268. *The Preparation and Nuclear Magnetic Resonance Spectra of the N-Acetyl Derivatives of Imidazoles, Benzimidazoles, and Purines.*

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A number of *N*-acetyl derivatives of imidazoles, purines, and benzimidazoles have been prepared by a simple method of rather wide applicability. The nuclear magnetic resonance (proton) spectra of these *N*-acetyl products in chloroform solution have been studied in detail and compared with spectra of the corresponding parent heterocyclic compounds. Spectral assignments and effects of *N*-acetyl and methyl substituents upon the spectral parameters are presented and discussed with reference to implications for the chemical behaviour of these ring systems.

Various *N*-acyl- and *N*-phosphoryl-imidazoles have been implicated as possible important unstable intermediates in certain biological processes, *e.g.*, in the catalytic activity of hydrolytic enzymes containing the histidine grouping.[1] Spectroscopic evidence points also to the occurrence of *N*-acetylimidazole during the imidazole-catalyzed hydrolysis of *p*-nitrophenyl acetate, considered as a possible model for enzymic activity.[2] Hitherto, however, no spectroscopic evidence has been obtained for the occurrence of such intermediates in actual enzymic systems,[3] which may, of course, be due to the low concentrations of the proposed transitory species.

The widespread interest [1] in these acylimidazoles has prompted us to prepare and examine a number of *N*-acetyl-imidazoles, -purines, and -benzimidazoles by nuclear magnetic resonance spectroscopy. This approach lacks the sensitivity of ultraviolet

[1] Barnard and Stein, *Advances in Enzymology*, 1958, **20**, 51.
[2] Bender and Turnquest, *J. Amer. Chem. Soc.*, 1957, **79**, 1652.
[3] Dixon, Dreyer, and Neurath, *J. Amer. Chem. Soc* 1956. **78**. 4810.

absorption spectroscopy but is capable of providing more detailed and diagnostically reliable structural information pertinent to chemical behaviour and biological activity. Thus, for example, it has been shown that acetylation of purine does not occur with equal readiness at the two imidazole-nitrogen atoms and that in 6-methylpurine acetylation occurs exclusively at the 9-nitrogen atom.

No satisfactory general preparative methods seem to have been reported for the laboratory preparation of *N*-acylimidazoles,[4] although a few isolated syntheses of some complexity have appeared.[5,6] We have found, however, that treatment with acetic anhydride, followed by evaporation under a vacuum of the excess of acetic anhydride and the acetic acid produced, is a simple and virtually quantitative method for all cases attempted, except 2-methylimidazole which appears to form a 1 : 1 complex with acetic acid.

Experimental

Preparation of N-*Acetylimidazoles.*—Commercial imidazole, purine, benzimidazole, and 6-methylpurine were used without purification. Pure samples of 2- and 4-methylimidazole were provided by Southern Research Institute, Birmingham, Alabama, and 4,5-dimethylimidazole was prepared in this laboratory.[7]

These compounds were dissolved in an excess of acetic anhydride, and the residual anhydride and the acetic acid produced were evaporated under a vacuum. In all cases, except that of 2-methylimidazole, the *N*-acetylation was essentially quantitative and yielded no undesirable by-products, as shown by the nuclear magnetic resonance spectra of the final products. When 2-methylimidazole, prolonged evacuation of the reaction mixture yielded an equimolar combination of the *N*-acetyl derivative and acetic acid, as indicated by the equal intensities of *N*-acetyl and acetyl-methyl peaks in the nuclear magnetic resonance spectrum of the product. Since repetition of the procedure led to the same results, it was concluded that *N*-acetyl-2-methylimidazole forms a salt or complex with acetic acid which is not decomposed by evaporation. No attempts were made to isolate free *N*-acetyl-2-methylimidazole since treatment with alkali or other reagents to decompose the salt or complex would also have hydrolyzed the acetyl derivative.

Acetylation occurs at the N-1, but because of the mobility of the N-1 proton, two *N*-acetyl derivatives are in principle possible for unsymmetrically substituted imidazoles. Thus, in purine the presence of the unsymmetrical pyrimidine ring leads to two *N*-acetyl derivatives, the 7- and the 9-position being acetylated in about 55 : 45 ratio. The proportion of these two isomers does not change appreciably when the reaction mixture is heated before evaporation under a vacuum. On the other hand, 6-methylpurine is acetylated at only the 9-position, and 4-methylimidazole only at the 1-position. In spite of the implications of steric hindrance in the above cases, 4,5-dimethylimidazole is acetylated quantitatively. The significance of these results is considered below.

Nuclear Magnetic Resonance.—Spectra were obtained on a Varian Associates model 4300B high-resolution spectrometer operating at 40 Mc./sec., and equipped with a flux stabilizer. The solvent used in most cases was deuterochloroform, with a small amount of tetramethylsilane as internal reference. For the less soluble benzimidazole the solvent was a mixture of deuterochloroform and dimethyl sulphoxide, with tetramethylsilane as internal reference.

Measurements were performed in relatively dilute solutions (*ca.* 25%), and concentration effects on the chemical shifts are accordingly expected to be negligible. Calibrations were effected by the superposition technique in the case of isolated single peaks, and by interpolation in the case of the more complex spectral patterns. The calibrations are accurate to $\sim\pm 0{\cdot}5$ c./sec. The relative shifts of imidazole and *N*-acetylimidazole were verified by calibrating the spectrum of a 1 : 1 mixture of the two compounds in chloroform.

All chemical shifts are expressed in τ values.

[4] Hofmann, in A. Weissberger's " The Chemistry of Heterocyclic Compounds," Vol. VI Imidazole and its Derivatives, Part I. Interscience Publ., Inc., New York, 1953.
[5] Boyer and Straw, *J. Amer. Chem. Soc.*, 1952, **74**, 4506.
[6] Boyer, *J. Amer. Chem. Soc.*, 1952, **74**, 6274.
[7] Brederick and Theilig, *Chem. Ber.*, 1953, **86**, 88.

Spectral Results and Assignments

Tables 1 and 2 summarize the chemical shifts and coupling constants obtained for the *N*-acetyl derivatives of the imidazoles (cf. *A*), purines (cf. *B*), and benzimidazole (*C*). The values in parentheses are the chemical shifts for the same position in the corresponding unacetylated parent compound. These values were obtained by first-order analysis of the spectral patterns, since, in all cases, the spin–spin coupling constants are small compared with differences in the chemical shifts of the coupled protons. Tables 3 and 4 present the *N*-acetyl substituent effects at the various positions, measured relative to the corresponding unacetylated parent

Table 1.

Chemical shifts (τ) and coupling constants (c./sec.) for *N*-acetylimidazoles.*

Ac deriv. of	2	4	5	Ac †	J_{24}	J_{25}	J_{45}
Imidazole	1·85	2·92	2·54	4·40	0·8	1·5	1·6
(parent)	(2·30)	(2·87)	(2·87)		(1·0)	(1·0)	
2-Methylimidazole	7·41	3·08	2·75	7·35	—	—	1·8
(parent)	(7·58)	(3·06)	(3·06)				
4-Methylimidazole	1·95	7·74	2·83	7·46	0·4	1·2	1·2
(parent)	(2·44)	(7·73)	(3·25)			(1·1)	(1·0)
4,5-Dimethylimidazole	2·06	7·84	7·64	7·42	—	—	—
(parent)	(2·41)	(7·81)	(7·81)				

* Values in parentheses are those for the corresponding unacetylated compound, where available (spectra analysed in these laboratories). For methyl substituents at a given ring position the entry under that position refers to the methyl protons. † Methyl protons of *N*-acetyl group.

Table 2.

Chemical shifts (τ) for *N*-acetyl derivatives of purines and benzimidazole.*

As deriv. of	Posn. of acetyl	2	6	8	Ac
Purine	9	0·80	0·94	1·21	6·92
(parent)		(0·94)	(1·11)	(1·66)	
Purine	7	0·44	0·80	1·25	7·17
(parent)		(0·94)	(1·11)	(1·66)	
6-Methyl purine	9	0·37	7·11	1·27	6·95
(parent)		(1·05)	(7·17)	(1·77)	
				(posn. 2) †	
Benzimidazole	1	- -	- -	1·45	- -
(parent)				(1·92)	
2-Methylbenzimidazole	1	—	—	7·19	7·26
(parent)				(7·35)	

* See footnote to Table 1. † Corresponds to position 8 of purine.

Table 3.

Acetyl substituent effects on chemical shifts (τ) in the imidazoles.*

	Position of protons					
Ac deriv. of	2	4	5	2-CH_3	4-CH_3	5-CH_3
Imidazole	−17·7	+2·0	−13·4	—	—	—
2-Methylimidazole	—	+0·8	−12·1	−6·9	—	—
4-Methylimidazole	−19·3	—	−16·7	—	+1·3	—
4,5-Dimethylimidazole	−14·0	—	—	—	+1·0	−7·0

* Values are in c./sec. at 40 Mc./sec. relative to the corresponding protons in the unacetylated parent compound (analysed in these laboratories).

compound. The signs of the coupling constants were not determined. The substituent effects are essentially independent of the tetramethylsilane reference since solvent and dilution effects are believed to be negligible under the conditions employed.

The spectrum of *N*-acetylimidazole (Fig. 1) has a first-order pattern, so the spacings in each proton group provided the coupling values without a detailed analysis and the centres of each

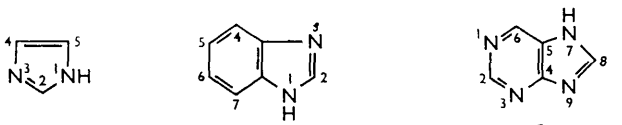
TABLE 4.

Acetyl substituent effects on chemical shifts (τ) in the purine and benzimidazole.*

Ac deriv. of	Posn. of acetyl	Position of protons: 2	6	8	6-CH_3
Purine	9	−5·3	−7·1	−18·0	—
Purine	7	−19·8	−12·3	−16·3	—
6-Methylpurine	9	−6·0	—	−20·0 (posn. 2) †	−2·0
Benzimidazole	1	—	—	−19·0	—
2-Methylbenzimidazole	1	—	—	−6·5	—

* See footnote to Table 3. † Corresponds to position 8 of purine.

group were taken as the chemical shifts of the corresponding protons. The assignments are based on those for imidazole, shown in parentheses, which are straightforward. The lowest shift in *N*-acetylimidazole clearly corresponds to the lowest value in imidazole and must, therefore, belong to H_2 (A in Fig. 1). Of the remaining two protons one is displaced up-field and the other down-field relative to the imidazole protons. For 4-methylimidazole and its acetylated derivative, since the primary effect of the methyl group should be steric, acetylation probably occurs at the nitrogen farther from it, indicating that this product is 1-acetyl-4-methylimidazole and not the 5-isomer. The 5-proton peak is shifted down-field (3·25 to 2·83 τ) on acetylation, allowing assignment of chemical shifts and spin–spin coupling constants for *N*-acetylimidazole by analogy.



(A) (B) (C)

For *N*-acetyl-2-methylimidazole (Fig. 2) the lowest-field peak (A) has an intensity equivalent to about one proton, and has been assigned as the hydroxyl proton of the acetic acid, which appears to form a salt (or 1 : 1 complex) with this acetylimidazole. The portion of the spectrum designated B corresponds to H_4 and H_5, and the small peaks X are believed to belong to a small amount of unacetylated 2-methylimidazole present. The separation in each doublet of B, 1·8 c./sec., is taken as the coupling constant J_{45}. Identification of H_4 and H_5 follow from the same considerations as were employed for imidazole, which indicates that the lower-field doublet corresponds to H_5. In the methyl region (C) there occur three peaks of almost equal intensity, the lowest-field peak of these being sharp, falling in the same region as the acetyl-methyl peak in imidazole, and being similarly identified in this case. The interior peak in the methyl region must be due to the 2-methyl group of the *N*-acetyl derivative, by comparison with the parent compound. The presence of some unchanged 2-methylimidazole accounts for the slightly greater intensity of the interior peak than of the other two of this group, since the methyl peak of the parent is very near that of the acetyl derivative. The remaining peak in the group (toward highest field) is assigned to the methyl group of acetic acid, since the addition of a small amount of the free acid increases its intensity. Finally, the down-field displacement of the 2-methyl shift, *ca.* −7 c./sec. on acetylation, corresponds well with the substituent effect on the H_2 shift, *ca.* −18 c./sec. (see Table 3).

In *N*-acetyl-4,5-dimethylimidazole (cf. Fig. 4), there is only one vinyl-proton (H_2) and this shifts to lower field on acetylation (see Tables 1 and 2), as in the previous cases. In the methyl region the peak at lower field is lower than the methyl peaks in the unacetylated parent compound and is accordingly assigned to the 5-position. The remaining peak (4-methyl) is about 1·0 c./sec. higher than the methyl peaks in 4,5-dimethylimidazole, in accord with the behaviour noted for 4-methylimidazole.

The spectrum (Fig. 5) of *N*-acetylbenzimidazole, the methyl region consists of a single peak, but the vinyl region is a complex pattern arising from the 4-spin ABCD system on the benzene ring. Peak A, clearly not a part of the adjacent pattern, is assigned to H_2 (which corresponds to proton H_2 in imidazole). The remaining benzene proton pattern will be ignored as irrelevant

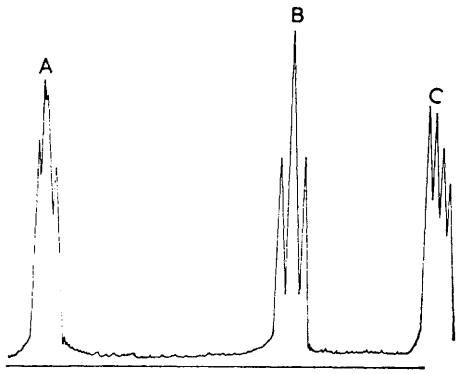


FIG. 1. Nuclear magnetic resonance spectrum of 1-acetylimidazole in $CDCl_2$.

(A) H_2. (B) H_5. (C) H_4. The methyl peak of the acetate group is not shown. In this and other Figures, increasing field strength is towards the right. The scale is to be inferred from the τ values given in the text for labelled peaks.

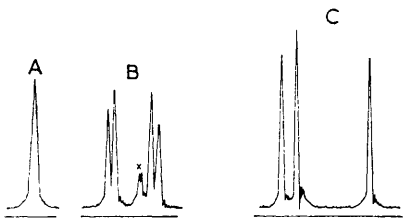


FIG. 2. Spectrum of 1-acetyl-2-methylimidazole in $CDCl_3$.

(A) N^+ proton. (B) H_4 and H_5. (C) Methyl groups. The compound is believed to be in the protonated form with acetic acid.

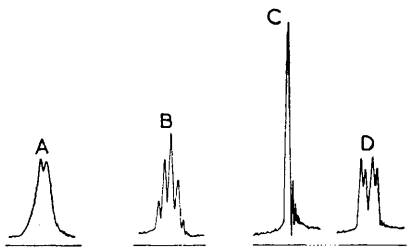


FIG. 3. Spectrum of 1-acetyl-4-methylimidazole in $CDCl_3$.

(A) H_2. (B) H_5. (C) Acetate-CH_3. (D) 4-CH_3.

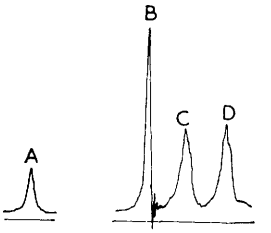


FIG. 4. Spectrum of 1-acetyl-4,5-dimethylimidazole in $CDCl_3$.

(A) H_2. (B) Acetate-CH_3. (C) 5-CH_3. (D) 4-CH_3.

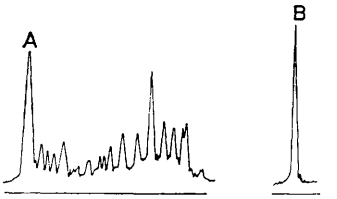


FIG. 5. Spectrum of 1-acetylbenzimidazole in $CDCl_3$.

(A) H_2. (B) Acetate-CH_3. The six-membered ring spectrum has not been analysed.

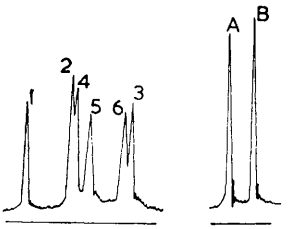


FIG. 6. Spectra of *N*-acetylpurines in $CDCl_3$.

Peaks 1, 2, 3, and B are due to the 7-acetyl derivative and peaks 4, 5, 6, and A to the 9-acetyl derivative.

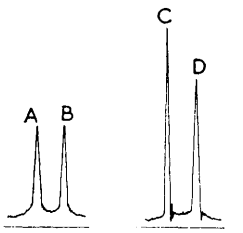


FIG. 7. Spectrum of 9-acetyl-6-methylpurine in $CDCl_2$.

(A) H_2. (B) H_8. (C) Acetate-CH_3. (D) 6-CH_3.

to this study. The symmetry of benzimidazole ensures that there will be only one *N*-acetyl derivative. The fact that H_2 shifts down-field by 19 c./sec. on acetylation is consistent with the behaviour of the corresponding proton (H_2) in the imidazoles upon acetylation.

For *N*-acetylpurine the spectrum shows that the two possible *N*-acetyl derivatives (7- and 9-) are both present in the reaction product. For example, the vinyl region contains two sets of three peaks each, of almost equal intensity, as also does the methyl region. The 7- and the 9-acetyl derivative are present in approximately 55 : 45 ratio, respectively, as determined from the integrated intensities of the two corresponding spectral patterns.

An assignment *a priori* of the patterns to the two derivatives is difficult. However, from the somewhat closer proximity of a 9-acetyl group to the nitrogen atom of the six-membered ring it is expected that the corresponding methyl peak would be at lower field than that of the 7-acetyl derivative.

The vinylic protons can be assigned on considerations of diamagnetic anisotropy. On inductive grounds, protons 2 and 8, both adjacent to two nitrogen atoms, should lie at about the same field, with H_6 somewhat higher. However, since it can be assumed that the " ring-current " anisotropy of the 6-membered ring will be greater than that of the 5-membered ring,[8] it follows that H_2 will be at lowest field, followed in order by H_8 and H_6. Support for this assignment is provided by the observation that the position of H_8 should not vary greatly for the two acetyl derivatives. On this basis, it seems reasonable to assign the peaks at 1·25 and 1·21 τ (see Table 2) to H_8 in the 7- and the 9-derivative, respectively. These assignments are also consistent with those in *N*-acetyl-6-methylpurine, where replacement of one proton by a methyl group has simplified the vinyl region (see below). The *N*-acetyl effect on the single remaining purine proton in the 6-methyl derivative can reasonably be used to assign the corresponding proton in purine itself. Table 4 shows that assignment of the low-field purine proton to H_2 leads to a consistent set of *N*-acetyl substituent effects.

The spectrum of 9-acetyl-6-methylpurine (Fig. 7) shows that only one acetyl derivative has been formed. The high-field peak in the vinyl region is due to H_8 and the low-field peak to H_2 for the same reasons as in the case of purine. In the methyl region the peak at 6·95 τ is assigned to the acetyl-methyl protons by comparison with the corresponding group in purine. The peak at 7·12 τ is then to be assigned to the 6-methyl group. It can be argued that acetylation occurs exclusively at the 9-position because of steric hindrance by the 6-methyl group. If so, the two acetylated derivatives of purine can be identified by comparing their acetyl-methyl shifts with that in the acetylated 6-methylpurine, since the 6-methyl substituent should affect a 9-acetyl group only slightly. The values of ν(Ac) in the last column of Table 2 show that the assignments chosen lead to very good agreement of ν(Ac) in the two 9-acetyl structures.

Discussion

In the nuclear magnetic resonance spectrum of the 1 : 1 complex of 2-methylimidazole and acetic acid the low-field peak, believed to be due to an OH or NH proton, is in the same region as the *N*-proton of imidazole at higher concentration. (The *N*-proton moves from about −3·75 τ below tetramethylsilane at about 14% in deuterochloroform to about 7·00 τ at infinite dilution and was apparently exchanged between imidazole molecules.[9]) It, therefore, seems that the complex is the protonated species (I) of the 1-acetyl-2-methyl-imidazole, with the proton exchanging between nitrogen and the acetic acid anion. The 2-methyl substituent increases the basicity of the nitrogen atoms slightly, which could account for the observed behaviour. In support of this explanation, it has also been observed that 2-methylimidazole itself initially forms a 1 : 2 complex with acetic acid which is converted into a 1 : 1 product (acetyl derivative) only upon prolonged evacuation.

Acetylation of 4-methylimidazole and 6-methylpurine yielded in each case only one

(I)

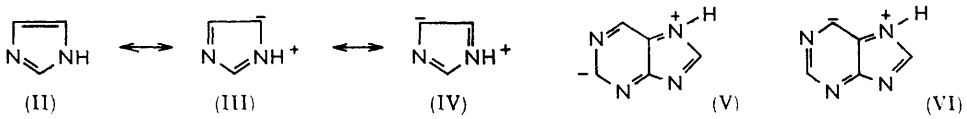
[8] Goldstein and Reddy, unpublished work.

[9] Reddy, Hobgood, Jr., and Goldstein, Abs. 140th Meeting Amer. Chem. Soc., Chicago, Sept. 3—8, 1961.

product in which the acyl group is as far removed from the methyl group as possible. The fact that 6-methylpurine is acylated exclusively at the 9-position is of interest since various 6-substituted purines appear to be linked through the 9-position in biological systems, *e.g.*, guanosine. However, in purine itself, substitution at the 7-position is slightly favoured over that at the 9-position.

The 6-methyl effect on the shift of H_2 in purine is +10·0 c./sec., which is comparable to that for pyrimidine.[10] An identical up-field shift is observed on methylating the *N*-acetyl derivative, which is in keeping with the previously postulated constancy of methyl substituent effects.[10,11] Also, the 9-acetyl effect of −7·0 c./sec. on H_2 in purine is duplicated with 6-methylpurine. The acetyl effect on H_2 in the imidazoles and benzimidazole and on the analogous proton, H_8, in purines is likewise constant. The shifts of the methyl protons at the same position are also constant and about one-third of the magnitude of the corresponding proton shift.

The constant down-field shift of about 19 c./sec. of the proton adjacent to the acetyl position in all these compounds can be explained, at least in part, by the electron-withdrawing effect of the acetyl group and in part by possible anisotropy effects of the carbonyl group. It is of interest that these differences of chemical shifts, at the various positions in these bases, on acetylation can be rationalized by resonance theory. For imidazole, for example, the extreme resonance form (III) would contribute more than (IV) to the resonance hybrid, since (III) has a linearly conjugated system and would thus be of



(II) (III) (IV) (V) (VI)

relatively lower energy than the cross-conjugated system (IV). Thus, on acetylation one would expect the 5-position to feel the electron-withdrawing effect of the acetyl group to a greater extent than the 4-position. The surprisingly slight up-field shift of the proton at the 4-position can be explained by a decrease in the ring-current anisotropy effect due to the general withdrawal of electrons from the ring by the acetyl group. The ring-current anisotropy would serve to deshield the nuclear protons,* and if this decrease were greater than the inductive deshielding effect of the acetyl group the small observed up-field shift would be explained.

Thus, of the two resonance extremes (V, VI) of the tautomeric precursor of 7-acetylpurine that influence the electron density of the 2- and the 6-position, form (V) should be of lower energy and consequently of greater import since in it electrons are sent to C-2 which, being adjacent to two heteroatoms, should be more electronegative than C-6. This, from an argument analogous to that for imidazole above, is in accord with the fact that acetylation at the 7-position affects the 2-position more than the 6-position.

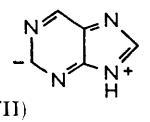
For 9-acetylpurine, the resonance extreme (VII) directs the charge to the more electronegative 2-position, yet form (VIII) comprises a linearly conjugated system (broken line) in contrast to the cross-conjugated system in (VII). It is not surprising, therefore, that in 9-acetylpurine the peaks for 2- and 6-substituents are shifted down-field by approximately equal amounts relative to purine.

The effects of methyl substitution on the chemical shifts in imidazole total 15—20 c./sec. in an up-field direction.[10] However, for *N*-acetylimidazoles (see Table 3) the total effect is much smaller and fails to show the expected considerable and consistent up-field trend. In fact, 2-methyl substitution in *N*-acetylimidazole is accompanied by a down-field

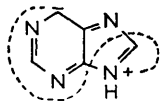
* The ring-current effect on both 2- and 4(5)-protons in imidazole has been obtained as ∼−38·0 c./sec. This effect on the methyl group in 2-methylimidazole is about −18·0 c./sec., which almost completely disappears in lysidine, 2-methyl-2-imidazoline.

[10] Reddy, Hobgood, Jr., and Goldstein, *J. Amer. Chem. Soc.*, 1962, **84**, 336.
[11] Reddy and Goldstein, *J. Amer. Chem. Soc.*, 1961, **83**, 2045, 5020.

displacement of 2 c./sec. in the acetyl-methyl shift (Table 1). The overall conclusion here is that charge transferred from the methyl group is probably largely localized in the carbonyl group or the N–CO bond, in which locations it cannot be determined directly by observation of proton resonance.



(VII)



(VIII)

The acetyl substituent effects in the purines (Table 4) show some interesting features. Although both 7- and 9-acetylpurine show the consistent and considerable down-field displacements to be associated with a primarily inductive withdrawal mechanism, the total effect is markedly larger for the latter compound, in spite of the rather close structural similarity between the two compounds. The difference in the shifts of H_2 in the two isomers is striking, *ca.* 15 c./sec. Comparison of the total acetyl effects for the imidazoles and purines suggests, moreover, that the latter are more polarizable.

The implications of the above observations for the chemical behaviour of the imidazoles and purines cannot be clarified without more detailed efforts to correlate these studies with other relevant data, which is beyond the scope of the present investigation. Nevertheless, the results obtained suggest ways in which both steric and electronic factors may be evaluated through analysis of nuclear magnetic resonance data.

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