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Structure Determination of the *N*-Methyl Isomers of 5-Amino-3,4-dicyanopyrazole and Certain Related Pyrazolo[3,4-*d*]pyrimidines

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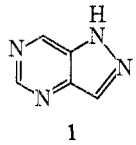
The position of N substitution of certain substituted 4-aminopyrazolo[3,4-*d*]pyrimidine derivatives has been studied by chemical and spectroscopic techniques and has resulted in the assignment of structures to the pyrazole precursors of these compounds. The more abundant pyrazole resulting from treatment of tetracyanoethylene with methylhydrazine [identical with the single pyrazole isomer originally isolated by the same condensation procedure, C. L. Dickinson, J. K. Williams, and B. C. McKusick, *J. Org. Chem.*, **29**, 1915 (1964), which was not characterized definitively with respect to the position of the N substituent] has thus been assigned as 3-amino-4,5-dicyano-1-methylpyrazole on the basis of its conversion to a pyrazolo[3,4-*d*]pyrimidine identical with authentic 4-amino-2-methylpyrazolo[3,4-*d*]pyrmidine, rather than with the authentic 1-methyl isomer. The assigned structure has been verified by X-ray crystallographic determination of 3-amino-4,5-dicyano-1-methylpyrazole. Because 3-amino-4,5-dicyano-1-methylpyrazole would not be expected to be the more abundant pyrazole on the basis of previous work, a mechanism is proposed which accounts for its formation. Also studied was the position of tautomeric equilibrium in 3-amino-4,5-dicyanopyrazole. A consideration of the ^{13}C NMR spectrum of 3-amino-4,5-dicyanopyrazole, relative to those of 5-amino-3,4-dicyano-1-methylpyrazole and 3-amino-4,5-dicyano-1-methylpyrazole, as well as the *N*-acetyl derivatives of all three, indicated that the major tautomer was 5-amino-3,4-dicyano-1*H*-pyrazole. A comparison of the ultraviolet spectrum of this pyrazole with those of the two methylated isomers led to the same conclusion.

The considerable biological and medicinal activities of substituted pyrazolo[4,3-*d*]pyrimidines (1) and pyrazolo[3,4-*d*]pyrimidines (2) as adenine analogs and antagonists has



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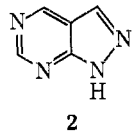

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contributed to the interest in the pyrazoles from which they are derived synthetically. Of special concern for many years has been a description of the position of N substitution in such pyrazoles. This information is usually not available by simple consideration of the reaction scheme by which a pyrazole is synthesized and appropriate methods for differentiation between such isomeric species are frequently less than obvious.

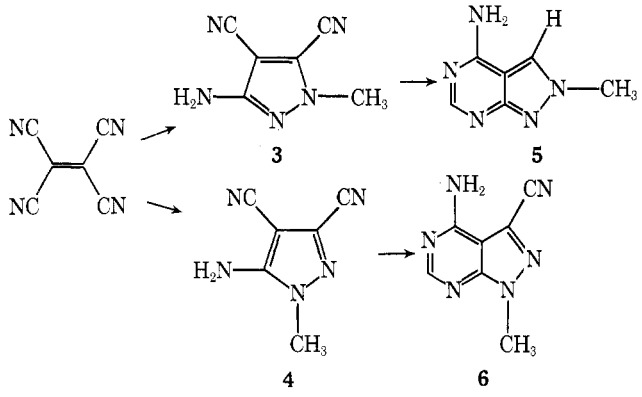
The question of the position of tautomeric equilibrium in pyrazoles which are not N substituted (e.g., **14a** ⇌ **14b**) has also been the subject of several studies,[1-3] as has the existence of individual tautomers as discrete substances.[4,5] Although the position of tautomeric equilibrium has been determined for several compounds by the use of molecular refractions or NMR spectroscopy, and the use of ultraviolet spectroscopy could be envisioned along similar lines, there is no well-established general method for such determinations.

This report is concerned with the chemical and spectroscopic determination of the position of N substitution in the isomeric N-methylated 5-amino-3,4-dicyanopyrazoles and compounds derived therefrom, as well as with the position of tautomeric equilibrium in these pyrazoles.

Results and Discussion

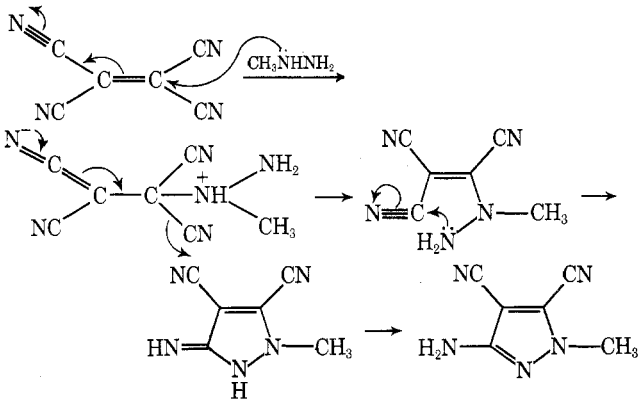
The difficulty in assigning correct structures to N-1- or N-2-substituted pyrazolo[3,4-*d*]pyrimidines can be attributed directly to the lack of available structural information concerning their pyrazole precursors. Substantial effort was expended in early studies in an attempt to prepare and characterize pyrazoles of authentic structure, including methods involving ring closures,[6,7] alkylations,[8,9] and selective dealkylations.[8,10,11] Subsequent studies, however, have rendered questionable many of the preparations of "authentic" samples. In addition, although at least three NMR studies have dealt with the problem of differentiating between isomeric pyrazoles,[2,3,12] the reported methods are not applicable in the present case.

A consideration of the mechanistic routes suggested in the literature for the formation of related pyrazoles illustrates the source of structural ambiguity in the formation of **3** and **4** from methylhydrazine and tetracyanoethylene. One might, for example, envision formation of the compound assigned structure **3** by conjugate addition of the more nucleophilic substituted nitrogen of methylhydrazine



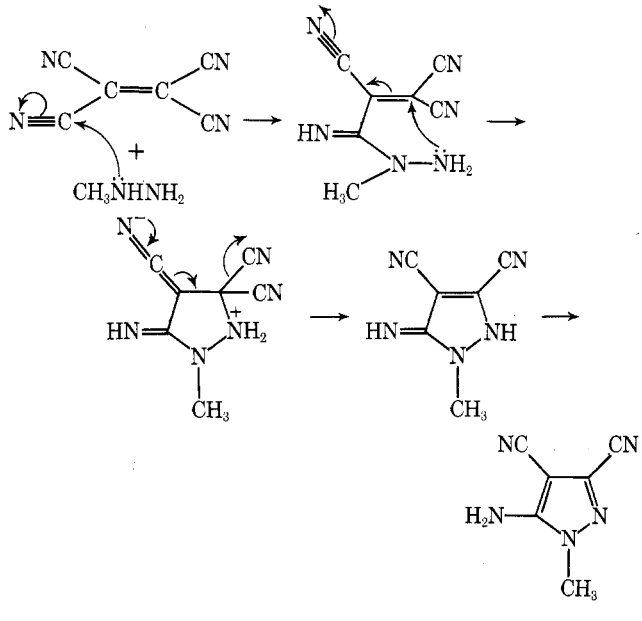
to tetracyanoethylene, followed by addition of the unsubstituted nitrogen of the hydrazine to a cyano group, affording the observed major isomer (Scheme I). Alternatively,

Scheme I



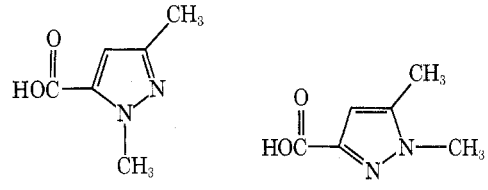
addition of the substituted nitrogen of methylhydrazine to the cyano group might occur first, followed by conjugate addition of the unsubstituted nitrogen to the intermediate olefin to afford pyrazole 4 (Scheme II).

Scheme II



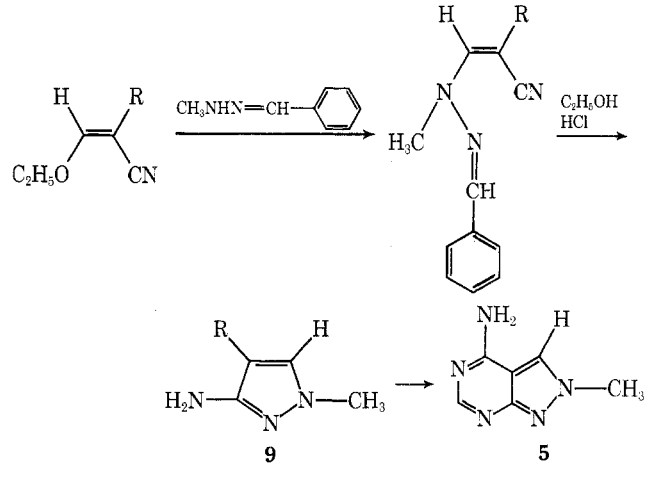
Moreover, although the substituted nitrogen atom in alkylhydrazines might be considered the better nucleophile in such additions,[13] it is also the more hindered nitrogen and reports have appeared in which the reaction products seem to be derived exclusively from initial nucleophilic attack by the unsubstituted nitrogen of methylhydrazine.[14-16] Since nucleophilic attack by the unsubstituted nitrogen atom according to Scheme I or II would afford the isomer opposite to that indicated in each case, the observed 2:1 ratio of **3:4** could ostensibly be due to a combination of initial nucleophilic 1,2 and 1,4 addition by a single nitrogen in methylhydrazine, or to nucleophilic attack by either nitrogen of methylhydrazine in a single type (1,2 or 1,4) of addition, or to some combination of these.

One of the earliest verified[2] examples of differentiation between two isomeric pyrazoles was recorded by von Auwers and Hollmann.[6,11] They were able to assign structures to 1,3-dimethylpyrazole-5-carboxylic acid (7) and 1,5-dimethylpyrazole-3-carboxylic acid (8) by virtue of the fact



that only one of the two respective 4-bromo derivatives could be esterified. That derivative was concluded to be related to compound **8**. In the present case, it was found that treatment of pyrazole **3** or 5-amino-3,4-dicyanopyrazole with acetic anhydride or pivaloyl chloride in pyridine at room temperature afforded the corresponding *N*-acetyl or *N*-pivaloyl derivatives in reasonable yield. Pyrazole **4** would not form the corresponding derivatives under the same conditions, but did form them at 50–60°. By analogy with the work of von Auwers and Hollmann,[6,11] this would suggest that the structural assignments for **3** and **4** should be 3-amino-4,5-dicyano-1-methylpyrazole and 5-amino-3,4-dicyano-1-methylpyrazole, respectively, since the amino group in the latter would be expected to be more hindered and therefore less reactive.

Schmidt and his coworkers[14,15] studied the reaction of ethyl ethoxymethylenecyanoacetate with the benzylidene adduct of methylhydrazine. This condensation must necessarily proceed by initial nucleophilic attack of the methyl-substituted nitrogen in methylhydrazine. Indeed, the initial adduct resulting from 1,4 addition was isolated and characterized, then shown to form 3-amino-4-carboethoxy-1-methylpyrazole (**9a**) when treated with ethanolic hydrochloric acid. The same reaction was carried out with ethoxymethylenemalononitrile to afford authentic 3-

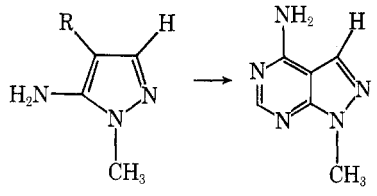


amino-4-cyano-1-methylpyrazole (**9b**). Analogous reaction utilizing methylhydrazine afforded, in each case, the isomeric pyrazoles, assumed to be 5-amino-4-carboethoxy-1-

Table I
Ultraviolet Spectra of Methylated 4-Aminopyrazolo[3,4-*d*]pyrimidines

Compd	λmax Acid	λmax Neutral	λmax Base
5[a]	268	289, 280 (sh), 266 (sh)	288, 281 (sh), 267 (sh)
4-Amino-1-methylpyrazolo[3,4-*d*]pyrimidine[b]	259	277, 268, 261	275, 262
4-Amino-2-methylpyrazolo[3,4-*d*]pyrimidine[b]	268	287, 270 (sh)	287

[a] Spectrum recorded in absolute ethanol. [b] Reference 18.



10 11

a, R = $COOC_2H_5$
b, R = CN

methylpyrazole (**10a**) and 5-amino-4-cyano-1-methylpyrazole (**10b**), respectively. Cyclizations of **9b** and **10b** were carried out[14,16] to afford authentic samples of 4-amino-2-methylpyrazolo[3,4-*d*]pyrimidine (**5**) and 4-amino-1-methylpyrazolo[3,4-*d*]pyrimidine (**11**), respectively. Montgomery et al.[17] have also reported the syntheses of the 1- and 2-β-D-ribofuranosyl derivatives of 4-aminopyrazolo[3,4-*d*]pyrimidine, which were assigned structures by comparison of their ultraviolet spectra with those of **5** and **11**.[18]

Treatment of tetracyanoethylene with methylhydrazine was reported to afford one *N*-methylpyrazole.[13] In our hands, the reaction afforded both possible *N*-methyl isomers, with the major product (53%) being the same as that reported in the literature. The two isomers were separated by chromatography on silica gel and the minor isomer was treated successively with triethyl orthoformate and ammonia,[19] thus effecting conversion to 4-amino-3-cyano-1-methylpyrazolo[3,4-*d*]pyrimidine (**6**). Similar ring closure of the more abundant pyrazole afforded a compound whose elemental analysis was consistent with the formula $C_7H_6N_6 \cdot C_2H_5OH$. The absence of a nitrile absorption in the infrared spectrum and the presence of a peak at *m/e* 220 in the mass spectrum suggested that this compound may have been ethyl 4-amino-2-methylpyrazolo[3,4-*d*]pyrimidine 3-carboximidate rather than 4-amino-3-cyano-2-methylpyrazolo[3,4-*d*]pyrimidine itself. This species was treated with aqueous sodium hydroxide to afford the corresponding 3-carboxylate, which was then fused to effect decarboxylation, thus yielding 4-amino-2-methylpyrazolo[3,4-*d*]pyrimidine (**5**). The ultraviolet spectrum of **5** was compared with those reported for authentic samples of 4-amino-1-methylpyrazolo[3,4-*d*]pyrimidine[16] and 4-amino-2-methylpyrazolo[3,4-*d*]pyrimidine.[14] As shown in Table I, the ultraviolet spectrum of **5** closely resembled that of the compound believed to be authentic 4-amino-2-methylpyrazolo[3,4-*d*]pyrimidine, rather than that of the authentic 1-methyl isomer. This may be more fully appreciated by a comparison of the ultraviolet spectrum of **5** with those of ribonucleoside analogs **12** and **13** (Figure 1), which were originally prepared by Montgomery et al.[17] and assigned structures by correlation of their ultraviolet spectra with

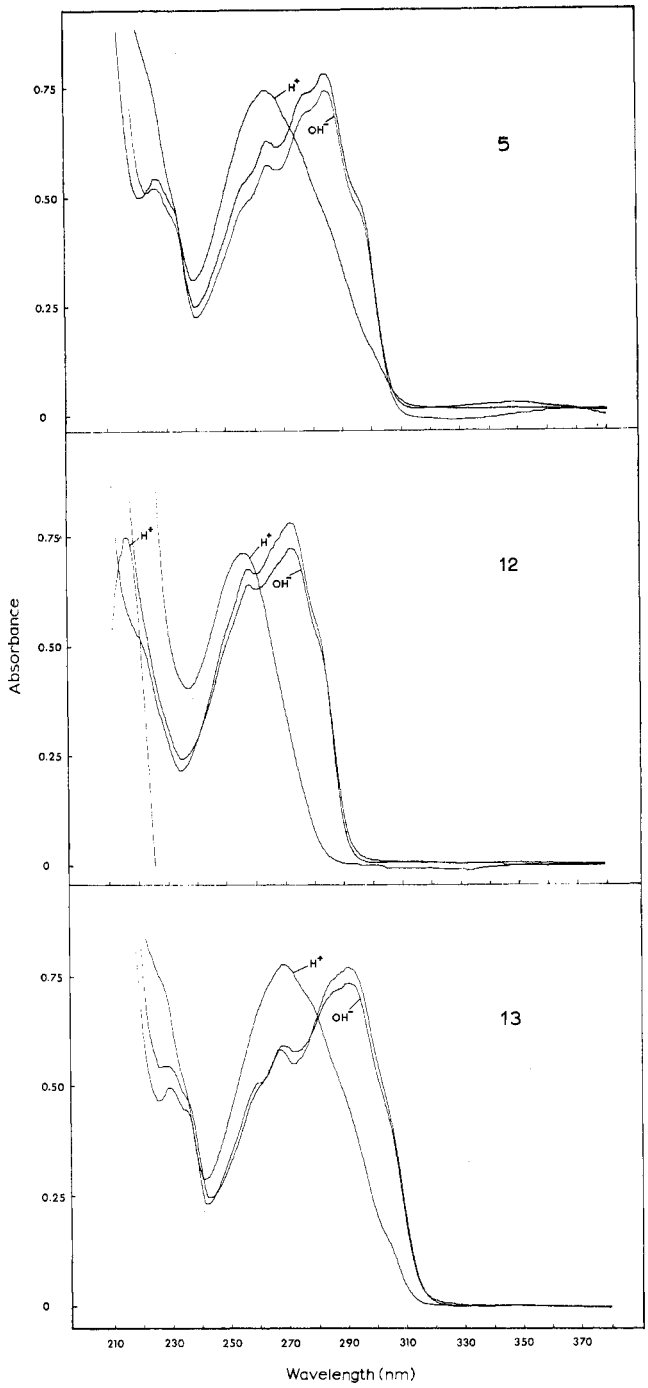
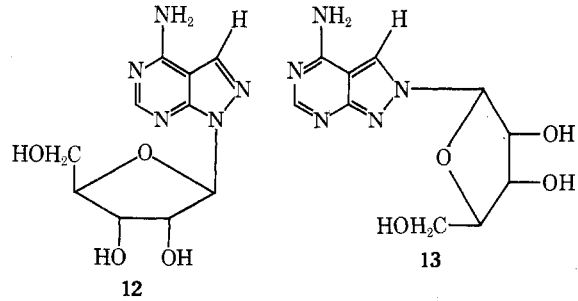


Figure 1. Comparison of the ultraviolet spectra of 4-amino-2-methylpyrazolo[3,4-*d*]pyrimidine (**5**), 4-amino-1-(β-D-ribofuranosyl)pyrazolo[3,4-*d*]pyrimidine (**12**) and 4-amino-2-(β-D-ribofuranosyl)pyrazolo[3,4-*d*]pyrimidine (**13**) in water at pH 1, 7, and 12.



12 13

those of authentic **5** and **11**.[20] This suggested strongly that the compound assigned structure **5** was best represented as 4-amino-2-methylpyrazolo[3,4-*d*]pyrimidine and that **3** was

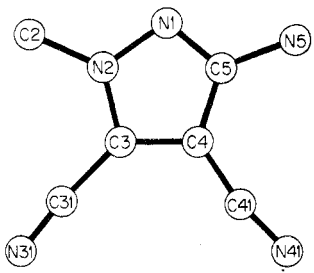


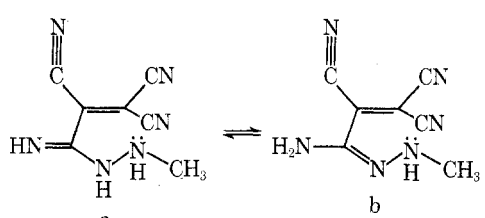
Figure 2. Relative positions of the nonhydrogen atoms in compound **3**, as determined by X-ray structure analysis.

3-amino-4,5-dicyano-1-methylpyrazole. The less abundant pyrazole was therefore assigned as 5-amino-3,4-dicyano-1-methylpyrazole (**4**) and its corresponding cyclized derivative **6** as 4-amino-3-cyano-1-methylpyrazolo[3,4-*d*]pyrimidine.[25]

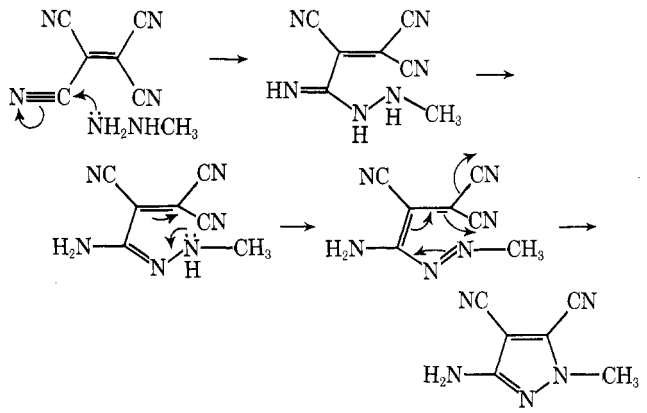
To verify the structures determined for the isomeric N-methylated pyrazoles, an X-ray structure analysis was carried out on the lower melting isomer of *N*-methyl-5-amino-3,4-dicyanopyrazole, which was identical with that isolated previously by Dickinson et al.[13] The X-ray structure determination indicated that the compound was 3-amino-4,5-dicyano-1-methylpyrazole (Figure 2), in agreement with the results obtained from chemical and spectroscopic studies.[27]

Previous studies of the condensation of monoalkyl and aryl hydrazines with ethoxymethylenemalonitrile and ethyl ethoxymethylenecyanoacetate indicated in each case the formation of pyrazoles identical with those which would form if the condensation were initiated by conjugate addition of the unsubstituted nitrogen of the alkyl or aryl hydrazine.[14-16] These findings were inconsistent with the prediction that alkyl and aryl hydrazines should afford pyrazoles of opposite N substitution, derived from initial conjugate addition by the more nucleophilic substituted and unsubstituted nitrogens, respectively.[13] The latter prediction finds superficial support in the present case, since the formation of 3-amino-4,5-dicyano-1-methylpyrazole (**3**) as the major condensation product of tetracyanoethylene and methylhydrazine would not be expected by analogy with the work of Schmidt et al.[14,15] and Cheng and Robins.[16]

However, the formation of **3** according to Scheme I, as indicated by Dickinson et al.,[13] would seem even less favorable than the corresponding additions of methylhydrazine to ethyl ethoxymethylenecyanoacetate and ethoxymethylenemalonitrile in the sense that the latter two involve additions to less hindered, more polarized double bonds and afford intermediate anions which are less electron deficient. That the additions of methylhydrazine to ethyl ethoxymethylenecyanoacetate and ethoxymethylenemalonitrile do not proceed via initial conjugate addition of the substituted nitrogen would seem to exclude the possibility that the less favorable addition to tetracyanoethylene can occur in this sense. The formation of compound **3** might also be thought to proceed by 1,2 addition of the unsubstituted nitrogen of methyl hydrazine to afford species a followed by cyclization via conjugate addition. Although this scheme is certainly plausible, the conjugate addition must

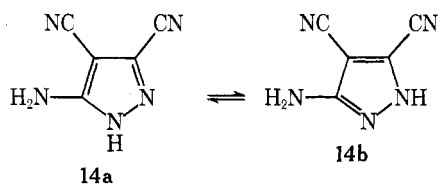


Scheme III

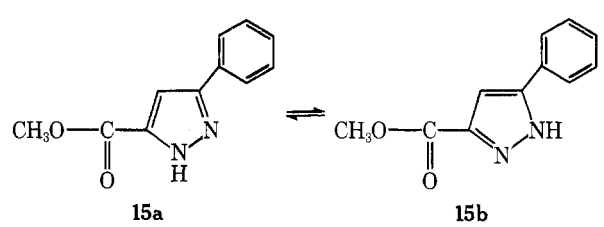


take place in the less favorable sense and the nucleophilicity of the methylated nitrogen is diminished by whatever tautomerization occurs between forms a and b. What may represent a more reasonable pathway to explain the formation of **3** is outlined in Scheme III, in which the condensation is initiated by 1,2 addition of the unsubstituted nitrogen of methylhydrazine to tetracyanoethylene.

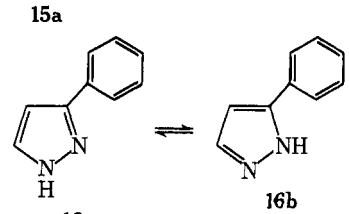
Also of interest for many years has been a description of the relationship between pyrazole tautomers (e.g., **14a** and **14b**). In the early literature, unsuccessful attempts to isolate individual tautomers were reported and these prompted the conclusion that the two forms were indistinguishable. In spite of the work of von Auwers,[1] which indicated that **15a** and **16a** were the predominant tautomeric species



14a **14b**



15a **15b**



16a **16b**

and that the 3 and 5 positions were therefore not equivalent, the belief that pyrazoles were properly represented as species in which the nitrogens were identical continued for some time. Hayes and Hunter,[4] e.g., indicated that pyrazoles existed as aggregates over which individual N-bound hydrogen atoms were delocalized and Hunter[5] later formulated this principle in general terms as "mesohydric tautomerism". Although evidence does exist for intermolecular hydrogen bonding in pyrazoles,[2,4] such bonds would not be symmetrical with respect to the heteroatoms to which they are attached so that the existence of distinct tautomers is possible.

The study of pyrazole tautomerism might in principle be facilitated by the use of ultraviolet spectroscopy, providing only that the particular isomers of interest have distinctly

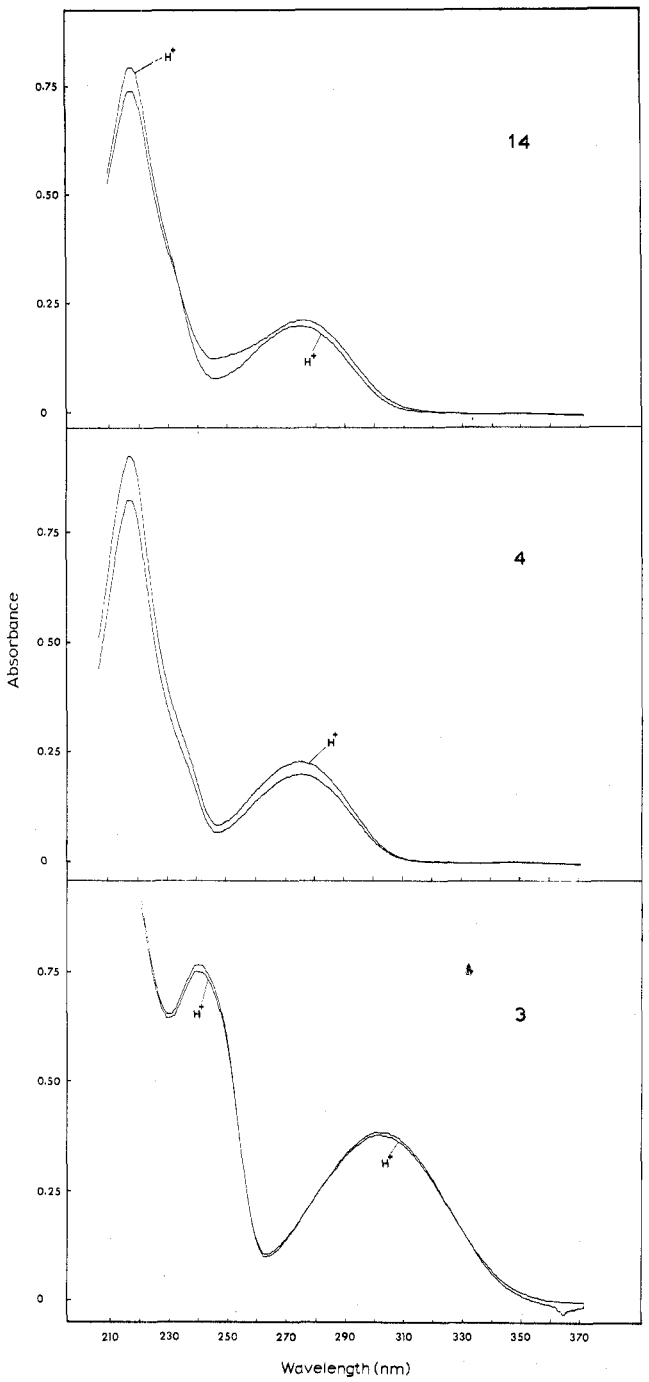


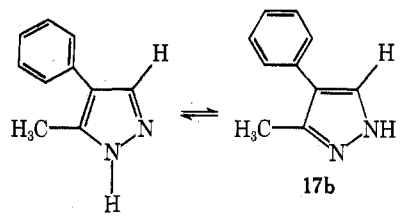
Figure 3. Ultraviolet spectra of 5-amino-3,4-dicyanopyrazole (14), 5-amino-3,4-dicyano-1-methylpyrazole (4), and 3-amino-4,5-dicyano-1-methylpyrazole (3). The spectra were determined in aqueous acid, pH 1, and in 0.01 *M* phosphate buffer, pH 6.7.

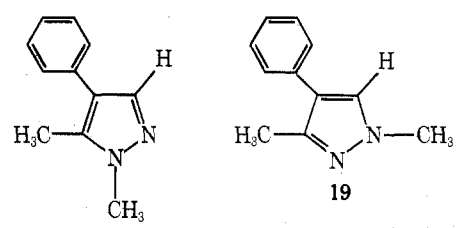
different spectra. Unfortunately, the differences between such isomers have sometimes been inadequate.[2] It has also been shown that proton magnetic resonance spectroscopy can be utilized to identify the major tautomeric species. Habraken and Moore,[2] e.g., showed that the chemical shift differences between the 3(5)-H proton absorption and those due to methyl and phenyl resonances in **17** were closer in magnitude to the corresponding chemical shift differences in **18** than to those in **19**, thus providing evidence that **17a** was the major tautomeric form of **17**. The original assignment of tautomeric preference to several 3(5)-phenylpyrazoles by von Auwers[1] was based on the molecular refractions of these pyrazoles as compared with those derived from the two N-alkylated derivatives of each 3(5)-phenylpyrazole.

Table II
Carbon-13 Chemical Shifts

	Compd					
Carbon atom	14	4	3	20	21	22
C-3	128.3	126.5	120.6	128.7	128.3	120.6
C-4	78.5	78.9	84.6	86.7	94.1	92.1
C-5	156.3	155.1	160.3	146.6	145.3	147.3
CN	115.6[b]	115.1[b]	111.6[b]	114.1[b]	113.4[b]	108.3[b]
CN	115.6[b]	115.1[b]	114.8[b]	115.1[b]	114.3[b]	110.8[b]
NCH_3		38.8	41.9		40.9	39.6
$CO^{13}CH_3$				26.0	25.9	22.3
CO				172.5	172.0	168.8

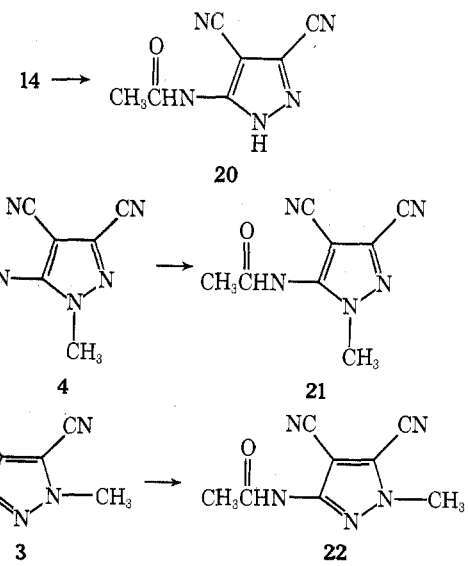
[a] Downfield from tetramethylsilane. [b] No attempt was made to assign these resonances to a specific CN group.





In the present case the study of the tautomeric equilibrium was facilitated by the characteristic chromophores associated with the methylated pyrazoles **3** and **4**. As shown in Figure 3, the ultraviolet spectra of **14** and **4** were essentially identical in the position and intensity of absorption maxima at pH 1 and 6.7, while that of **3** was different from **14** and **4** at both measured pH values. The similarity of the spectra of **14** and **4** suggested that the major tautomeric form of **14** was **14a**.

Also considered in this context were the ^{13}C NMR spectra of compounds **3**, **4**, **14**, and **20–22**. The obvious similarity of the spectra of pyrazoles **14** and **4** and their acetylated derivatives **20** and **21** (Figure 4, Table II), as compared



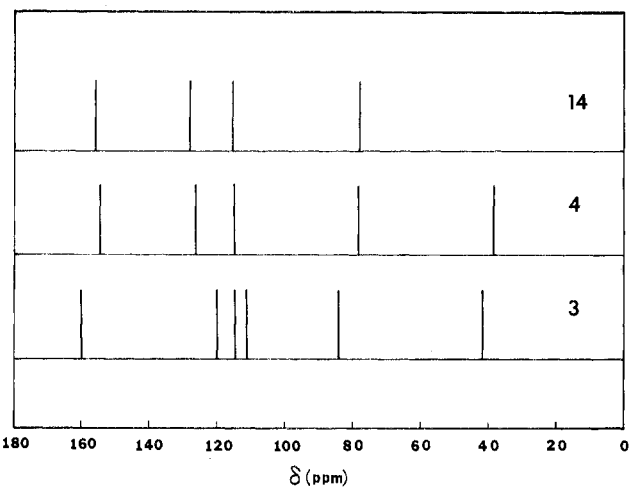


Figure 4. [^{13}C] NMR spectra of 5-amino-3,4-dicyanopyrazole (**14**), 5-amino-3,4-dicyano-1-methylpyrazole (**4**), and 3-amino-4,5-dicyano-1-methylpyrazole (**3**). The chemical shifts are given with respect to external TMS. Positive values represent decreased shieldings.

with the spectra of **3** and **22,** respectively, supported the hypothesis that **14a** was the major tautomer. The similarity of the spectra, of course, must necessarily be regarded in light of the spectral change which one would expect to accompany the formal N-methylation of a pyrazole.[28] For the unsubstituted pyrazole case, "methylation" changed the chemical shifts of carbons 3, 4, and 5 by a total of 9.4 ppm in absolute terms. This compared somewhat more favorably with the observed absolute change in chemical shifts accompanying the formal methylation of **14** to give **4** (3.4 ppm, 4.4 ppm with the cyano groups) than for absolute change associated with the formal conversion of **14** to **3** (17.8 ppm, 22.6 ppm with the cyano groups) and suggested that pyrazole **4** was structurally related to the major tautomer of **14.** A more definitive result was obtained corresponding to the formal methylation of **20** to yield **21** (9.1 ppm, 10.6 ppm with the cyano groups) and **22** (14.2 ppm, 24.3 ppm with the cyano groups), suggesting that **21** was structurally related to the major tautomer of **14.**

The known chemical shift differences of C-3, C-4, and C-5 in the spectra of pyrazole and 1-methylpyrazole may also be used to calculate the spectra of **4** and **3** from the recorded spectrum of **14.** Representation of the major tautomer as **14a** afforded calculated C-3, C-4, and C-5 values for **4** of 133.2, 79.0, and 152.3 ppm (Table II), based on the expected chemical shift differences[28] of 4.9, 0.5, and −4.0 ppm, respectively. This would imply that carbons 3, 4, and 5 in compound **3** must be related to carbons 5, 4, and 3 in the major tautomer, so that the calculated values of C-3, C-4, and C-5 for **3** (numbered as in **3**) would be 124.3, 79.0, and 161.2 ppm, based on expected respective shifts of −4.0, 0.5, and 4.9 ppm. The total difference between these six values and the observed values was 19.8 ppm. If, on the other hand, it was assumed that **14b** was the major tautomer, the total difference between the observed and calculated values of the six chemical shifts in **4** and **3** was 34.6 ppm. The better agreement between observed and calculated spectra in the former case again suggested that **14a** was the major tautomer. The same calculations, when carried out on the acetyl derivative **20,** afforded a value of 28.1 ppm on the assumption that the *N*-acetyl derivative of **14a** was the major tautomer and 39.3 ppm if the other tautomer was assumed to predominate, consistent with the results obtained for compounds **14, 4,** and **3.**

Elguero et al.[29] have recently reported on the ^{13}C NMR spectra of a number of azoles, including several pyrazoles, and concluded that the ^{13}C chemical shifts were of limited value in ascertaining the position of tautomeric equilibrium. Interestingly, when the additivity relationship presented here for such determinations was applied to the data reported by Elguero et al.,[29] a definite tautomeric preference was indicated, e.g., for 3(5)-methylpyrazole, 3(5)-phenylpyrazole, and 3(5)-methyl-5(3)-phenylpyrazole. The first two were predicted to exist as the 3-substituted 1*H*-pyrazoles and the third as 3-phenyl-5-methyl-1*H*-pyrazole. Although no independent verification can be made of the assignment of tautomeric preference to 3(5)-methylpyrazole, the latter two assignments are consistent with those made by von Auwers,[1] who reached the same conclusion by a study of the molecular refractions of these two compounds and their *N*-methyl and *N*-ethyl derivatives.

Experimental Section

Ultraviolet spectra were recorded on a Cary 15 uv spectrophotometer; measurements in ethanol at low or high pH were taken after the addition of 1 *N* aqueous HCl or 4 *N* aqueous NaOH, respectively. Infrared spectra were recorded on a Perkin-Elmer 457A spectrophotometer and mass spectra on a Perkin-Elmer Hitachi RMU-6 spectrometer using a direct inlet. Melting points were determined on a Thomas-Hoover apparatus and are corrected. Elemental analyses were determined by Chemalytics, Inc., or by Scandinavian Microanalytical Laboratory.

^{1}H NMR spectra were recorded on a Varian Associates T-60 NMR spectrometer. The carbon-13 NMR spectra of **1–6** were obtained at 22.63 MHz using a Bruker HFX-90 interfaced with a Digilab FTS/NMR-3 data system. Either DMSO or DMF was employed as the solvent and the reference.[30] The chemical shifts reported in Table I are in parts per million with respect to external tetramethylsilane (TMS) and were converted from the original data using $\delta_{DMSO} = 40.4$ or $\delta_{DMF} = 162.4$. Positive values represent decreased shieldings.

X-Ray Structure Analysis. A sample suitable for X-ray structure analysis, identical with the pyrazole originally isolated from the condensation of tetracyanoethylene with methylhydrazine,[13] was obtained by crystallization of the lower melting *N*-methylpyrazole from 50% aqueous methanol. Preliminary film data indicated the systematic absences $h0l$, $l = 2n + 1$, and $0k0$, $k = 2n + 1$; hence the space group was $P2_1/c$. The cell constants, determined from 12 medium angle reflections measured on a Picker automatic diffractometer with nickel filtered Cu Kα ($\lambda = 1.5418$ Å) radiation, were found to be $a = 6.199 \pm 0.003$ Å, $b = 15.168 \pm 0.005$ Å, $c = 7.646 \pm 0.003$ Å, and $\beta = 91.95 \pm 0.04°$. Complete three-dimensional intensity data were collected on the diffractometer up to a 2θ of 128°, employing the θ–2θ scan technique. A total of 1062 independent reflections were recorded of which 920 were considered observed [$I > 1.5\ \sigma\ (I)$] and were used in the structure analysis. The structure was solved by employing a combination of direct methods, trial and error, and difference Fourier techniques. The *R* value after block-diagonal least-squares refinement using anisotropic temperature factors for the nonhydrogen atoms and isotropic temperature factors for the hydrogen atoms was 0.07. The X-ray structure conforms to the chemical structure 3-amino-4,5-dicyano-1-methylpyrazole.

3-Amino-4,5-dicyano-1-methylpyrazole (3) and 5-Amino-3,4-dicyano-1-methylpyrazole (4). To a solution of 4.03 g (85.4 mmol) of methylhydrazine in 160 ml of water was added 10.93 g (87.6 mmol) of tetracyanoethylene in one portion. The resulting suspension was stirred at 0° for 1 hr and then heated on a steam bath for 45 min. The cooled solution was refrigerated for several hours and the precipitated solid was separated by filtration and air dried. The crude product, containing both **3** and **4,** was purified by chromatography on silica gel (3 × 9.5 cm) and elution with 9:1 chloroform–ethyl acetate to remove the major isomer (**3**) and then with ethyl acetate to remove the more polar isomer (**4**). The major isomer, mp 133–134°, was further purified by crystallization from water to afford **3** as colorless needles: yield 6.66 g (53%); mp 135–135.5° (lit.[13] mp 131.5–133°); λ_{max} (EtOH) (pH 1) 302 nm (ϵ 4700) and 239 (9300), λ_{min} 264 (800) and 229 (8100); λ_{max} (EtOH) (pH 7) 302 (4700) and 239 (9300), λ_{min} 263 (800) and 229 (8300); λ_{max} (EtOH) (pH 11) 298 (3700) and 240 (8400), λ_{min} 267 (2200) and 231 (7700); MS *m/e* 147, 122, 121, 120, 119, 104, 77, and 76; ir (Nujol) 3440, 3350, 3220, 2955, 2920, 2850, 2245, 2225, 1630, 1550, and 1520 cm^{-1}.

The more polar isomer, mp 238–242°, was further purified by preparative TLC on silica gel, elution with ethyl acetate, and finally by crystallization from ethanol and from ethyl acetate to afford **4** as colorless crystals: yield 3.38 g (27%); mp 244–247° (lit.[13] mp 243–245°); λ_{max} (EtOH) (pH 1) 275 nm (ϵ 4800) and 218 (21,000), λ_{min} 246 (2000); λ_{max} (EtOH) (pH 7) 274 (4600) and 218 (21,000), λ_{min} 247 (1700); λ_{max} (EtOH) (pH 11) 275 (4400), λ_{min} 257 (2700); MS *m/e* 147, 121, 120, 119, 104, 77, and 76; ir (Nujol) 3400, 3340, 3255, 3220, 2960, 2920, 2850, 2255, 2230, 1650, 1585, and 1510 cm^{-1}.

Anal. Calcd for $C_6H_5N_5$: C, 48.97; H, 3.42. Found: C, 48.76; H, 3.58.

4-Amino-2-methylpyrazolo[3,4-*d*]pyrimidine (5). A solution of 1.62 g (10.9 mmol) of 3-amino-4,5-dicyano-1-methylpyrazole (**3**) in 17 ml of triethyl orthoformate was heated to reflux for 7 hr. Excess orthoformate was removed under diminished pressure and the crystalline residue was dissolved in 35 ml of absolute ethanol and added dropwise at room temperature to a stirred solution of ethanol previously saturated with ammonia. The reaction mixture was stirred for 24 hr and the white precipitate which formed in quantitative yield was filtered: mp 238–242° dec; λ_{max} (EtOH) (pH 1) 295 nm, 281 (sh), and 248, λ_{min} 256 and 236; λ_{max} (EtOH) (pH 7) 308, 280, 269, and 252, λ_{min} 286, 275, 266, and 239; λ_{max} (EtOH) (pH 10) 305, 279, and 248, λ_{min} 285, 276, and 241 (Figure 2); MS *m/e* 220, 174, 158, 147, and 146; ir (Nujol) 3170, 1690, 1640, 1590, 1530, and 1480 cm^{-1}.

Anal. Calcd for $C_7H_6N_6 \cdot C_2H_5OH$: C, 49.07; H, 5.49. Found: C, 49.03; H, 5.15.

A suspension of this material (84 mg, 0.49 mmol) in 6 ml of 5% sodium hydroxide solution was warmed to 50° for 4 hr. Filtration afforded a clear solution which was acidified with dilute hydrochloric acid. After a few hours 4-amino-3-carboxy-2-methylpyrazolo[3,4-*d*]pyrimidine separated as colorless crystals: yield 73 mg (90%); mp > 300°; λ_{max} (EtOH) (pH 1) 313 nm, 302, 280 (sh), and 244, λ_{min} 310, 258 and 227; λ_{max} (EtOH) (pH 7) 297 (sh), 290, 280 (sh), and 240, λ_{min} 255 and 229, λ_{max} (EtOH) (pH 10) 302, 270 (sh), and 243, λ_{min} 258 and 233 (Figure 2); MS *m/e* 193, 149, 133, and 122.

Anal. Calcd for $C_7H_7N_5O_2 \cdot H_2O$: C, 39.80; H, 4.29. Found: C, 40.15; H, 4.35.

A portion of the carboxylic acid was pyrolyzed to afford a dark brown residue which was triturated with hot water, treated with charcoal to afford a clear solution, and then concentrated to give a white solid in 43% yield: mp >300° dec; λ_{max} (EtOH) (pH 1) 268 nm, λ_{min} 242; λ_{max} (EtOH) (pH 10) 288, 281 (sh), and 267 (sh), λ_{min} 242; MS *m/e* 149, 122, 107, 104, and 94.

4-Amino-3-cyano-1-methylpyrazolo[3,4-*d*]pyrimidine (6). Compound **6** was synthesized from 5-amino-3,4-dicyano-1-methylpyrazole and triethyl orthoformate by analogy with the synthesis of **5**: yield 75%; mp 309–312°; λ_{max} (EtOH) (pH 1) 286 nm (sh), 276 (ϵ 9900), 270 (sh), 241 (sh), 235 (12,000), and 228 (sh), λ_{min} 250 (5900) and 218 (10,800), λ_{max} (EtOH) (pH 7) 293 (sh), 286 (12,600), 241 (sh), and 237 (9700), λ_{min} 252 (4300) and 226 (7500); λ_{max} (EtOH) (pH 10) 292 (sh), 283 (11,100), and 243 (10,300), λ_{min} 258 (6700) and 226 (8100) (Figure 2); ir (Nujol) 3435, 3320, 3060, 2920, 2235, 1665, 1590, 1530, and 1515 cm^{-1}.

Anal. Calcd for $C_7H_6N_6$: C, 48.25; H, 3.47. Found: C, 48.34; H, 3.19.

5-Acetamido-3,4-dicyanopyrazole (20). To a solution of 400 mg (3.0 mmol) of 5-amino-3,4-dicyanopyrazole (**14**)[13] in 10 ml of pyridine was added 8.6 ml of acetic anhydride. The reaction mixture was maintained at room temperature for 16 hr, then concentrated under diminished pressure to afford a solid residue which was crystallized from ethanol (decolorization) to give colorless crystals of **20**: yield 364 mg (69%); mp 266.5–267° dec (lit.[13] mp 250° dec); λ_{max} (EtOH) (pH 1) 243 nm and 215, λ_{min} 231; λ_{max} (EtOH) (pH 7) 243 and 216, λ_{min} 230; ir (Nujol) 3270, 3120, 3070, 2960, 2920, 2855, 2250, 1710, 1705, 1685, and 1590 cm^{-1}.

5-Acetamido-3,4-dicyano-1-methylpyrazole (21). To a solution of 147 mg (1.0 mmol) of 5-amino-3,4-dicyano-1-methylpyrazole (**4**)[13] in 3.4 ml of pyridine was added 2.8 ml of acetic anhydride. The reaction mixture was maintained at room temperature for 24 hr, which afforded no change, and then at 50° for 14 hr. The solution was concentrated under diminished pressure to give a yellow solid which was decolorized with charcoal and then purified by chromatography on silica gel and crystallization from ethanol to afford **21** as colorless crystals: yield 127 mg (67%, 84% based on consumed starting material); mp 167°; λ_{max} (EtOH) (pH 10) 258 nm, λ_{min} 250 nm; MS *m/e* 189, 174, 161, 148, 147, and 146; ir (Nujol) 3180, 2960, 2920, 2850, 2245, 1685, and 1545 cm^{-1}.

Anal. Calcd for $C_8H_7N_5O$: C, 50.79; H, 3.73. Found: C, 50.53; H, 3.64.

The isomeric 3-acetamido-4,5-dicyano-1-methylpyrazole (**22**) was obtained from 3-amino-4,5-dicyano-1-methylpyrazole (**3**) as above, except that the reaction mixture was maintained at room temperature for 24 hr or at 60° for 5 hr. The product was obtained as colorless crystals from ethanol (decolorization): yield 41%; mp 172–173°; λ_{max} (EtOH) (pH 1) 271 nm and 223, λ_{min} 257; λ_{max} (EtOH) (pH 7) 271 and 223, λ_{min} 257; λ_{max} (EtOH) (pH 10) 305 and 237, λ_{min} 283 and 231; ir (Nujol) 3200, 2960, 2920, 2850, 2235, 1725, 1675, 1585, and 1510 cm^{-1}.

3,4-Dicyano-5-trimethylacetamidopyrazole. A solution of 408 mg (3.06 mmol) of 5-amino-3,4-dicyanopyrazole[13] in 10 ml of pyridine was added dropwise to a cooled, stirred solution of 406 mg (3.35 mmol) of pivaloyl chloride in 5 ml of pyridine. The combined solution was stirred at room temperature for 14 hr and then concentrated under diminished pressure to afford a yellow, crystalline product which was recrystallized from ethanol (decolorization) to give colorless crystals of the pivaloyl derivative: yield 380 mg (66%); mp 199–199.5°; λ_{max} (EtOH) (pH 1) 298 nm, 262 (sh), and 256, λ_{min} 272 and 239; λ_{max} (EtOH) (pH 7) 298, 262 (sh), and 256, λ_{min} 272 and 239; λ_{max} (EtOH) (pH 10) 277 and 246, λ_{min} 264 and 236; ir (Nujol) 3440, 3310, 3235, 3160, 2960, 2920, 2855, 2235, 2230, 1725, 1715, 1635, 1550, and 1505 cm^{-1}.

Anal. Calcd for $C_{10}H_{11}N_5O$: C, 55.29; H, 5.10. Found: C, 55.23; H, 4.93.

3,4-Dicyano-1-methyl-5-trimethylacetamidopyrazole. To a solution containing 179 mg (1.22 mmol) of 5-amino-3,4-dicyano-1-methylpyrazole (**4**) in 5 ml of pyridine was added dropwise a solution containing 202 mg (1.66 mmol) of pivaloyl chloride in 3 ml of pyridine. The reaction mixture was warmed to 50° for 3 hr, which afforded no change, and then to 60° for 13 hr. The solution was concentrated under diminished pressure to afford a residue which was crystallized from ethanol (decolorization) to afford 67 mg of **4**. The mother liquors were purified by preparative silica TLC and elution with ethyl acetate, to afford an additional 20 mg of **4** as well as the desired product, which gave colorless crystals from ethanol: yield 87 mg (31%, 60% based on consumed **4**); mp 174.5–176°; λ_{max} (EtOH) (pH 10) 262 nm, λ_{min} 252; MS *m/e* 231, 230, 217, 216, 203, 202, 188, 187, 174, 173, 172, 148, 147, and 146; NMR (DMSO-d_6) δ 1.33 (s, 9 H) and 3.80 (s, 3 H).

The isomeric 4,5-dicyano-1-methyl-3-trimethylacetamidopyrazole was obtained from 3-amino-4,5-dicyano-1-methylpyrazole (**3**) as above, except that the reaction mixture was maintained at room temperature for 14 hr. The product was obtained as white crystals after recrystallization from ethanol (decolorization): yield 80%; mp 174.5–175°; λ_{max} (EtOH) (pH 1) 265 nm and 222, λ_{min} 258; λ_{max} (EtOH) (pH 7) 265 and 222, λ_{min} 258; MS *m/e* 231, 217, 216, 203, 189, 188, 174, 160, 148, 147, and 146; NMR (DMSO-d_6) δ 1.25 (s, 9 H) and 4.00 (s, 3 H).

Anal. Calcd for $C_{11}H_{13}N_5O$: C, 57.13; H, 5.67. Found: C, 56.96; H, 5.63.

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Registry No.—**3,** 54385-48-7; **4,** 50680-85-8; **5,** 21230-48-8; **6,** 42204-41-1; **12,** 3258-05-7; **13,** 3258-06-8; **14,** 54385-49-8; **20,** 54385-50-1; **21,** 54385-51-2; **22,** 54385-52-3; methylhydrazine, 60-34-4; tetracyanoethylene, 670-54-2; triethyl orthoformate, 122-51-0; ethyl 4-amino-2-methylpyrazolo[3,4-*d*]pyrimidine 3-carboximidate, 54385-53-4; 4-amino-3-carboxy-2-methylpyrazolo[3,4-*d*]pyrimidine, 54385-54-5 3,4-dicyano-5-trimethylacetamidopyrazole, 54385-55-6; pivaloyl chloride, 3282-30-2; 3,4-dicyano-1-methyl-5-trimethylacetamidopyrazole, 54385-56-7; 4,5-dicyano-1-methyl-3-trimethylacetamidopyrazole, 54385-57-8.

Supplementary Material Available. A table of atomic positional coordinates for compound **3** will appear following these pages in the microfilm edition of this volume of the journal. Photocopies of the supplementary material from this paper only or microfiche (105 × 148 mm, 24× reduction, negatives) containing all

of the supplementary material for the papers in this issue may be obtained from the Journals Department, American Chemical Society, 1155 16th St., N.W., Washington, D.C. 20036. Remit check or money order for $4.00 for photocopy or $2.50 for microfiche, referring to code number JOC-75-1815.

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(20) Because all of these structural assignments rested ultimately on the correctness of the structures of 4-amino-1-methylpyrazolo[3,4-*d*]pyrimidine[16] and 4-amino-2-methylpyrazolo[3,4-*d*]pyrimidine,[14] independent verification of the structures assigned to the "authentic" compounds was sought. On the assumption that the isomer structurally related to adenosine would more nearly resemble that nucleoside in its activity in certain bioassays, **12** and **13** were synthesized and compared for biological activity in those bioassays.[21] Compound **12** (but not **13**) was a substrate for adenosine deaminase and (as the diphosphate) polynucleotide phosphorylase. Compound **12** was also found to be cytotoxic to mouse fibroblasts, while compound **13** was inactive (Hecht et al., in preparation).
(21) Although the compound presumed to be 4-amino-1-(β-D-ribofuranosyl)pyrazolo[3,4-*d*]pyrimidine has been shown to be a substrate for certain adenosine-utilizing enzymes, e.g., adenosine deaminase[22] and adenosine kinase,[23] the isomeric ribofuranoside has not been tested for comparative purposes. Since it has been shown that isoadenosine [6-amino-3-(β-D-ribofuranosyl)purine] is a (weak) substrate for adenosine deaminase[24] and adenosine kinase,[23] it was obviously important to compare the activities of isomers **12** and **13** in bioassays before using the results to assign structures.
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A Chemical and Carbon-13 Nuclear Magnetic Resonance Reinvestigation of the *N*-Methyl Isomers Obtained by Direct Methylation of 5-Amino-3,4-dicyanopyrazole and the Synthesis of Certain Pyrazolo[3,4-*d*]pyrimidines[1]

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A reinvestigation of the structural assignments for the isomeric *N*-1- and *N*-2-methyl derivatives of 5-amino-3,4-dicyanopyrazole (**1**), obtained by direct methylation, according to the published procedure, has been accomplished. The higher melting isomer (**2**, mp 243–245°) was annulated to give 4-amino-3-cyano-1-methylpyrazolo[3,4-*d*]pyrimidine (**4**) and alkaline peroxide converted **4** into 4-amino-1-methylpyrazolo[3,4-*d*]pyrimidine-3-carboxamide (**7a**). Hydrolysis of the cyano group of **4** under more vigorous conditions gave 4-amino-1-methylpyrazolo[3,4-*d*]pyrimidine-3-carboxylic acid (**7b**), which was subsequently decarboxylated in hot sulfolane to afford 4-amino-1-methylpyrazolo[3,4-*d*]pyrimidine (**7c**) of established structure. This established the structure of the *N*-methylpyrazole (mp 243–245°) as 5-amino-3,4-dicyano-1-methylpyrazole (**2**) and reversed the structural assignment previously reported for **2**. A similar reaction sequence converted the lower melting isomer (**3**, mp 128–130°) into 4-amino-2-methylpyrazolo[3,4-*d*]pyrimidine (**8d**) and established the structure of **3** as 5-amino-3,4-dicyano-2-methylpyrazole. ^{13}C NMR spectroscopy has furnished additional corroboration for these structural reassignments.

We have been involved for some time in the synthesis of nucleosides which are related to the naturally occurring pyrazolo[4,3-*d*]pyrimidine nucleosides[2] formycin and formycin B and the pyrrolo[2,3-*d*]pyrimidine nucleosides[3a] tubercidin, toyocamycin, and sangivamycin. The significant antitumor activity reported[3b] for these nucleoside antibiotic analogs (vide supra) prompted us to extend our investigation into the pyrazolo[3,4-*d*]pyrimidine area. It was during this phase of our research that we synthesized a ribofuranosyl derivative of 4-amino-3-cyanopyrazolo[3,4-*d*]pyrimidine (**12**).[4] This required the preparation of the *N*-1- and *N*-2-methyl derivatives of 4-amino-3-cyanopyrazolo[3,4-*d*]pyrimidine (**12**) so that an unequivocal assignment for the site of ribosylation could be made on the basis of uv spectral data.[5,6] However, a survey of the literature revealed that these *N*-methyl derivatives of **12** had not yet been reported. The most obvious approach to the synthesis of these desired model methyl compounds appeared to be a ring closure of the known[7] *N*-1- and *N*-2-methyl derivatives[8] of 5-amino-3,4-dicyanopyrazole (**2** and **3**, respectively). However, assignments[7] for the actual sites of methylation for **2** and **3** were found on closer examination to be equivocal and this prompted us to initiate the present study which was designed to unequivocally establish the actual sites of methylation.[5]

It was reported that the reaction of methylhydrazine with tetracyanoethylene gave a single *N*-methyl derivative of 5-amino-3,4-dicyanopyrazole which was reported[7] to be the *N*-1-methyl derivative **2**. On the other hand, reaction of 5-amino-3,4-dicyanopyrazole (**1**) with dimethyl sulfate was