)] have suggested that cytosine exists in the *imino* form in the deoxyriboside, but not in the riboside. This, if true, would imply a striking effect of the sugar on the basicity of the ring nitrogens. However, peating their experiments in dimethyl sulfoxide (DMS) we find *no difference* in the spectra of cytidine and deoxycitidine (or cytidylic and deoxycytidylic acid) if the HCl or H_2SO_4 present in some preparations of deoxycytidine (1 equivalent of acid per mole of nucleoside) is removed prior to dissolving the material in DMS. (This does not appear to have been done in the experiments of Gatlin and Davis.) The spectrum of acid-free cytidine *or* deoxycytidine in DMS contains a well defined $—NH_2$ peak. The peak disappears upon addition of acid and is replaced by two single proton peaks at somewhat lower fields. In addition, a very broad peak appears in the $—NH^+$ region (not seen by Gatlin and Davis). These observations are readily interpreted if it is assumed that N_1 is protonated by the acid and the N_1H^+ forms an ion pair with the anion, which is stable in anhydrous DMS. The two amino protons will then be unshielded to a different extent because of the difference in their distance from the anion, with the result that the $—NH_2$ resonance line is split into two components of equal intensity. The observations reported by Gatlin and Davis do not, therefore, constitute evidence either for the existence of the inino form in deoxycytidine or for any effect of the sugar on the basicity of the ring nitrogens.