18-Carboxy-17,18-secoyohimban-17-oic Acid (4).—A mixture of 10.52 g. (0.030 mole) of yohimbinone (2), 3.0 g. of potassium hydroxide, and 75 ml. of absolute ethanol was warmed gently for 2 hr. (not refluxing) and was then refluxed for 2 hr. The solvent was removed *in vacuo* and the residue was dissolved in 50 ml. of water. The mixture was chilled overnight and filtered. The precipitate was washed thoroughly with water and recrystallized from ethanol–water to give 0.13 g. (1.4%) of yohimban-17-one. The filtrate was chilled and brought to pH 7.0 with glacial acetic acid. Chilling and filtering gave 8.4 g. (78%) of **4** as tan crystals, m.p. 195–200° (with previous sintering). Recrystallization of a sample twice from ethanol–water (2:3) with the aid of Darco gave white crystals: these change to a viscous mass at 204–207° and then slowly melt; $[\alpha]^{25}D$ +19° (*c* 1.0, pyridine); ν_{max}^{KBr} 1721 (s) and 1565 (s) cm.$^{-1}$.

Anal. Calcd. for $C_{20}H_{24}N_2O_4$: C, 67.4; H, 6.79; N, 7.86. Found: C, 67.4; H, 6.99; N, 7.46.

Yohimban-17-one (3) from Yohimbinone (2).—A mixture of 2.10 g. of **2** hydrochloride, 100 ml. of 3 *N* hydrochloric acid, and 25 ml. of glacial acetic acid was refluxed for 4 hr. The mixture was cooled and poured into a mixture of ice and 75 ml. of concentrated ammonium hydroxide. Filtration gave a tan solid which was dissolved in ethanol–dichloromethane and the solution was concentrated *in vacuo*. The residue was triturated with methanol to give 0.90 g. (57%) of **3**, m.p. 292–295° dec. A second crop (0.65 g., m.p. 285–288° dec.) was obtained from the filtrate. The solids were combined and recrystallized by dissolving in hot methanol–dichloromethane and boiling off the dichloromethane. There was obtained 1.30 g. (82%) of light tan crystals, m.p. 298–303° dec.

Acknowledgment.—We wish to express our thanks to Mr. L. M. Brancone and staff for elemental analyses, Mr. W. Fulmor and staff for spectral determinations, and Mr. C. Beck for a large-scale preparation.

Correlation of the Proton Magnetic Resonance Chemical Shifts of Substituted Purines with Reactivity Parameters. I. 2,6-Disubstituted Purines

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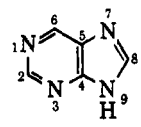
Spin–spin coupling of the 2- and 6-protons of purine has been observed in trifluoroacetic acid solutions ($J_{2,6}$ = 1.05 ± 0.05 c.p.s.) and in aqueous acid ($J_{2,6}$ = 1.05 ± 0.05 c.p.s.). Protonation at N-1 in acid solutions, reducing the asymmetry of the electric field about N-1 and thus allowing spin–spin coupling between H-2 and H-6 to take place without quadrupole relaxation, is offered as an explanation for the observation of splitting in acid but not in neutral solution. Long-range coupling of H-6 and H-8 is also observed in acid, $J \cong 0.3$ c.p.s. Study of the proton magnetic resonance spectra of sixteen 2,6-disubstituted purines in dimethyl sulfoxide solution yielded a linear correlation of the chemical shift of the 8-proton with Brown's electrophilic substituent constant, $\delta_{H\text{-}8} = (8.658 \pm 0.017) + (0.342 \pm 0.016)\Sigma_{2,6}\sigma_p^+$, where $\delta_{H\text{-}8}$ is in parts per million downfield from internal tetramethylsilane. The standard deviation in $\delta_{H\text{-}8}$ is ±0.066 p.p.m. and the correlation coefficient is 0.984. No other substituent parameter tried fit the data as well as σ_p^+.

Recently, several workers have attempted to predict on the basis of theoretical considerations the relative positions of the proton magnetic resonance (p.m.r.) absorption peaks of the three C–H protons of purine (I).[1,2,3a] Experimental determinations of the correct sequence (H-6 at lowest field and H-8 at highest field) in aqueous solutions have been reported by Matsuura and Goto,[4] by Schweizer, Chan, Helmkamp, and Ts'o,[3] and by Bullock and Jardetzky.[5a] Although these theoretical predictions have been successful in rationalizing the relative order of the C–H peaks in the spectrum of the parent purine molecule, especially when compared with the more intuitive predictions of Jardetzky and Jardetzky[5b] and of Reddy, Mandell, and Goldstein,[6] only moderate success may be claimed for the correlation of the chemical shifts of substituted purines with excess charge density.[1]



I

In the course of the routine examination of the p.m.r. spectra of potential anticancer agents, we have obtained the spectra of a number of simply substituted purines. In view of the difficulties of theoretically estimating the effect of substituents in the 2- and 6-positions on the chemical shift of the 8-proton, we have examined several empirical correlations of chemical shifts with reactivity parameters, such as the Hammett σ constants. This report summarizes the results of such correlations for 2,6-disubstituted purines, together with some additional p.m.r. data on unsubstituted purine.

Experimental

Solvents.—Dimethyl sulfoxide-d_6 was obtained from either Merck Sharp and Dohme of Canada Limited or from Stohler Isotope Chemicals, Montreal. Ordinary dimethyl sulfoxide was employed in some cases, where no interference from solvent absorption was expected, and was obtained from Matheson Coleman and Bell. Both of these solvents were dried over a Linde 4A Molecular Sieve before use. Trifluoroacetic acid was an Eastman Kodak reagent and was used without prior treatment. Deuterium oxide was obtained from General Dynamics Corporation.

Purines.—Purine-d_0 was bought from Francis Earle Laboratories, Inc., and was found to be chromatographically homogeneous.

The preparations of most of the substituted purines have been previously reported. One exception is 2-chloro-6-methoxypurine. A solution of 2,6-dichloropurine (1 g., 5 mmoles) in 1 *N* sodium

(1) A. Veillard, *J. chim. phys.*, **59**, 1056 (1962).
(2) P. G. Lykos and R. L. Miller, *Tetrahedron Letters*, **No. 25**, 1743 (1963).
(3) (a) M. P. Schweizer, S. I. Chan, G. K. Helmcamp, and P. O. P. Ts'o, *J. Am. Chem. Soc.*, **86**, 696 (1964); (b) S. I. Chan, M. P. Schweizer, P. O. P. Ts'o, and G. K. Helmcamp, *ibid.*, **86**, 4182 (1964).
(4) S. Matsuura and T. Goto, *Tetrahedron Letters*, **No. 22**, 1499 (1963).
(5) (a) F. J. Bullock and O. Jardetzky, *J. Org. Chem.*, **29**, 1988 (1964); (b) C. D. Jardetzky and O. Jardetzky, *J. Am. Chem. Soc.*, **82**, 222 (1960).
(6) G. S. Reddy, L. Mandell, and J. H. Goldstein, *J. Chem. Soc.*, 1414 (1963).

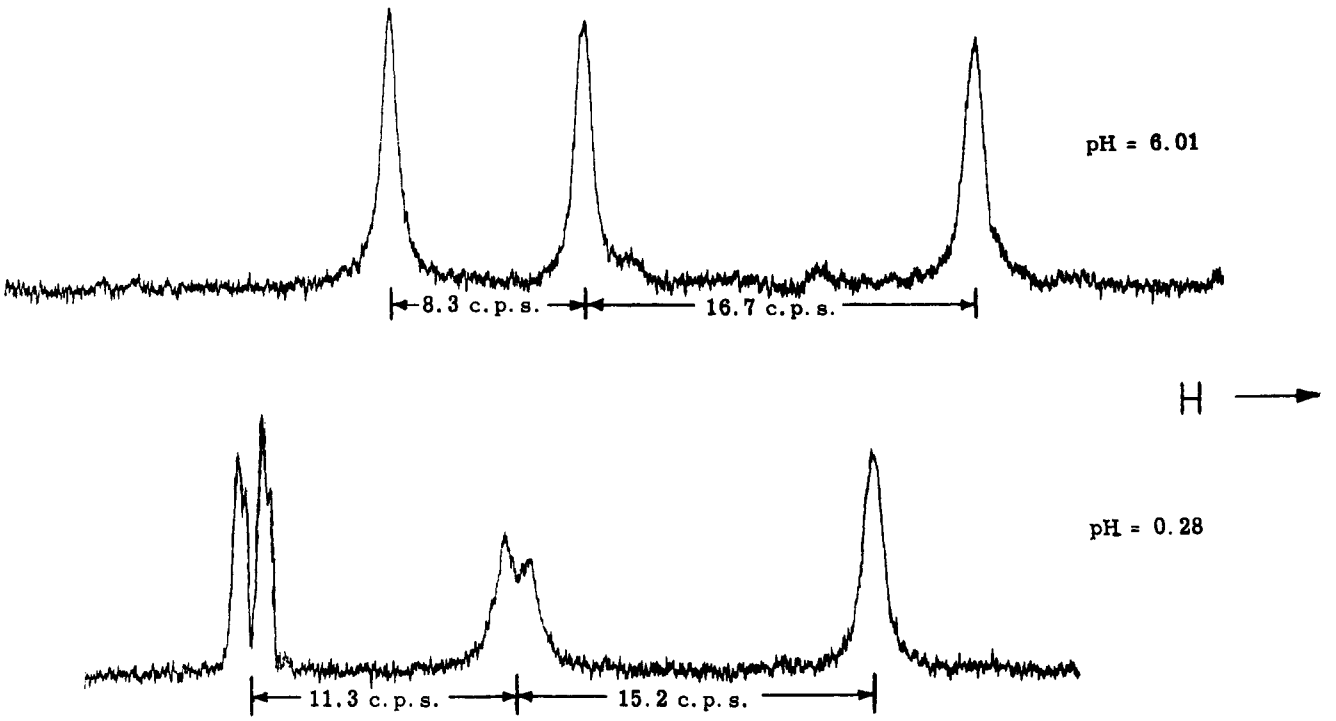


Figure 1.—P.m.r. spectra of purine in water at 38° C.: top, neutral molecule; bottom, cation; left-hand peaks, H-6; center peaks, H-2; right-hand peaks, H-8. Solute concentration was 0.42 *M* in each case (not referenced).

methoxide (50 ml.) was refluxed for 20 hr. The reaction solution was clarified by filtration and the filtrate was neutralized (pH 6–7) with glacial acetic acid. The crude product (910 mg.) that precipitated in two crops from the neutral solution on concentration was collected by filtration and washed with water. Recrystallization of this product from ethanol (50 ml.) with Norit treatment gave the pure material: yield 490 mg (52%); m.p. (Heizbank) 254° (with sublimation above 230°); λ_{max} 259 mμ (ε 10,300) at pH 1, 259 (10,100) at pH 7, 267 (10,200) at pH 13. The material was shown by thin layer chromatography to be homogeneous (silica gel H and ethyl acetate).

Anal. Calcd. for $C_6H_5ClN_4O$: C, 39.04; H, 2.73; N, 30.35. Found: C, 39.51; H, 3.02; N, 30.53.

Purine-2-*d* and purine-6-*d* were prepared from the corresponding chloropurines as follows. Palladium–charcoal catalyst (5%, 0.4 g./gram of chloropurine) was added to the chloropurine (1.4–2 mmoles) suspended in deuterium oxide (30–75 ml. or sufficient volume to dissolve the deuterated product), and the resulting mixture was stirred under deuterium gas at atmospheric pressure. After deuterolysis was complete, the catalyst was removed by filtration in a nitrogen atmosphere, and the filtrate was neutralized (pH 5–6) with Amberlite IR 4 B(OH) (3–4 equiv./equivalent of chloropurine), which had previously been washed with water and then with deuterium oxide. The resin was removed by filtration in a nitrogen atmosphere, and the filtrate was evaporated to dryness by freeze drying. The resulting residue was triturated with ether, and the ether-insoluble solid was dried under vacuum. The identity and homogeneity of the isolated material were established by thin layer chromatography using the starting chloropurine and purine-d_0 as standards. The deuterolysis of 6-chloropurine required 2.5 hr. at 25° and gave a 70% yield of purine-6-*d*. The p.m.r. spectrum showed little exchange at the 2- or 8-positions and no evidence of the presence of 6-chloropurine. The deuterolysis of 2-chloropurine required 2 hr. at 25° and gave a 55% yield, assuming the product to be purine-2-*d*. However, the p.m.r. spectrum showed that considerable exchange had occurred at the 6-position, and that the product could be more accurately described as purine-2,6-d_2.

Spectra.—^{1}H, 60-Mc./sec. n.m.r. spectra were obtained on a Varian Associates A-60 spectrometer. The compounds were investigated over a period of 2 years, during which the probe temperature was changed several times over a range of 30–38°. Tetramethylsilane (TMS) was used as internal reference in solutions in dimethyl sulfoxide (DMSO) and in trifluoroacetic acid (TFAA). In aqueous solutions, sodium 3-trimethylsilylpropane-1-sulfonate (TSPS) was used as internal reference.[7] All chemical shifts, δ, are reported in parts per million downfield from the position of the internal reference. The estimated accuracy of δ is ±0.04 p.p.m.

The majority of the compounds were studied at only one concentration, usually 5 g./dl. However, measurements on purine at three concentrations in DMSO, Table I, indicate that the change in δ with concentration is small for the three C–H protons, but is considerable for the N–H proton, usually designated H-9,[8a] probably caused by association effects. Chan, Schweizer, Ts'o, and Helmcamp[8b] have proposed a model of vertically stacked rings for the association of purine and of 6-methylpurine in aqueous solutions, based on the large upfield chemical shifts with increasing concentration that they observed for these compounds. They report small upfield shifts for H-2, H-6, and H-8 in DMSO solution but report no observation of H-9. Our observation of a large downfield shift of H-9 with increasing concentration is in accord with normal horizontal association through N–H···N hydrogen bonds. Perhaps the reason Chan and co-workers did not observe a peak attributable to H-9 was the presence of moisture in their DMSO; this solvent is notoriously hygroscopic. H-9 may have been exchanging with the water present. This would also explain the small upfield shift of H-2, H-6, and H-8 that they report in DMSO, since our observed shifts for these protons with increasing concentration are in the opposite direction.

TABLE I

EFFECT OF CONCENTRATION ON CHEMICAL SHIFTS OF PURINE IN DIMETHYL SULFOXIDE

Concn., g./dl.	δ, p.p.m. H-2	H-6	H-8	H-9[a]
1.0	8.95′	9.16	8.63	7
5.0	8.99	9.19	8.68	11
12.0	9.01	9.23	8.71	12.3

[a] H-9 position is less precise because peak was very broad.

Ultraviolet spectra were recorded on a Cary Model 14 spectrophotometer.

(7) G. V. D. Tiers and R. I. Coon, *J. Org. Chem.*, **26**, 2097 (1961).

(8) (a) A. Albert in "Physical Methods in Heterocyclic Chemistry," Vol. I, A. R. Katritzky, Ed., Academic Press Inc., New York, N. Y., 1963, Chapter 1, p. 50; (b) p. 99.

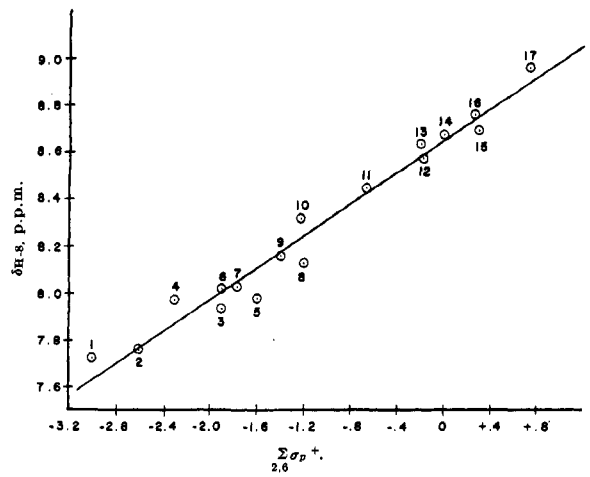


Figure 2.—δ_{H-8} of 2,6-disubstitut ed purines as a function of $\Sigma\sigma_p^+$. To identify points see the compound numbers given in Table II.

pH values were measured at 28° on a Beckman Model H-2 pH meter standardized at pH 4.01 with Beckman 3506 buffer solution and at pH 1.09 with 0.10 *N* hydrochloric acid.[9]

Results

Purine Spin–Spin Coupling.—In DMSO solution the peaks assigned to H-2 and H-8 of purine, when observed at the reduced sweep rate of 0.1 c.p.s./sec., are noticeably broader than the peak assigned to H-6, presumably because of the quadrupole broadening effect of the two adjacent nitrogens on H-2 and H-8. In TFAA, however, spin–spin coupling is observable; H-2 and H-6 appear as an AB pair with $\nu_0(\delta_6 - \delta_2) = 22.63 \pm 0.05$ c.p.s. and $J_{2,6} = 1.05 \pm 0.05$ c.p.s. The observation of spin–spin coupling in TFAA, where purine is most probably protonated at N-1,[8a] prompted us to run purine in aqueous acid solution to determine whether splitting might not be observable here also, although it had not been previously reported. Our results in neutral deuterium oxide (20 mg./0.4 ml. of deuterium oxide) showed no splitting (δ values in parts per million from TSPS: H-2, 8.88; H-6, 9.01; H-8, 8.59). We accordingly measured the p.m.r. spectra of purine in water at several pH values between 6 and <0. Since pK_{a_1} for purine in water at 20° is 2.39 and pK_{a_2} is 8.39,[8b] at pH 6.01 the predominant species is the neutral molecule, for which no spin–spin splitting is observed. At pH 2.4 approximately half of the solute molecules must be protonated, and splitting begins to be observed as indicated by broadening of the H-2 and H-6 peaks. Below pH 0.5 the predominant species is the cation and splitting is clearly observable. Spectra at pH 6.01 and 0.28 are shown in Figure 1. These spectra were scanned at a reduced sweep rate of 0.1 c.p.s./sec. Not only was H-6 found to be spin–spin coupled to H-2 ($J = 1.05 \pm 0.05$ c.p.s.), but H-6 shows an additional splitting of about 0.3 c.p.s., which was shown to be caused by spin–spin coupling with H-8. The assignments of $J_{2,6}$ and $J_{6,8}$ were established by measuring the spectra of purine-6-*d* and purine-8-*d* under identical conditions. These results suggest that the coupling between H-2 and H-6 in the neutral species is collapsed because of the electric quadrupole moment of N-1, but, when the symmetry of the electric field at N-1 is made more nearly spherical by protonation, coupling is observed. Because of the nitrogen quadrupole broadening of the resonances of H-2 and H-8, it is not possible to tell whether long-range coupling takes place between these two protons without a double-resonance experiment, nor, for the same reason, can we be certain that the coupling between H-6 and H-8 does not take place in neutral solution.

2,6-Disubstituted Purines.—Our results for 2,6-disubstituted purines are most complete for DMSO solutions and are shown in Table II. Since only one of the three C–H ring protons is present in this series, its assignment is certain. The data in Table II are arranged in order of increasing δ, *i.e.*, decreasing shielding. It is immediately apparent that the strongly electron-donating substituents, such as amino and dimethylamino, are increasing the shielding of H-8.

TABLE II
CHEMICAL SHIFTS OF 2,6-DISUBSTITUTED PURINES IN DIMETHYL SULFOXIDE

Compound	Substituents 2	Substituents 6	Concn., g./dl.	δ_{H-8}
1	$-NMe_2$	$-NH_2$	5.0	7.72
2	$-NH_2$	$-NH_2$	5.0	7.76
3	$-NH_2$	-SMe	5.0	7.93
4	-SMe	$-NMe_2$	5.0	7.97
5	$-NH_2$	-Me	2.6	7.98
6	-SMe	$-NH_2$	5.0	8.02
7	-F	$-NMe_2$	5.0	8.03
8	-Cl	$-NH_2$	<2.5[a]	8.13
9	-F	$-NH_2$	5.0	8.16
10	-SMe	-SMe	5.0	8.32
11	-Cl	-OMe	5.0	8.45
12	-Et	-Cl	10.0	8.58
13	-Cl	-Me	10.0	8.64
14	-H	-H	5.0	8.68
15	-Br	-Br	5.0	8.70
16	-Cl	-Cl	10.0	8.77
17	$-CF_3$	-Cl	5.0	8.97

[a] Saturated solution.

We attempted to correlate δ_{H-8} with several of the quantities commonly used as measures of the electron-withdrawing power of substituents. The most satisfactory measure we found was Brown's[10] electrophilic substituent constant, σ_p^+, considered to be a measure of substituent effects when there is considerable direct resonance interaction between the substituent and a ring carbon atom, in this case C-8. The 17 compounds in Table II fit eq. 1 with a correlation coefficient $r =$

$$\delta_{H-8} = (8.655 \pm 0.018) + (0.353 \pm 0.029)\sigma_{p_2}^+ + (0.332 \pm 0.029)\sigma_{p_6}^+ \quad (1)$$

0.984, and a standard deviation in δ_{H-8} of ±0.066 p.p.m.[11] Since this multiple regression equation shows the effects of the two substituents on δ_{H-8} to be equal within the indicated precision of the fit, the relation may be more simply represented as is shown in eq. 2 and in Figure 2.

(9) R. A. Robinson and R. H. Stokes, "Electrolyte Solutions," 2nd Ed., Butterworth and Co. (Publishers) Ltd., London, 1959, p. 546.

(10) H. C. Brown and Y. Okamoto, *J. Am. Chem. Soc.*, **80**, 4979 (1958).

(11) The data were fitted to this multiple linear regression equation by the method of least squares. The calculations were performed on a General Precision LGP-30 digital computer using a program of statistical subroutines written by Mr. W. F. Burggrabe, Jr., Compumatix, Inc., Clayton, Mo.

$$\delta_{H\text{-}8} = (8.658 \pm 0.017) + (0.342 \pm 0.016)\Sigma_{2,6}\sigma_p^+ \quad (2)$$

This line also fits the data with a correlation coefficient of 0.984, and the standard deviation in $\delta_{H\text{-}8}$ is also ±0.066 p.p.m., about twice our estimated error in the measurement of $\delta_{H\text{-}8}$.

We tried several other measures of substituent effects on $\delta_{H\text{-}8}$; among them were Hammett's σ_m and σ_p, Taft's σ_I and σ_R, and Wilmshurst's group electronegativities.[12] Hammett's σ_p gave a fair fit, eq. 3, with

$$\delta_{H\text{-}8} = (8.49 \pm 0.03) + (0.60 \pm 0.08)\sigma_{p_2} + (0.62 \pm 0.08)\sigma_{p_6} \quad (3)$$

$r = 0.960$ and a standard deviation in $\delta_{H\text{-}8}$ of ±0.10 p.p.m. However, the intercept in this case, $\delta_{H\text{-}8} = 8.49$ p.p.m., does not agree well with the experimental value found for purine (8.68 p.p.m.). The intercept found in eq. 1 and 2, 8.66 p.p.m., agrees within experimental error with the value observed for purine and thus provides additional support for the choice of σ_p^+ as the best measure of substituent effects on $\delta_{H\text{-}8}$. The fit with σ_R gave $r = 0.912$ and a standard deviation in $\delta_{H\text{-}8}$ of ±0.15. Little correlation, $r \cong 0.5$, was found with σ_I or with group electronegativity.

Only limited data on 2,6-disubstituted purines are available in TFAA. Values of $\delta_{H\text{-}8}$ for six compounds plus purine are shown in Table III. From these limited data it may be seen that the two amino-containing compounds have abnormally high values of $\delta_{H\text{-}8}$. This deshielding effect is probably caused by protonation of the amino group in this solvent.

TABLE III

CHEMICAL SHIFTS OF 2,6-DISUBSTITUTED PURINES (5 g./dl. in TFAA)

Substituents			
2	6	$\Sigma_{2,6}\sigma_p^+$	$\delta_{H\text{-}8}$
-SMe	-SMe	−1.208	9.01
-Cl	$-NH_2$	−1.2	9.21
-SMe	$-NH_2$	−1.9	9.31
-Et	-Cl	−0.181	9.41
-H	-H	0	9.41
-Cl	-Cl	0.228	9.55
$-CF_3$	-Cl	0.726	9.71

(12) J. K. Wilmshurst, *J. Chem. Phys.*, **27**, 1129 (1957).

Discussion

From Figure 2 it is evident that points representing the chemical shifts of purines containing amino and dimethylamino groups fit the regression equation less well than points representing other purines.

When these compounds are eliminated from the data used to derive eq. 1, the equation becomes that shown in eq. 4. The correlation coefficient improves to $r =$

$$\delta_{H\text{-}8} = (8.688 \pm 0.013) + (0.361 \pm 0.041)\sigma_{p_2}^+ + (0.313 \pm 0.041)\sigma_{p_6}^+ \quad (4)$$

0.991, and values of $\delta_{H\text{-}8}$ calculated from eq. 4 agree with experimental values for $\delta_{H\text{-}8}$ within experimental error. However, compounds containing these two groups are not far enough off the line to justify their removal, and the range of σ_p^+ is considerably reduced if they are removed. In the case of the amino group, at least, the value of σ_p^+ which would be predicted from the p.m.r. data appears to vary with the electron-donating ability of the other substituent, as is shown in Table IV. In this table σ_p^+ (X) is the value of σ_p^+ for the other substituent, given in ref. 10, and σ_p^+ (NH_2) represents the p.m.r.-predicted value. Brown and Okamoto[10] point out that their values of σ_p^+ for $-NH_2$ and $-NMe_2$ are less reliable, as they were not based on the solvolysis of *t*-cumyl chlorides, their standard reaction, as were the other values of σ_p^+ we have employed. These data may indicate that there is no unique value of σ_p^+ for the amino group (nor perhaps for the dimethylamino group, though we have not enough data to substantiate this).

TABLE IV

PREDICTED VALUES OF σ_p^+ FOR THE AMINO GROUP

Purine	σ_p^+ (X)	σ_p^+ (NH_2)
2-NH_2-6-NH_2	...	−1.3
2-Cl-6-NH_2	0.114	−1.8
2-F-6-NH_2	−0.073	−1.5
2-NH_2-6-Me	−0.311	−1.7
2-SMe-6-NH_2	−0.604	−1.4
2-NH_2-6-SMe	−0.604	−1.6
2-NMe_2-6-NH_2	−1.7	−1.1