TABLE III

N.M.R. SPECTRA OF GLAZIOVINE AND TETRAHYDROGLAZIOVINE

Group	No. of protons	δ-Values[a] Glaziovine	Tetrahydroglaziovine
N-CH_3	3, singlet	2.4	2.35
O-CH_3	3, singlet	3.85	3.75
OH	1, broad singlet	6.0	4.5–5.3
Aromatic proton	1, singlet	6.65	6.5
Vinyl protons	4, two AB-type quartets with fine structure[b]	6.25–6.6(α,α') 6.7–7.3(β,β')	... ...

[a] Measured at 60 Mc. in $CDCl_3$; values relative to $SiMe_4$ as an internal standard, $\delta = 0$. [b] The α- and α'-protons show marked interaction across the ring; this is not observed in the case of β,β'-protons.

of this extract (100 g.) were dispersed in dilute aqueous tartaric acid (pH 4, 1 l.) and filtered, and the filtrate was extracted continuously with chloroform. The chloroform solution on evaporation yielded 1–2 g. of crude alkaloid which, on recrystallization from ether, ether–methanol, and ethyl acetate, gave **glaziovine (III)** as colorless needles, m.p. 235–237° dec., $[\alpha]D$ +7° (*c* 1.0, chloroform); $\nu_{max}^{CHCl_3}$ 1657 (s), 1619 (s) cm.$^{-1}$.

Anal. Calcd. for $C_{18}H_{19}NO_3$: C, 72.7; H, 6.4; N, 4.7; 1 OCH_3, 10.4; mol. wt., 297. Found: C, 72.4; H, 6.2; N, 5.0; OCH_3, 10.5; mol. wt., 297, mass spec.[19]

The picrate crystallized from ethanol and had m.p. 199–203°.

Anal. Calcd. for $C_{18}H_{19}NO_3 \cdot C_6H_3N_3O_7$: N, 10.4. Found: N, 10.6.

Further chloroform extractions of the above aqueous solution at pH 5 and 6 resulted in the isolation of further small amounts of glaziovine. The aqueous solution was then made alkaline with ammonium hydroxide and extracted with ether. Evaporation of the ethereal extract gave crude **3,5-dihydroxy-6-methoxyaporphine (I)**, but as this suffered ready air oxidation, a preferred method of isolation involved the addition of a few drops of concentrated hydrochloric acid to the ethereal extract, which resulted in the precipitation of the hydrochloride, dec. >300°, from which the free base I was isolated as colorless crystals of monohydrate (0.5 g.), m.p. 149–152° dec., $[\alpha]^{26}D$ −35° (*c* 0.2, chloroform); ν_{max}^{Nujol} 3460 (w), 3300 (m), 1600 (m), 1302 (m), 1136 (m) cm.$^{-1}$; λ_{max}^{EtOH} 218, 266, 275, and 307 mμ, ε 38100, 10200, 13400, 9040; $\lambda_{max}^{EtOH-NaOH}$ 340 mμ, ε 9590[7]; neutral spectrum unchanged by the presence of boric acid buffered with sodium acetate.[12]

Anal. Calcd. for $C_{18}H_{19}NO_3 \cdot H_2O$: C, 68.6; H, 6.7; N, 4.4; 1 OCH_3, 9.8. Found: C, 68.9; H, 6.5; N, 4.2; OCH_3, 9.8.

(19) The mass spectrum of glaziovine was measured by C. Djerassi and H. Budzikiewicz, Stanford University, and showed peaks at the following positions, M^+, 297; M − 17; M − 29; M − 43; M − 58; M − 86; *m/e* 165.

Compound I did not show fluorescence in ultraviolet light when treated with 10% aqueous ethylenediamine,[20] demonstrating the absence of a catechol grouping.

With methyl iodide, compound I gave a crystalline methiodide, m.p. 251–253°, which darkened rapidly in air. A solution of the methiodide in aqueous methanol was stirred with freshly prepared silver chloride for 1 hr. and filtered.[21] The filtrate was evaporated and the residue recrystallized from methanol–acetone giving **I·methochloride·hydrate**, m.p. 226–229°.

Anal. Calcd. for $C_{19}H_{22}ClNO_3 \cdot H_2O$: C, 62.4; H, 6.6. Found: C, 62.4; H, 6.8.

3,5,6-Trimethoxyaporphine Methosulfate.—3,5-Dihydroxy-6-methoxyaporphine (I, 226 mg.) was boiled during 72 hr. with dimethyl sulfate (0.1 ml.) in acetone (10 ml.) in the presence of anhydrous potassium carbonate. The mixture was filtered and the filtrate concentrated, whereupon 3,5,6-trimethoxyaporphine methosulfate (172 mg.) crystallized. The salt was recrystallized from acetone–hexane; m.p. 189–202° dec.; ν_{max}^{KCl} 1608, 1582, 1506, 1420 cm.$^{-1}$, identical with a sample prepared similarly from tuduranine (II).[9]

Tetrahydroglaziovine (IV).—Glaziovine (100 mg.) was hydrogenated in acetic acid (10 ml.) in the presence of platinum oxide (50 mg.), until two molar proportions of hydrogen had been absorbed. The solution was filtered and evaporated and the crude **tetrahydroglaziovine** (96 mg.), m.p. 105–120°, crystallized from benzene to give colorless crystals, m.p. 112–116°; $\nu_{max}^{CHCl_3}$ 3520, 1699 cm.$^{-1}$.

Anal. Calcd. for $C_{18}H_{21}NO_3 \cdot {}^1/_6C_6H_6$: C, 73.1; H, 7.1; N, 4.5. Found: C, 73.3; H, 7.6; N, 4.3.

5-Hydroxy-6-methoxyaporphine (V).—Glaziovine (100 mg.) was stirred in 50% aqueous methanol (10 ml.) with sodium borohydride (130 mg.) during 45 min. The solution was then acidified with diluted hydrochloric acid, alkalinized with ammonia, and extracted with ether. Addition of a drop of concentrated hydrochloric acid resulted in the slow crystallization of 5-hydroxy-6-methoxyaporphine hydrochloride hemihydrate (116 mg.), dec. >220°; ν_{max}^{Nujol} 1610 (m), 1577 (w), 1499 (m), 781 (m), 752 (s) cm.$^{-1}$.

Anal. Calcd. for $C_{18}H_{20}ClNO_2 \cdot {}^1/_2H_2O$: C, 66.2; H, 6.2. Found: C, 66.7; H, 6.0.

The free base V liberated with ammonia had m.p. 167–169°; R.D.[22] in dioxane (*c* 0.17): $[\alpha]_{589}$ −150°, $[\alpha]_{400}$ −360°, $[\alpha]_{340}$ −560°, $[\alpha]_{318}$ −600°, $[\alpha]_{310}$ −520°, $[\alpha]_{305}$ 0°, $[\alpha]_{300}$ +2560°, $[\alpha]_{285}$ +7680°, $[\alpha]_{270}$ 0°, $[\alpha]_{250}$ −22,940°; λ_{max}^{EtOH} 271 and 310 mμ, ε 13700, 3900; $\lambda_{max}^{EtOH-KOH}$ 270 and 345 mμ, ε 7100, 4850. Methylation of V (10 mg.) with diazomethane in ether–methanol during 2 weeks followed by evaporation of the solution and purification of the product by thin layer chromatography gave nuciferine (5,6-dimethoxyaporphine, VI) identical with an authentic sample[13] by infrared and chromatographic comparison.

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(21) M. Tomita and I. Kikkawa, *Pharm. Bull. Japan*, **4**, 230 (1956).

(22) Measured by C. Djerassi and R. Records with an automatically recording spectropolarimeter.

[CONTRIBUTION FROM THE DEPARTMENT OF CHEMISTRY, UNIVERSITY OF CALIFORNIA, RIVERSIDE, CALIF., AND THE DIVISION OF BIOLOGY, CALIFORNIA INSTITUTE OF TECHNOLOGY, PASADENA, CALIF.]

An Experimental Assignment of the Proton Magnetic Resonance Spectrum of Purine[1]

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The assignment of the proton n.m.r. spectrum of purine has been made on the basis of specific deuteration procedures. In aqueous solution, the spectrum consists of three lines. The high field peak was shown to arise from the 8-proton on the imidazole ring, for desulfurization of 8-mercaptopurine with deuterated Raney nickel, or exchange of that proton with deuterium resulted in the decrease of intensity of that peak. The low field peak has been assigned to the 6-position of the pyrimidine ring by a similar desulfurization of 6-mercaptopurine and by reduction of 6-iodopurine with Adams catalyst and deuterium in methanol-*d*. The middle peak was assigned to the 2-proton on the pyrimidine ring by difference. This assignment of the proton n.m.r. spectrum of purine can be rationalized on the basis of an improved theory relating π-electron densities to proton chemical shifts in aromatic systems.

Introduction

In an investigation of the association of purine in aqueous solution,[3] we have been concerned with the assignment of the protons observed in its n.m.r. spectrum. The actual assignment was critical for the interpretation of the solute–solute interaction. Several modes of interaction were conceivable, and n.m.r. spectral results could be properly assessed in terms of a

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(3) S. I. Chan, G. K. Helmkamp, M. P. Schweizer, and P. O. P. Ts'o, paper presented at the Symposium on Molecular Structure and Spectroscopy, Columbus, Ohio, June 10–14, 1963.

physical model only with a correct assignment of protons.

Previous assignments of spectral peaks to the purine protons have been made. Jardetzky and Jardetzky[4] utilized the effects of substituents and charge density considerations at the appropriate carbon atoms for assignments. From the effect of substitution at N-9 on the shifts in 6-blocked derivatives, they concluded that H_8 occurs at higher field than does H_2. The highest field peak was assigned to H_6 because of less inductive electron withdrawal due to adjacent nitrogen atoms compared to that expected at positions 2 and 8. Reddy, Mandell, and Goldstein,[5] on the other hand, relied primarily on ring current anisotropy differences between the five- and six-membered rings. On this basis and from a study of N-acetylpurines, they assigned the high field peak to H_8. The low and intermediate field peaks were assigned to H_2 and H_6, respectively, of the pyrimidine ring. On the basis of inductive effects, H_2 was placed below H_6.

Because of the above disagreement in the literature concerning the assignment of the proton n.m.r. spectrum of purine, we have embarked on an experimental verification. The results of this work now contradict both previous assignments.

The importance of a correct assignment can hardly be overemphasized. As any quantitative interpretation of the chemical shifts rests with a knowledge of the electronic distribution within the molecule, the present work is significant in that the experimental results will provide a framework for future theoretical study and understanding of the electronic structure of purine and its derivatives.

Results and Discussion

a. Experimental Assignment.—In aqueous solution, the n.m.r. spectrum of purine consists of three peaks (Fig. 1a). The N-9 proton was not observed because of exchange.

The desulfurization of 8-mercaptopurine with deuterated Raney nickel in dioxane containing 1% D_2O yielded a partially deuterated product in which the relative area of the high field peak was diminished by 48% (Fig. 1b). When purine itself was treated with deuterium oxide for 4 hr. at 105°, the high field peak disappeared completely,[6] and the other peaks were unaffected (Fig. 1c). The exchange reaction was 50% complete at the end of 45 min.

In the desulfurization of 6-mercaptopurine with deuterated Raney nickel, the relative area of the low field peak was reduced by 47% (Fig. 1d). Better deuterium substitution was accomplished, however, by the reduction of 6-iodopurine with deuterium and Adams catalyst in methanol-*d* solvent (Fig. 1e). In this case, the low field peak disappeared completely, and the intensities and relative areas of the remaining peaks were only slightly affected. The possibility of exchange in the reduction was further excluded by treating purine under the reaction conditions. No deuterium was taken up and only slight reduction of the high field peak was evident in the spectrum of the recovered product.

Some exchange of purine hydrogen took place under desulfurization reaction conditions. The 8-proton was reduced about 20% and the 6-proton about 10%. Little exchange took place if pure dioxane instead of the 1% D_2O in dioxane was used as the solvent, even though the catalyst contained an excess of 80% deuterium.[7] However, reduction of the mercaptopurines with deuterated catalyst in pure dioxane yielded product purine in which no peak reduction was apparent. If the mercaptan had been previously equilibrated at room temperature with deuterium oxide, desulfurization in pure dioxane led to the replacement of the deuterated sulfhydryl group by deuterium without exchange at the other positions.

The spectra in Fig. 1 for normal purine and its deuterated derivatives were all measured in neutral solution. Since Goldstein's results were obtained in chloroform and Jardetzky's measurements were made in basic aqueous media (pH 14), our present contradiction of these earlier assignments would appear to be more convincing if the spectra of purine and its deuterated derivatives were also recorded in the solvent systems of these earlier workers. Unfortunately, we have not been able to dissolve sufficient purine in chloroform to record an n.m.r. spectrum. Goldstein[8] has indicated to us that the concentration of 25% by weight cited in his article is most likely in error and suggested that it might be 2.5%. However, our own solubility studies indicated that the solubility of purine in chloroform is less than 2.5% by weight.

In basic solution, all three spectral peaks were shifted to higher fields. When the pH was 14 (1 *M* NaOD in D_2O), H_8 was shifted upfield by 9 c.p.s., H_2 by 6 c.p.s., and H_6 by 2 c.p.s. Under these conditions, purine presumably exists as the anion. No change in the relative positions of the proton resonance peaks was indicated by the spectra of 8-deuteriopurine and 6-deuteriopurine run under identical conditions.

The proton n.m.r. spectra of purine and its deuterated derivatives have also been investigated in acid solution. At pH 1 (0.1 *M* DCl in D_2O), all three resonances were shifted downfield by about 150 c.p.s. They were approximately evenly spaced (~12 c.p.s. apart) and were considerably broader. Here again, there was no evidence for cross-over of the proton resonances.

The n.m.r. spectrum of purine exhibits a strong concentration dependence even in dilute aqueous solution. This behavior has now been studied on a quantitative basis and the results will be reported in a later communication. Pertinent to the present discussion is the approximate parallel concentration effect found for all three protons. Thus, despite the large solute–solute interaction, there is no chance for an incorrect assignment due to cross-over of the proton resonances. The spectra presented in Fig. 1 were all measured at a concentration of 0.8 *M*. Because of the strong concentration effect, it was desirable to compare spectra corresponding to the same concentration.

The acetylation experiments of Reddy, Mandell, and Goldstein[5] deserve comment as their results are readily shown to be consistent with our present assignment of the proton n.m.r. spectrum. When purine was acetylated at N-9, the highest field peak was shifted downfield by 18.0 c.p.s., the middle peak by 7.1 c.p.s., and the lowest field peak by 5.3 c.p.s. Upon acetylation at N-7, the corresponding shifts were −16.3, −12.3, and −19.8 c.p.s., respectively. On the basis of these substituent effects, it would appear reasonable to assign the highest field peak to H_8, the center peak to H_2, and the lowest field peak to H_6. Reddy, *et al.*, on the other hand, have assigned the center peak to H_6 and the lowest field peak to H_2.

b. Theoretical Rationalization.—Now that an experimental assignment has been obtained, it would seem appropriate to rationalize the relative positions of the proton resonances on the basis of existing theories. A number of factors are known to influence proton chemi-

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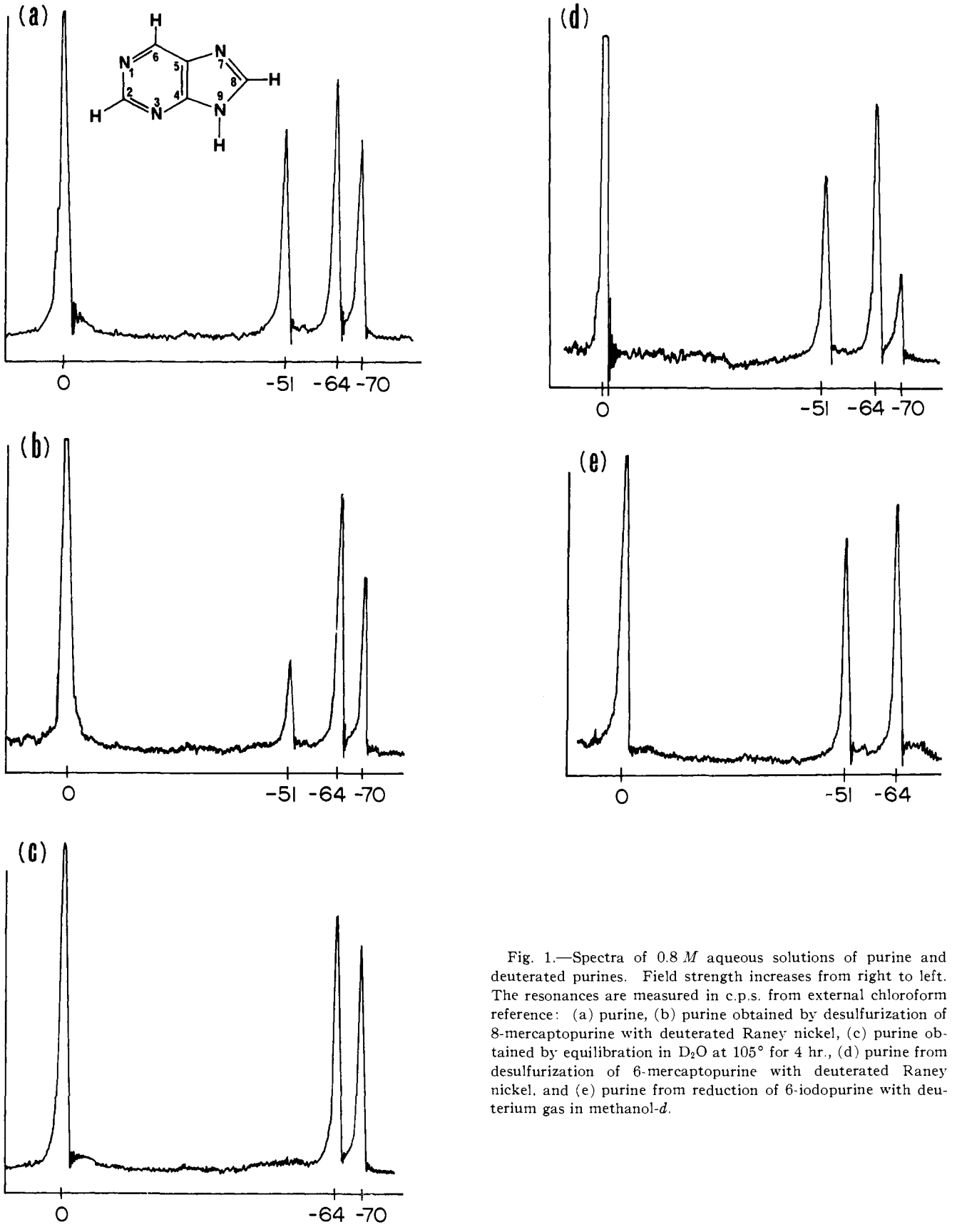

Fig. 1.—Spectra of 0.8 M aqueous solutions of purine and deuterated purines. Field strength increases from right to left. The resonances are measured in c.p.s. from external chloroform reference: (a) purine, (b) purine obtained by desulfurization of 8-mercaptopurine with deuterated Raney nickel, (c) purine obtained by equilibration in D_2O at 105° for 4 hr., (d) purine from desulfurization of 6-mercaptopurine with deuterated Raney nickel, and (e) purine from reduction of 6-iodopurine with deuterium gas in methanol-d.

cal shifts. Aside from different electron densities at the various protons, the following perturbing effects are also expected to contribute to the proton shifts in purine: (a) the ring current effect associated with the mobile π-electron cloud; (b) the magnetic anisotropy of the hetero atoms in the aromatic rings; (c) solvent effects; (d) solute–solute interactions. These effects will now be discussed.

It is tempting to relate the charge density at each proton to the π-electron density at the carbon atom to which the proton is bonded. That a linear correlation exists between proton chemical shifts and "local" excess

π-electron densities in aromatic systems has been recognized for some time.[9-11] Schaefer and Schneider[12] have also recently examined the experimental basis for this relationship more closely by applying it to a wide variety of normal aromatic systems. The extension of this correlation to heterocyclic aromatic molecules, however, should not be made without some further considerations. Owing to the inductive behavior of the hetero atoms, much larger excess π-charge densities are encountered here than in normal aromatic hydrocarbons. In addition to a term linear in the excess charge density, a quadratic term perhaps should also be included. The higher excess charge densities at each atom throughout the aromatic π-system also questions the validity of a simple correlation of proton resonance shifts to the π-charge density at the carbon atom to which the proton is directly bonded. Just as the proton resonance responds to the charge density on the carbon atom due to the electrostatic polarization of the C–H σ-bond, so should it respond similarly to the excess charge densities on the other atoms in the ring. This point has been discussed by Schaefer and Schneider.[12] However, a quantitative treatment to include these additional effects was not given.

The effects of charge densities on proton resonance shifts may be treated in terms of a field effect. In fact, it is readily shown that the equation for field effects[13-15] predicts the above-mentioned linear correlation of proton resonance shifts with excess π-electron densities. This field approach has the advantage that it is easily adaptable to include the additional polarization of the C–H σ-bond by excess charge densities on atoms other than the one to which the proton is bonded.

In this work, the effect of the π-charge densities on the proton resonance shifts has been treated in this manner and has been estimated using the expression

$$\Delta\sigma = 12.5 \times 10^{-6} \sum_i \frac{\Delta\rho_i}{R_i^2} \cos\theta_i - 17.0 \times 10^{-6} \left(\sum_i \frac{\Delta\rho_i}{R_i^2}\right)^2 \quad (1)$$

$\Delta\rho_i$ is the excess π-electron density at the ith atom, R_i is the distance (Å.) from that atom to the proton in question, and θ_i is the angle of the field vector with respect to the C–H bond axis for the proton under consideration. This expression was obtained from the familiar equation for field effect calculations

$$\Delta\sigma = -aE_z - bE^2 \quad (2)$$

For our case, a has been chosen to fit the linear correlation found experimentally by Schaefer and Schneider.[12] This value of a is 2.60×10^{-12}, which is close to the value of 2.0×10^{-12} obtained by Buckingham[14] and 2.9×10^{-12} by Musher.[15] Musher's value for b, which was obtained from Marshall and Pople's calculation, was used. The results were not too sensitive to the choice of b since contributions from the second term in eq. 1, while not negligible, were found to be an order of magnitude smaller than the contributions from the first term. For charge densities, the results of several investigators have been considered; namely those of (a) Mason[16]; (b) Pullman and Pullman[17]; (c) Miller, Lykos, and Schmeising[18,19]; and (d) Veillard and Pullman.[20] However, it is felt that the calculations of Miller, *et al.*, and those of Veillard and Pullman, being self-consistent field treatments, are more reliable. The calculations of Miller, *et al.*, were carried out within the framework of the open-shell SCF–MO method due to Roothaan[21] and Huzinaga,[22] while Veillard and Pullman treated purine using the self-consistent field method of Pariser and Parr.[23] Even though these two calculations yielded somewhat different sets of π-electron densities and in particular predicted different orders for the basicities of the nitrogens and for the relative charge densities at C_2, C_6, and C_8, it will be shown that they lead to similar n.m.r. results within the framework of our present treatment. The charge densities from the other MO calculations, which are simple Hückel treatments, however, yield chemical shift values which are inconsistent with our present assignment. Our calculations employing Miller's charge densities indicated that H_8 should be upfield relative to H_2 by 0.43 p.p.m. and H_2 upfield relative to H_6 by 0.50 p.p.m. With the charge densities of Veillard and Pullman, H_2 was predicted to be more shielded relative to H_6 by 0.28 p.p.m. and H_8 slightly deshielded relative to H_2 by 0.01 p.p.m.

These results must be modified to include contributions from the other perturbing effects mentioned above. The effect of the ring current magnetic anisotropy will first be considered. Purine is a bicyclic aromatic molecule, and thus in addition to the ring current effect associated with the ring to which the proton is attached, a contribution from the neighboring ring must also be taken into consideration. A simple calculation based upon the dipole model due to Pople[24] indicated that H_6 is expected to experience a larger ring current magnetic anisotropy than H_2 by 0.18 p.p.m. The same calculation showed that both H_2 and H_8 experience a similar ring current effect.

Contributions to the resonance shifts from the magnetic anisotropy of the nitrogen atoms cannot be determined on an equally reliable basis as the charge density and ring current effects. However, this effect may be estimated from the chemical shifts of H_2 and H_6 in pyrimidine.[25] The effect of charge densities on the proton shifts in pyrimidine can be treated in an analogous way to purine as Miller[19] and his co-workers as well as Veillard and Pullman[20] have made in each case a similar MO calculation for pyrimidine. With Miller's charge densities, H_2 should resonate at a lower field than H_6 by 0.29 p.p.m., whereas Veillard and Pullman's charge densities would predict a corresponding value of 0.26 p.p.m. The ring current effect is the same for both protons. Experimentally, $\delta H_2 - \delta H_6 = -0.50$ p.p.m. If the remaining 0.21 or 0.24 p.p.m. can be attributed to a difference in the effects of the nitrogen magnetic anisotropy at the two protons, one can compute a magnetic anisotropy for the nitrogen atoms in the pyrimidine ring. Assuming that this result is transferable to the purine molecule and that it is an average magnetic anisotropy for N_1, N_3, and N_7, it is readily shown that with the results of Miller, *et al.*, the effect under consideration would lead to a deshielding of H_2 relative to H_6 by 0.17 p.p.m. and an additional shielding of H_8 relative to H_2 by 0.18 p.p.m. If the charge densities of Veillard and Pullman were employed, H_2 would be deshielded relative to H_6 by 0.20 p.p.m. and H_8 would be

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shielded relative to H_2 by an additional 0.20 p.p.m. The effect of N_9 has been neglected in these calculations.

Both solvent and concentration effects can be controlled experimentally. Concentration effects can also be accounted for by the extrapolation of measured shifts to infinite dilution. In this work, the proton magnetic resonance spectrum of purine was measured in aqueous solution only. Although the spectra in both acid and basic aqueous solution were also considered, it is likely that these results correspond to the protonated species and the negative anion, respectively, and are therefore not exactly pertinent within this discussion. A less polar solvent presumably could have been used to study the neutral molecule, subject to solubility limitations. However, a change in solvent would not be expected to shift the order of the proton resonances, judging from our spectral results in acid and basic media. Nevertheless, the results of our present computations should be compared with chemical shifts obtained in less polar solvents. The chemical shifts of purine have been measured in chloroform by Reddy and co-workers.[5] Even though we have not been able to duplicate their results, we have shown that the results of their acetylation studies are consistent with our present assignment of the proton resonances. If their assignment is corrected to correspond to our present observations, then, according to their measurements in chloroform, $\delta H_8 - \delta H_2 = 0.55$ p.p.m. and $\delta H_2 - \delta H_6 = 0.17$ p.p.m. The results of our present calculations are: with Miller's charge densities, $\delta H_8 - \delta H_2 = 0.61$ p.p.m. and $\delta H_2 - \delta H_6 = 0.50$ p.p.m.; with the charge densities of Veillard and Pullman, $\delta H_8 - \delta H_2 = 0.20$ p.p.m. and $\delta H_2 - \delta H_6 = 0.25$ p.p.m. While the agreement with experiment is not exactly quantitative, the order of the proton resonances is predicted. In aqueous solution, $\delta H_8 - \delta H_2 = 0.25$ p.p.m. and $\delta H_2 - \delta H_6 = 0.11$ p.p.m. from our measurements.

So far, we have been concerned with relative shifts. Our calculations also yield absolute shifts. Thus, relative to benzene, the following chemical shifts were obtained. With Miller's charge densities: H_8, -1.49 p.p.m.; H_2, -2.10 p.p.m.; and H_6, -2.60 p.p.m. With Veillard and Pullman's charge densities: H_8, -1.73 p.p.m.; H_2, -1.93 p.p.m.; and H_6, -2.18 p.p.m. These shifts are to be compared with the values of -1.08, -1.63, and -1.80 p.p.m. observed in chloroform and -1.40, -1.78, and -1.95 p.p.m. observed in aqueous solution. These observed shifts are referred to benzene in a 2% CCl_4 solution. Where applicable bulk susceptibility corrections have been made, and for the measurements in aqueous solutions, the resonance shifts have also been extrapolated to infinite dilution to correct for the large concentration effect. Since the excess π-charge densities account for the major portion of these calculated shifts, more satisfactory agreement with experiment is conceivable with more accurate charge densities and with a more accurate theory to account for the charge density effect.

Finally, it is noteworthy to point out that the proton n.m.r. assignment of Jardetzky was recently invoked to discredit the SCF charge densities of Miller and his co-workers and to defend the Hückel and SCF charge densities of Pullman and his co-workers.[26] In the light of our present experimental assignment as well as our theoretical rationalization using both sets of charge densities, it is felt that the criticisms were not entirely justified.

Experimental

Materials.—Methanol-*d* was prepared from dry sodium methoxide and deuterium oxide. The product was removed from the reaction mixture by heating at 140° under vacuum. It was distilled through a Holtzmann column. Purine was obtained from California Corp. for Biochemical Research, Los Angeles, and from Cyclo Chemical Corp., Los Angeles. The 6- and 8-mercaptopurines were also purchased from Cyclo. Powdered nickel–aluminum alloy was obtained from Harshaw Chemical Co., lithium aluminum hydride and sodium hydride from Metal Hydrides, Inc., Beverly, Mass., and deuterium oxide and platinum oxide from Matheson Coleman and Bell. The above compounds were of the highest purity commercially available and were used without further purification. All other chemicals were reagent grade.

Since the present proton assignment rests with the validity of the starting materials, the ultraviolet spectra of the mercaptopurines and 6-iodopurine were compared with those reported in the literature. At pH 1.0, the observed λ_{max} and ϵ_{max} for 6-mercaptopurine agreed with the values reported by Bendich, Russell, and Fox.[27] For 8-mercaptopurine at pH 5.5, the data were in accord with those of Brown and Mason[28] reported at pH 4.5. At pH 1.0, 6-iodopurine displayed the same spectrum as that reported by Elion and Hitchings.[29] The melting point was also confirmed.

Instrumentation.—All n.m.r. spectra were taken with a Varian Associates V-4300 spectrometer operating at 56.4 Mc. The probe operating temperature was 25 ± 1°. The spectrometer utilized V-4365 field homogeneity coils with a V-K3506 flux stabilizer for maximum field homogeneity. Peak positions were measured using a Hewlett-Packard wide-range audiooscillator and a HW 521C electronic counter monitored the audiofrequency output.

Chemical shifts were measured using the standard audio sideband technique. The accuracy of these shifts, measured in c.p.s. from external chloroform, was well within 0.5 c.p.s.

Exchange of the 8-Proton in Deuterium Oxide.—A solution of 0.26 g. (0.0022 mole) of purine in 5 ml. of 99.5 mole % deuterium oxide was heated at 105° up to 4 hr. Samples were withdrawn at various intervals for n.m.r. analysis.

Reduction of 6-Iodopurine with Deuterium.—A methanol-*d* solution of 6-iodopurine was reduced with deuterium gas in the presence of Adams catalyst. After 1 equivalent of deuterium was consumed, the reaction ceased. Freshly precipitated silver oxide, washed free of water with dioxane and methanol-*d*, was added to the well-stirred reaction mixture until silver iodide no longer formed. At this point the pH of the solution was about 5.5. The catalyst and silver iodide were removed by filtration. The amount of purine recovered, determined by ultraviolet absorption, was virtually quantitative and the spectrum was identical with that of known purine. The melting point, taken after evaporation of the solvent and sublimation at 120°, was the same as that of purine.

Raney Nickel.—The procedure of Billika and Adkins[30] was used for the preparation of W-6 Raney nickel. To assay the activity of the catalyst, preliminary desulfurizations were run on 8-mercaptopurine in both dioxane and distilled water. The procedure of Bendich, Russell, and Fox[27] was followed. Catalyst was added portionwise until an aliquot showed no ultraviolet absorption above 300 mμ. In order to effect complete desulfurization, it was necessary to use 3 equivalents of Raney nickel in aqueous solution or 5 equivalents in dioxane solution. A 2 hr. reflux period was convenient, but shorter times were adequate if proper initial amounts of active catalyst were present. The product was purified by sublimation at 120°.

Deuteration of Raney nickel was carried out by the procedure of Bonner.[31]

Desulfurization of Mercaptopurines.—Desulfurizations were carried out as before, but more catalyst was necessary because its activity had been reduced during the deuteration process. Yields were about 50% after sublimation. After triple sublimation, the melting points of product purines from 6- and 8-mercaptopurine were 218–218.5° and 217–217.5°, respectively. Mixture melting points with purine showed no lowering. Ultraviolet absorption spectra could not be distinguished from that of purine.

Infrared spectra (in paraffin oil) of the two deuterated products and also of the purine from reduction of 6-iodopurine displayed weak bands in the region of aromatic C–D stretching (2200–2300 cm.$^{-1}$). The remainder of these spectra differed from that of known purine in the region 700–1600 cm.$^{-1}$, primarily in band intensities, although several frequencies were also different. However, purine synthesized *via* desulfurization of 8-mercaptopurine with hydrogenated Raney nickel and commercial purine show the same spectra. It might be expected that deuteration would alter the infrared-active vibrations due to isotope effects.

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