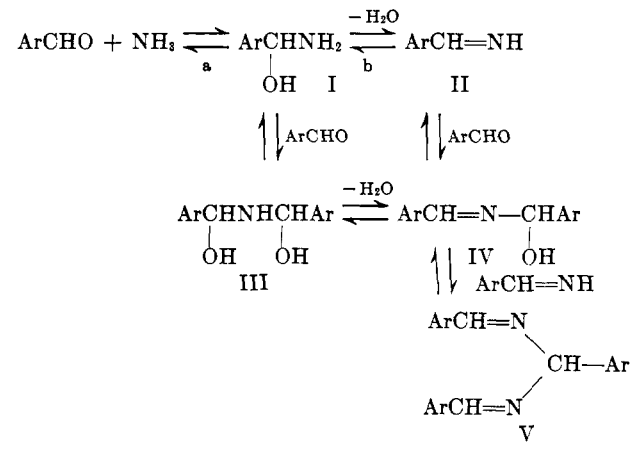
the hydrobenzamide, and suggest that the rate-determining step shifts from addition to dehydration as suggested by Jencks in the formation of oximes and semicarbazones.[19]

The Reaction Mechanisms.—The results in the reaction of benzaldehydes with ammonia in methanol are summarized (1–7) and suggest Scheme I.

(1) The over-all reaction is reversible, and the addition of water makes the reverse reaction appreciable.
(2) Since the reaction is second order, the rate-determining step may be the addition of ammonia with aldehyde or the dehydration of the resulting α-aminobenzyl alcohol.
(3) The rate of the formation of hydrobenzamide decreases slightly with increasing molar ratio of the initial concentration of ammonia *vs.* that of benzaldehyde.
(4) The addition of potassium hydroxide retards the rate of the reaction and the amount of intermediate may increase with increasing time.
(5) A positive Hammett's ρ-value was obtained with electron-releasing p-substituents in benzaldehyde and a negative ρ-value with the electron-withdrawing substituents.
(6) An induction period was observed in the formation of hydrobenzamides with electron-withdrawing substituents.
(7) The rate of the consumption is faster with p-cyanobenzaldehyde than with benzaldehyde, while the rate of the formation of hydrobenzamides from p-cyanobenzaldehyde is slower than that from benzaldehyde.

The steps leading to benzylidenimine (II) have been reported in very dilute solution.[5] As stated above, the rate-determining step may be the formation of α-aminobenzyl alcohol I (step a) or the dehydration to II (step b). The formation of hydrobenzamide V from II should be fast, since the rate is second order. The dehydration (b) may be rate determining in weakly basic media as reported by Jencks and others.[18,20]

SCHEME I



The mechanism explains the facts that a constant amount of intermediates exists (Fig. 1), that the rate of the formation of hydrobenzamide decreases on addition of alkali by retarding acid-catalyzed dehydration, and that electron-withdrawing substituents increase the rate of the consumption of the reactants and reduce the rate of the formation of the products because of the elongation of induction period. However, in the reaction of benzaldehydes with electron-releasing groups the rate-determining step may be the addition (a) because of its positive ρ-value and the absence of induction period. When the initial concentration of benzaldehyde was higher than that of ammonia, the reaction of I with benzaldehyde yielding α,α'-dioxydibenzylamine (III) isolated by Francis[6] became appreciable and caused a little increase in the rate constant (Table II). There was no evidence for the formation of 2,4,6-triphenyl-1,3,5-hexahydrotriazine[8] under these conditions. Although the formation of hydrobenzamide from II or III seems to be fast, these steps are still obscure with the present data.

Acknowledgment.—This work was supported in part by Asahi Glass Company research fund.

(19) B. M. Anderson and W. P. Jencks, *J. Am. Chem. Soc.*, **82**, 1773 (1960).

(20) E. H. Cordes and W. P. Jencks, *ibid.*, **84**, 830 (1962).

Proton Magnetic Resonance Studies of Purines and Pyrimidines. XII. An Experimental Assignment of Peaks in Purine Derivatives[1a]

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A facile hydrogen for deuterium exchange in the purine nucleus and an unambiguous synthesis of 6- and 8-deuteriopurine have enabled us to determine the assignments for the proton magnetic resonance spectra of several purines and their nucleosides. An inversion of the order of peaks in passing from alkaline to acidic solutions occurs for several 6-substituted purines. A qualitative explanation for this effect is presented.

In a previous paper of this series[2a] assignments of the aromatic protons in the p.m.r. spectra of several purine derivatives have been proposed. These assignments were based solely on a comparison of different spectra and are therefore unsatisfactory for any detailed study. In the present investigation they were tested by the examination of p.m.r. spectra of several deuterated derivatives. 6-Deuterio- and 8-deuteriopurine were prepared by direct, unambiguous synthesis. A convenient method for the preparation of other 8-deu-

(1) (a) Supported in part by U. S. Public Health Service Grants GM-0951 and GM-K3 and by National Science Foundation Grant G-19296; (b) postdoctoral fellow of the U. S. Public Health Service.

(2) (a) C. D. Jardetzky and O. Jardetzky, *J. Am. Chem. Soc.*, **82**, 222 (1960). (b) NOTE ADDED IN PROOF.—A study of purine assignments similar to ours was recently reported by M. P. Schweizer, S. I. Chan, G. K. Helmkamp, and P. O. Is'o [*J. Am. Chem. Soc.*, **86**, 696 (1964)].

terated derivatives arose from the observation made in this laboratory that the proton at position 8 of the purine nucleus is readily exchanged for deuterium by heating the material in D_2O at 90 to 100° for 10 to 20 min. The exchange is reversible in H_2O under similar conditions. The site of exchange was confirmed on the directly synthesized 8-deuterated purine. It is reasonable to assume that the proton exchangeable under these conditions is at position 8 in all the purine derivatives studied. Using this method for preparing 8-deuterated compounds we have been able to confirm and extend several previously proposed assignments.[2b]

Results

The spectra for purine and the 6- and 8-deuteriopurine, together with the assignments, are presented in Fig. 1. It was found that in this case the correct assignments are different from those made previously.[2a,3,4] The assignments and chemical shifts for other compounds studied are indicated in Table I

TABLE I

CHEMICAL SHIFTS[a]

Compd.	Concn.	Position C-8	C-2	C-6
Purine	0.1 *M* in 3 *M* NaOD	522	542	554
	0.1 *M* in D_2O	527	545	555
	0.1 *M* in 0.6 *M* D_2SO_4	563	579	589
Adenine[b]	0.1 *M* in 3 *M* NaOD	503*	512	
	0.05 *M* in D_2O, *ca.* 70°	(508)	(510)	
	0.1 *M* in 0.6 *M* D_2SO_4	532*	527	
Adenosine[c]	0.1 *M* in D_2O	509*	498	
	0.1 *M* in 0.6 *M* D_2SO_4	533*	527	
Hypoxanthine[d]	0.1 *M* in 3 *M* NaOD	495*	507	
	0.1 *M* in D_2O, *ca.* 70°	(509)	(511)	
	0.1 *M* in 0.6 *M* D_2SO_4	569	522	
Inosine[e]	0.1 *M* in D_2O	514	507	
	0.1 *M* in 0.6 *M* D_2SO_4	578	522	
Guanosine[f]	0.05 *M* in D_2O, *ca.* 80°	510		
	0.1 *M* in 0.6 *M* D_2SO_4	566		
6-Chloropurine	0.1 *M* in D_2O, 40% CH_3OH	525	531	
	0.1 *M* in 0.6 *M* D_2SO_4	568	549	

[a] All chemical shifts are in c.p.s. from hexamethyldisiloxane as external standard; * indicates confirmation of previous assignments (ref. 2a). Bulk diamagnetic susceptibility corrections are small (ref. 2a) and have been neglected. The correction for the methanol solution is +1.5 c.p.s. and has not been made. [b] 6-Aminopurine. [c] 6-Amino-9-β-D-ribofuranosyl-9H-purine. [d] Purin-6(1H)-one. [e] 9-β-D-Ribofuranosyl-9H-purin-6(1H)-one. [f] 2-Amino-9-β-D-ribofuranosyl-9H-purin-6(1H)-one.

together with the conditions of pD. Only the assignments for adenine and hypoxanthine in D_2O at neutral pD must still be considered tentative. Even in warm D_2O it was not possible to dissolve enough partially deuterated material to enable the peak of the C-8 proton to be clearly seen. Consequently it was necessary to compare in a separate experiment the shift of the single observed peak to a control. Since the peaks are separated by only 2 c.p.s. and the experimental error is possibly ±0.5 c.p.s., the conclusions are somewhat uncertain. On the other hand, the

(3) G. S. Reddy, L. Mandell, and J. H. Goldstein, *J. Chem. Soc.*, 1414 (1963).

(4) After this work was completed, the communication of S. Matsura and T. Goto, [*Tetrahedron Letters*, **No. 22**, 1499 (1963)] appeared. These workers have synthesized 2- and 6-deuteriopurine and independently reached the same conclusion.

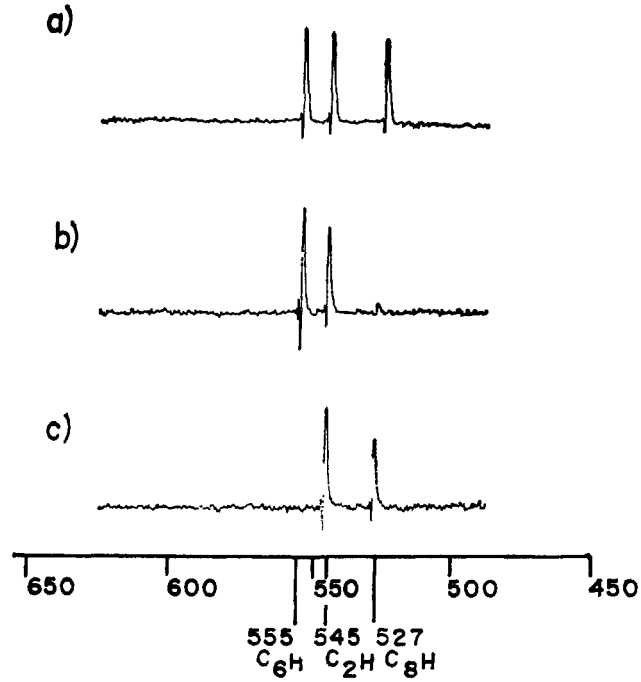


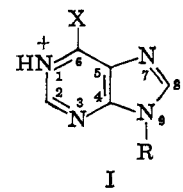
Fig. 1.—The spectra are for 0.1 *M* solutions and were determined at 60 Mc./sec.: (a) purine, (b) 8-deuteriopurine, (c) 6-deuteriopurine. (It is apparent that a small amount of exchange occurred at position 8 during the preparation of this compound.)

assignments for the acidic and basic solutions are not subject to this uncertainty. For reasons of solubility, the spectra of 6-chloropurine were run in aqueous methanol (3:2).

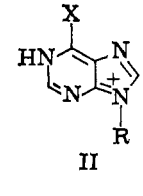
Of particular interest is the contrasting behavior of purine and the 6-substituted purines in alkaline and acidic solutions. In the 6-substituted purines there is a crossover of peaks, the peak due to the C-8 proton appearing at high field in basic solutions, but at low field in acidic solutions. This behavior is illustrated for adenine in Fig. 2. For purine, the C-8 proton is at high field under all conditions of acidity. In the nucleosides, the deshielding effect of the ribose causes the C-8 proton to appear at low field even in neutral solution.[2a] Consequently, the crossover phenomenon is not observed.

Discussion

Several studies indicate that many purines are protonated in the pyrimidine ring most probably at N-1.[2a,5,6] Yet even for purines known to be protonated at N-1, shifts of the C-8 protons comparable with, or greater than, those of the protons at C-2 or C-6 suggest that in acid solution there is some delocalization of



I



II

X = H, NH_2, Cl

(5) F. Bordwell and G. Cooper, *J. Am. Chem. Soc.*, **74**, 1058 (1952).

(6) (a) M. Tsuboi, Y. Kyogoky, and T. Shimanouchi, *Biochim. Biophys. Acta*, **55**, 1 (1962); (b) H. C. Börresen, *Acta Chem. Scand.*, **17**, 921 (1963); (c) W. Corchran, *Acta Cryst.*, **4**, 81 (1951).

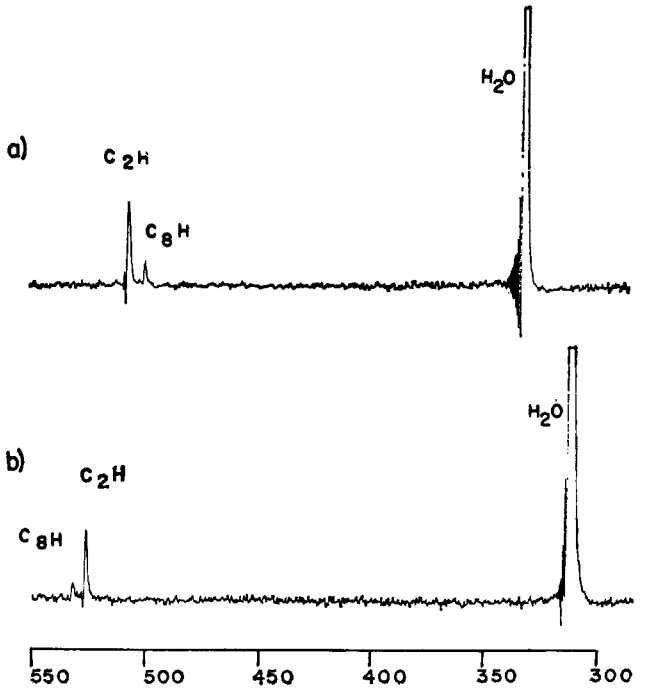
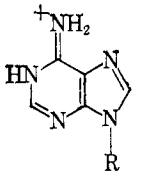


Fig. 2.—Adenine partially deuterated at position 8: (a) in 3 *M* NaOD, (b) in 0.6 *M* D_2SO_4.

charge into the imidazole ring. This may be accounted for by considering the cation as a linear combination of resonance structures I and II.

It is apparent from Table I that the C-2 protons of the 6-substituted purines undergo smaller shifts in passing from neutral to acidic solutions than is the case for purine itself. It is generally recognized that chemical shifts in aromatic molecules are a function of intra- and intermolecular ring current effects, magnetic anisotropy effects, and solvent effects. In several instances,[7-9] a relationship has been observed between the chemical shift and the charge density at the carbon to which the proton is bonded. In related isoelectronic aromatic species, such as a base and its cation or an acid and its anion, linear correlations have been made between changes in chemical shifts and charge densities.[10-12] It is our feeling that the chemical shift *differences* which we have observed between a purine base and its cation may be attributed in great part to changes in π-electron density at the carbon to which the observed proton is bound. These changes are caused by the inductive effect of a positively charged nitrogen in the ring and should be large at positions *ortho* to this nitrogen. The crossover of peaks observed in the 6-substituted purines might then be explained in either of two ways: (1) a larger contribution of structure II in the cation, or (2) contributions from structures such as III,[13] where the charge is further delocalized from C-2. This could lead to a smaller downfield shift of the C-2 proton as compared with that of the C-8 proton. The first possibility seems ruled out for adenine by the fact that the shift of the C-8 proton on cation formation is actually somewhat less than that of purine.[14]



III

Experimental

The n.m.r. spectra were recorded at 60 Mc./sec., using a Varian V-4310 instrument and calibrated by the standard side-band method.[15] The chemical shifts are accurate to ± 0.5 c.p.s. In preparing the deuterated purines by direct exchange, the base (100 mg.) was heated in 5 ml. of D_2O (or just sufficient D_2O to dissolve it at 100° if it were only partially soluble in this amount of D_2O) for 1 to 1.5 hr.; then the deuterated material was isolated by cooling the solution or evaporating the solvent *in vacuo*. 6-Chloropurine was exchanged in D_2O containing 40% methanol by refluxing for 2.5 hr. The material was about 30% deuterated by this procedure. Melting points were determined in unsealed capillary tubes using a Mel-Temp apparatus.

8-Deuteriopurine.—This material was prepared by a modification of the classical method of Traube[16] as has been described for purine.[17] 4,5-Diaminopyrimidine obtained from the Sigma Chemical Co. (0.5 g.) was dissolved in 7.0 ml. of D_2O and warmed for 10 min. The solvent was removed by lyophilization and the process was repeated. A sample of this material (200 mg.) was dissolved in 1.5 ml. of DCO_2D (Volk), and heated at 105° for 30 min. The bath temperature was raised to 205° during 50 min. and maintained at this temperature for 15 min. After cooling to 120°, the formic acid was evaporated in a stream of CO_2. Sublimation of the residue at 140° (1.5 mm.) yielded 98 mg. of material, m.p. 200–203°. Resublimation raised the melting point to 215–216°.[18] It did not depress the melting point of purine.

6-Deuteriopurine.—This method is a modification of a method previously used to prepare purine from 6-chloropurine.[17] The reduction was carried out with a Parr apparatus, Series 3910, in a 500-ml. pressure bottle. The storage tank was not used, the reaction vessel being charged directly through the valve assembly. 6-Chloropurine purchased from the Sigma Chemical Co. (1 g.) was suspended in 25 ml. of D_2O and shaken for 30 min. with 0.5 g. of 10% palladium-on-charcoal catalyst after the vessel had been charged to 7 p.s.i. with deuterium. After filtering the catalyst, the solution was neutralized by careful addition of sodium peroxide and the solvent was removed by lyophilization. Sublimation of the residue at 140° (1.5 mm.) yielded 490 mg. of material,[18] m.p. 215–216°. It did not depress the melting point of purine.

(7) P. L. Corio and B. P. Dailey, *J. Am. Chem. Soc.*, **78**, 3034 (1956).

(8) G. Fraenkel, R. E. Carter, A. MacLachlan, and J. H. Richards, *ibid.*, **82**, 5846 (1960).

(9) H. Spiesecke and W. G. Schneider, *J. Chem. Phys.*, **35**, 731 (1961).

(10) R. B. Moodie, T. M. Connor, and R. S. Stewart, *Can. J. Chem.*, **37**, 1402 (1959); T. Schaefer and W. G. Schneider, *ibid.*, **41**, 966 (1963).

(11) I. C. Smith and W. G. Schneider, *ibid.*, **39**, 1158 (1961).

(12) V. R. Sandel and H. H. Freedman, *J. Am. Chem. Soc.* **85**, 2328 (1963).

(13) Resonance contributions of this type are of course well known. E. S. Gould, "Mechanism and Structure in Organic Chemistry," Henry Holt, New York, N. Y., 1959, p. 219.

(14) We have performed calculations of the changes in π-density at the C-2 and C-8 carbons when the purines are protonated, using the constant 10 p.p.m./electron. The results appear tenable, but as there is as yet no evidence whether the constant is also applicable to a five-membered heterocyclic ring, we shall not present them now.

(15) J. T. Arnold and M. G. Packard, *J. Chem. Phys.*, **19**, 1606 (1951). We use hexamethyldisiloxane as reference since it is less volatile than tetramethylsilane. It is 2 c.p.s. downfield from tetramethylsilane.

(16) W. Traube, *Ber.*, **33**, 1371 (1900).

(17) A. Bendich, P. J. Russell, and J. J. Fox, *J. Am. Chem. Soc.*, **76**, 6073 (1954).

(18) This material also was compared with a sample of purine by descending paper chromatography using the solvent systems: 80% satd. $(NH_4)_2SO_4$, 2% isopropyl alcohol, 18% 1 *M* sodium acetate, and butanol saturated with NH_4OH–H_2O (1:4 v./v.). It was found to be identical.