7548-94-9; **12c**, 51464-63-2; **13a**, 2140-46-7; **13b**, 10525-22-1; **14**, 2665-04-5; **15**, 10525-24-3; **16b**, 24281-79-6; **17**, 51373-32-1; **18a**, 22145-68-2; **18b**, 24281-78-5; **18c**, 34679-19-1; **19**, 51373-33-2.

References and Notes

(1) This work was supported by Grants AM 12156, CA 13369, and GM 19882 from the National Institutes of Health and by Grants GB 36201 and GB 23801-A1 from the National Science Foundation.
(2) (a) Extracted in part from the thesis of J. P. M. to be submitted to the University of Orleans, Orleans, France, in partial fulfillment of the requirements for a Doctorat d'Etat. (b) Postdoctoral Fellow, 1969–1970.
(3) E. Caspi and P. J. Ramm, *Tetrahedron Lett.*, 181 (1969).
(4) P. Bladon, J. M. Fabian, H. B. Henbest, H. P. Koch, and G. W. Wood, *J. Chem. Soc.*, 2402 (1951).
(5) J. M. Zander, and E. Caspi, *J. Biol. Chem.*, **245**, 1682 (1970), and references cited therein.
(6) E. Lederer, *Quart. Rev., Chem. Soc.*, **23**, 453 (1969).
(7) T. J. Scallen, *Biochem. Biophys. Res. Commun.*, **21**, 149 (1965).
(8) I. D. Frantz, and M. L. Mobberley, *Fed. Proc.*, **20**, 285 (1961); I. D. Frantz, J. E. Grev, and M. Marita, *Progr. Biochem. Pharmacol.*, **5**, 24. (1969).
(9) A. J. Birch and K. A. M. Walker, *J. Chem. Soc. C*, 1895 (1968).
(10) J. J. Pappas, W. P. Keaveney, E. Gancher, and M. Berger, *Tetrahedron Lett.*, 4273 (1966); K. Sakai and K. Tsuda, *Chem. Pharm. Bull.*, **11**, 529 (1963).
(11) J. Cason and J. S. Correia, *J. Org. Chem.*, **26**, 3645 (1961).
(12) J. Hooz and S. S. H. Gilani, *Can. J. Chem.*, **46**, 86 (1968).
(13) M. Andrac, *Ann. Chim. (Paris)*, (13) **9**, 287 (1964).
(14) C. Djerassi, *Pure Appl. Chem.*, **21**, 205 (1970).
(15) N. S. Bhacca and D. H. Williams, "Applications of NMR Spectroscopy in Organic Chemistry," Holden-Day, San Francisco, Calif., 1964.
(16) W. R. Nes and J. A. Steele, *J. Org. Chem.*, **22**, 1457 (1957).
(17) K. R. Varma, J. A. F. Wickramasinghe, and E. Caspi, *J. Biol. Chem.*, **244**, 3951 (1969).
(18) F. Reindel, E. Walter, and H. Rauch, *Justus Liebigs Ann. Chem.*, **452**, 42 (1927).
(19) R. Ikan, A. Markus, and Z. Goldschmidt, *J. Org. Chem.*, **37**, 1892 (1972).
(20) E. M. Burgess, H. R. Penton, Jr., and E. A. Taylor, *J. Amer. Chem. Soc.*, **92**, 5224 (1970); *J. Org. Chem.*, **38**, 26 (1973).
(21) P. Crabbé and C. Léon, *J. Org. Chem.*, **35**, 2594 (1970).
(22) S. Bernstein, L. S. Binovi, L. Dorfman, K. J. Sax, and Y. Subbarow, *J. Org. Chem.*, **14**, 433 (1949); A. E. Bide, H. B. Henbest, E. R. H. Jones, R. W. Peevers, and P. A. Wilkinson, *J. Chem. Soc.*, 1783 (1948).
(23) W. R. Nes, R. B. Kostic, and E. Mosettig, *J. Amer. Chem. Soc.*, **78**, 436 (1956).
(24) E. Caspi and G. F. Scrimshaw, "Steroid Hormone Analysis," H. Carstensen, Ed., Marcel Dekker, New York, N. Y., 1967, p 55.
(25) U. H. M. Fagerlund and D. R. Idler, *J. Amer. Chem. Soc.*, **79**, 6473 (1957).
(26) T. J. Scallen and W. Krueger, *J. Lipid Res.*, **9**, 120 (1968).

Pyrazolopyrimidine Nucleosides. V. Methylation of the C-Nucleoside Antibiotic Formycin and Structural Elucidation of Products by Magnetic Circular Dichroism Spectroscopy[1]

Leroy B. Townsend,*[2] Robert A. Long, James P. McGraw, Daniel W. Miles, Roland K. Robins, and Henry Eyring

Department of Chemistry and Department of Biopharmaceutical Sciences, University of Utah, Salt Lake City, Utah 84112

Received December 12, 1973

The direct methylation of formycin (**9**) has furnished the two monomethyl derivatives, 7-amino-1-methyl-3-(β-D-ribofuranosyl)pyrazolo[4,3-*d*]pyrimidine (**10**) and 7-amino-2-methyl-3-(β-D-ribofuranosyl)pyrazolo[4,3-*d*]pyrimidine (**12**). An unequivocal assignment of the above structures was made by a comparison of the magnetic circular dichroism (MCD) curves obtained for the model compounds 7-amino-2,3-dimethylpyrazolo[4,3-*d*]pyrimidine (**6**) and 7-amino-1,3-dimethylpyrazolo[4,3-*d*]pyrimidine (**7**) with the MCD spectra of **10** and **12**. The unequivocal synthesis of **6** and **7** was accomplished by ring annulation of the appropriately substituted pyrazole precursors. The synthesis of 1,3-dimethylpyrazolo[4,3-*d*]pyrimidin-7-one (**8**) and 2-methyl-3-(β-D-ribofuranosyl)-pyrazolo[4,3-*d*]pyrimidin-7-one (**11**) was accomplished by an unusual displacement of the exocyclic amino group in 1 *N* sodium hydroxide.

The antibiotics formycin and formycin B were isolated[3] from *Norcardia interforma* and found[4-6] to be C-nucleosides which were isomeric with the naturally occurring nucleosides adenosine and inosine, respectively. These antibiotics are of considerable interest since they are C-nucleosides and belong to the same class of compounds as showdomycin,[7] pseudouridine,[8] and pyrazomycin.[9] Formycin has demonstrated inhibition of Ehrlich carcinoma, mouse leukemia L-1210, Yoshida rat sarcoma, HeLa cells, and *Xanthomonas oryzae* as well as some antiviral activity.[3,12] Formycin 5′-triphosphate acts as a source of biological energy[13] and ribopolynucleotides with formycin replacing adenosine, at the binding site of t-RNA to ribosomes, have shown[14] no mistranslation of the messenger. In fact, formycin has shown the ability to act as a substrate for a number of enzymes specific for adenosine, including adenosine kinase[11] and, unfortunately, adenosine deaminase.[10] The resemblance of formycin to adenosine is thus apparent in many biological systems. Since formycin is such an excellent substrate for adenosine deaminase, this would suggest that although formycin hydrobromide has been found to exist in the syn conformation, there must be a population of formycin in the anti conformation in solution and *in vivo*. In fact, a recent X-ray study[15] has revealed that formycin, *per se*, exists on the average somewhere between the classical syn and anti forms (amphi form[16]) in the solid state. A recent study has established that adenosine derivatives in the syn conformation are not substrates for adenosine deaminase and this prompted us to initiate a study[17] designed to restrict rotation around the glycosyl (carbon–carbon) bond of formycin and increase the per cent of nucleoside in the syn conformation.

The isomeric purine nucleosides, when alkylated on an imidazole nitrogen, form salts with a positively charged heterocyclic ring[18] which can then undergo a facile ring opening.[19] However, formycin presents a unique opportunity to alkylate a ring nitrogen of a bicyclic nucleoside without the usual quaternization. These alkylated derivatives of formycin should be chemically very similar to formycin and yet the 2-alkyl derivative should exhibit

Table I
Ultraviolet Absorption Spectral Data for Some Substituted Pyrazolo[4,3-*d*]pyrimidines

Compd	pH 1 λ_{max}, nm	$\epsilon \times 10^{-3}$	pH 11 λ_{max}, nm	$\epsilon \times 10^{-3}$	MeOH λ_{max}, nm	$\epsilon \times 10^{-3}$
9	295	10.15	302	7.90	304	7.20
	232.5	8.28	234	17.90	293.5	10.55
					286[a]	9.88
					230	5.87
3	282	7.80	282	8.47	278	8.96
5			282.5	7.66	290	6.00
			230	13.70	230	6.88
7	305	9.45	315[a]	6.04	315[a]	6.04
	241.5	12.55	303	9.30	303	9.48
			295[a]	8.80	297[a]	9.08
					233	9.62
2	278	6.96	278	7.89	272	6.48
4			278	4.49	276	4.49
			255[a]	2.72	255[a]	3.00
6	305	9.80	316[a]	6.20	316[a]	6.54
	270[a]	4.90	304	10.10	304	10.10
	258	6.54	294	8.65	294[a]	8.65
	234	15.10	232	8.50	232	7.84
12	305	11.24	317[a]	8.45	317[a]	8.85
	270	5.90	305	12.90	305	13.80
	260	6.05	295[a]	11.24	295[a]	11.80
	231	10.95	237	5.61	237	6.05
10	302	6.32	314[a]	3.93	314[a]	3.65
	236	7.03	301	6.46	301	6.18
			293[a]	6.19	293[a]	5.75
			232	6.51	231	5.20
11	284.5	9.30	310[a]	8.60	283	10.15
			299	13.80		
			291[a]	12.70		
			228.5	7.05		

[a] Shoulder.

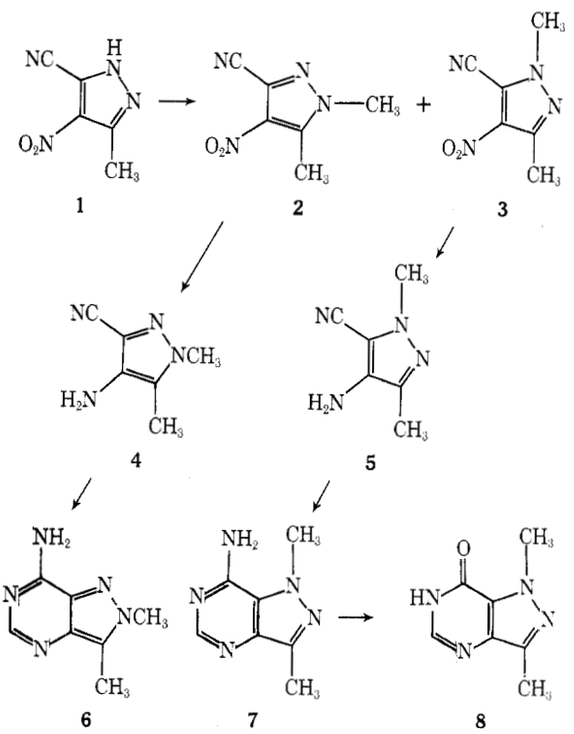
some steric restriction toward rotation around the glycosyl bond and decrease the population of nucleoside in the anti conformation.

Results and Discussion

Since alkylation of formycin could lead to a number of products, depending on the pH of the solution and the alkylating agent used, we selected conditions designed to facilitate preferential alkylation of the pyrazole moiety. The monosodium salt of formycin was prepared and then alkylated by the addition of excess methyl iodide. Chromatography (tlc) of the reaction mixture revealed the presence of three products. The two major components were isolated and purified by dry column chromatography while the third product was not isolated, since it was estimated to be present in only a very small quantity. Therefore, with the isolation of two products, we were required to establish the actual site of methylation for each product.

The initial structural elucidation studies[5] of formycin and formycin B confirmed the similarity of their ultraviolet spectra with the ultraviolet spectra of the corresponding 7-substituted 3-methylpyrazolo[4,3-*d*]pyrimidines.[20] This prompted us to synthesize the appropriate 3-methylpyrazolo[4,3-*d*]pyrimidines [7-amino-1,3-dimethylpyrazolo[4,3-*d*]pyrimidine (**7**) and 7-amino-2,3-dimethylpyrazolo[4,3-*d*]pyrimidine (**6**)] in order to establish the actual site of methylation of formycin (*vide supra*). It has been established that the most facile synthesis of the pyrazolo[4,3-*d*]pyrimidine moiety can be accomplished by ring annulation of the appropriately substituted pyrazole precursor.[20,21] Therefore, we selected 5-cyano-1,3-dimethyl-4-nitropyrazole[22] (**3**), which had been prepared by the nucleophilic displacement of a chloro group by cyanide, as our starting material for the synthesis of the model compound **7**. The nitro group was reduced with sodium hydrosulfite to furnish 4-amino-5-cyano-1,3-dimethylpyrazole (**5**) and treatment of **5** with formamidine acetate afforded a good yield of **7** (Scheme I).

Scheme I



The synthesis of 3-cyano-1,5-dimethyl-4-nitropyrazole (**2**) by a similar route was then initiated. The synthesis of 3-chloro-1,5-dimethyl-4-nitropyrazole was accomplished, but repeated attempts to displace the chloro atom with cyanide in dimethylformamide under the same and even more forceful conditions as those that yielded **3** were not successful. The synthesis of this compound (**2**) was finally accomplished by methylation of 5-cyano-3-methyl-4-nitropyrazole[21] (**1**). The isomeric 5-cyano-1,3-dimethyl-4-nitropyrazole (**3**) was also formed but only in a very low yield. The nitro group of **2** was reduced with sodium hydrosulfite and ring closure with formamidine acetate yielded **6**.

As stated above, when this investigation was initiated, we had expected to ascertain the actual site of methylation of formycin by a comparison of the ultraviolet spectra of the formycin derivatives with the ultraviolet spectra of the model compounds **6** and **7**. However, unlike the closely related pyrazolo[3,4-*d*]pyrimidine ring system, the ultraviolet spectra of the model compounds **6** and **7** were found to be very similar (Table I). Therefore, a comparison of the ultraviolet and pmr spectra of the monomethyl derivatives of formycin (*vide supra*) and the model compounds **6** and **7** allowed us to make only a very tentative assignment of structure for the specific methylformycins.

Formycin has been reported[13] to be fluorescent; therefore, owing to the difference in their structures, we expected the methylformycins to produce dissimilar fluorescent spectra. However, fluorescent spectra of the methylformycins and the model compounds **6** and **7** were obtained and no definitive conclusions could be drawn as to their unequivocal structural assignment.

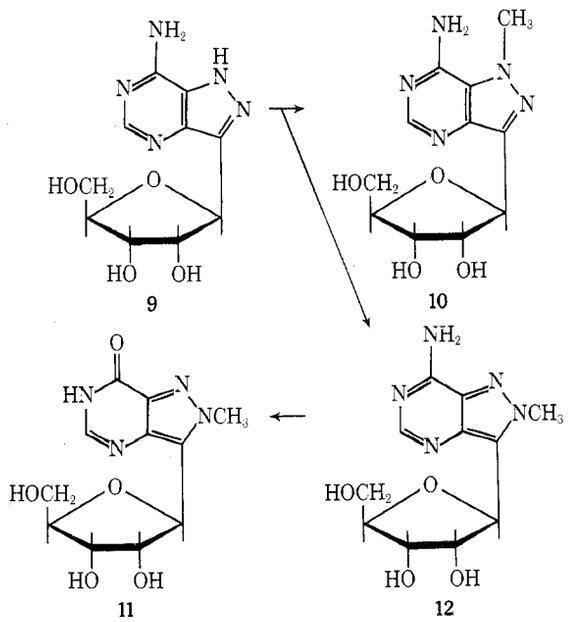
We have recently observed a very close similarity between the magnetic circular dichroism (MCD) spectra of 7-methylpurine[23] and 7-(β-D-ribofuranosyl)purine[24] in our laboratory. MCD should theoretically provide more information than that obtained by the usual spectrophotomet-

ric techniques owing to negative bonds and the increased sensitivity to changes in electronic structure. This sensitivity of MCD spectra to the precise chromophoric structure has been shown to provide definitive spectra for isomeric compounds, *e.g.*, the *N*-methyl (1, 3, 7, and 9) derivatives of purine. Therefore, although the MCD spectra of 7-methylpurine and 7-(β-D-ribofuranosyl)purine are very similar, there was observed a significant difference between 7-methylpurine and the isomeric 1-, 3-, and 9-methylpurines. This prompted us to obtain the MCD spectra of **6**, **7**, and the methylated formycins. The MCD spectra (Figure 1) have provided a very clear distinction between the two methylated derivatives of formycin and allowed us to make definite structural assignments.[25]

Of particular interest is the fact that the spectra observed for **7** and 1-methylformycin are very similar to the spectrum observed for formycin, *per se*. Since the spectra for **6** and 2-methylformycin are so dissimilar to those of formycin and 1-methylformycin, this would suggest that formycin in solution under the present conditions probably exists predominantly with the proton residing on the N-1 nitrogen of the pyrazole moiety. It is of interest that studies[26,27] on the tautomerism of formycin, *per se*, by ^{13}C nuclear magnetic resonance has revealed that prototropic tautomerism can be a function of temperature and solvent. Therefore, although MCD spectroscopy may be very useful in the elucidation of tautomeric structures, considerable effort must be expended in this area before any of these preliminary observations can be corroborated and firmly established.

On treatment of **6**, **7**, **10**, and **12** individually with 1 *N* sodium hydroxide at reflux temperature, the position of the ultraviolet maxima was found to shift to lower wavelengths in each case. In view of the structures assigned these compounds (Scheme II), a Dimroth rearrangement

Scheme II[a]



[a] All nucleosides have been drawn in the syn conformation, although **9** and **10** probably exist predominantly in either the anti or amphi conformations as per the Discussion Section.

was not deemed possible. This prompted us to conduct the reaction on a larger scale with **7** and **12** and the isolated products were identified as 1,3-dimethylpyrazolo[4,3-*d*]pyrimidin-7-one (**8**) and 2-methyl-3-(β-D-ribofuranosyl)-

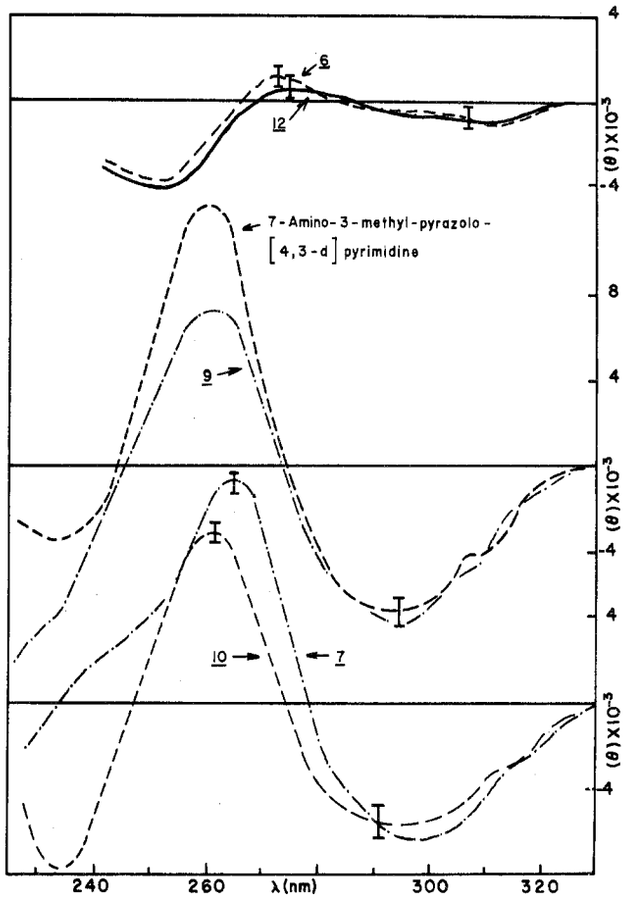


Figure 1. MCD spectra of some pyrazolo[4,3-*d*]pyrimidines.

pyrazolo[4,3-*d*]pyrimidin-7-one (**11**). The conversion of an amino group into a hydroxyl function under basic conditions is not normally observed in heterocyclic chemistry, although this conversion has been reported[28] to occur for 3-methylguanine *via* an apparent nucleophilic displacement. The possibility of a ring opening followed by ring closure could not be demonstrated, since chromatography (tlc) of the reaction solutions of **7** and **12** revealed only starting material and product. Apparently, in this case, a nucleophilic displacement of the amino group by base does occur.

It has been observed that **12** exhibited a higher T/C against leukemia L-1210 than **10**, although whether this difference is due to a difference in conformation (syn or anti) or their ability to act as substrates for specific catabolic and anabolic enzymes, etc., will be determined by additional studies.

Experimental Section[29]

3-Cyano-1,5-dimethyl-4-nitropyrazole (2). 5-Cyano-3-methyl-4-nitropyrazole (**1**, 5.0 g) was dissolved in water (100 ml) containing sodium hydroxide (1.4 g) and to this solution was added 3.76 ml of dimethyl sulfate. The solution was stirred for 45 min and then extracted with $CHCl_3$ (2 × 200 ml). The combined $CHCl_3$ extracts were washed with H_2O (100 ml), dried over magnesium sulfate, and evaporated to dryness *in vacuo*. The white solid was dissolved in a minimum amount of boiling MeOH and allowed to stand at 0° for 16 hr. The solid which had separated was removed by filtration. The filtrate was evaporated to *ca.* half volume and the solid was again collected by filtration. The presence of a very small amount of **2** and **3** was detected in the filtrate by tlc. The combined solid was dried at room temperature in a vacuum desiccator over phosphorus pentoxide to yield 3.78 g (69.2%) of **2**. An analytical sample was prepared by three recrystallizations from EtOH-H_2O, mp 113–114°.

Anal. Calcd for $C_6H_6N_4O_2$: C, 43.38; H, 3.64; N, 33.72. Found: C, 43.39; H, 3.98; N, 33.61.

4-Amino-3-cyano-1,5-dimethylpyrazole (4). 3-Cyano-1,5-dimethyl-4-nitropyrazole (**2**, 1.0 g) was treated by the same procedure (A) which yielded **5**. The product was recrystallized from H_2O for analysis to yield 0.23 g (28.8%) of **4**, mp 144–145°.

Anal. Calcd for $C_6H_8N_4$: C, 52.94; H, 5.93; N, 41.15. Found: C, 52.68; H, 6.13; N, 41.20.

4-Amino-5-cyano-1,3-dimethylpyrazole (5). Method A. 5-Cyano-1,3-dimethyl-4-nitropyrazole[30] (**3**, 2.5 g) was slurried in boiling H_2O (25 ml) and then stirred rapidly during the gradual addition of sodium hydrosulfite (8.7 g). The temperature of the reaction mixture was maintained between 75 and 80° by the rate of addition. After the final addition of sodium hydrosulfite, the solution was filtered immediately and then allowed to stand at 0° for 16 hr. The solid was collected by filtration and recrystallized from H_2O to yield 0.58 g (28.2%) of **5**. Recrystallization from H_2O produced an analytical sample which was dried *in vacuo* at 100°, mp 116–117°.

Anal. Calcd for $C_6H_8N_4$: C, 52.94; H, 5.93; N, 41.15. Found: C, 52.70; H, 5.91; N, 41.28.

Method B. A solution of **3** (1.66 g) in 100 ml of MeOH was hydrogenated at 1 atm over 5% Pd/C (0.8 g). After the calculated amount of hydrogen had been absorbed, the mixture was filtered through a Celite pad and the pad was washed with warm MeOH (2 × 50 ml). The filtrate and washings were combined and evaporated to dryness. The crude product (mp 115–118°) was recrystallized from H_2O to provide **5** (0.95 g, 69.8%), mp 117–119°, identical in all respects with the sample obtained from method A.

7-Amino-2,3-dimethylpyrazolo[4,3-*d*]pyrimidine (6). 4-Amino-3-cyano-1,5-dimethylpyrazole (**4**, 0.18 g) and formamidine acetate (0.2 g) were heated in EtOH (20 ml) at reflux temperature for 2 hr. The EtOH was removed *in vacuo* and the resulting solid was recrystallized from H_2O to yield 0.14 g (65.1%) of **6**. An analytical sample was prepared by two recrystallizations from H_2O, mp 289–290° dec.

Anal. Calcd for $C_7H_9N_5$: C, 51.52; H, 5.56; N, 42.92. Found: C, 51.75; H, 5.49; N, 43.20.

7-Amino-1,3-dimethylpyrazolo[4,3-*d*]pyrimidine (7). Method A. 4-Amino-5-cyano-1,3-dimethylpyrazole (**5**, 1.06 g) and formamidine acetate (1.15 g) were heated in EtOH (50 ml) at reflux temperature for 1 hr. The EtOH was removed *in vacuo*. EtOH (50 ml) was added, and again evaporated to dryness with this procedure being repeated three times. The residue was added to EtOAc (200 ml) at reflux temperature and the small amount of insoluble material was removed by filtration. The filtrate was reduced in volume to *ca.* 100 ml and allowed to stand at 5° for 16 hr. The yellow solid was collected by filtration and recrystallized from EtOAc to yield 0.6 g of **7**, mp 242–244°.

Anal. Calcd for $C_7H_9N_5$: C, 51.52; H, 5.56; N, 42.92. Found: C, 51.49; H, 5.50; N, 43.20.

Method B. 4-Amino-5-cyano-1,3-dimethylpyrazole (**5**, 0.5 g) and formamidine acetate (0.58 g) were dissolved in absolute EtOH (50 ml) and the solution was heated at reflux temperature. The reaction was monitored by tlc; after 3 hr an additional portion of formamidine acetate (104 mg) was added. The reaction mixture was heated at reflux temperature for an additional 1 hr (total 4 hr). The reaction mixture was evaporated to dryness and the residue was then dissolved in hot EtOAc (*ca.* 100 ml), filtered, and let stand at room temperature for 18 hr. The crystalline material was collected by filtration and air dried to yield **7** (0.53 g, 88.5%), mp 242–244°. This compound was identical in all respects with the sample obtained by method A.

1-3-Dimethylpyrazolo[4,3-*d*]pyrimidin-7-one (8). 7-Amino-1,3-dimethylpyrazolo[4,3-*d*]pyrimidine (**7**, 1.1 g) was added to 10 ml of 1 *N* sodium hydroxide and the solution was then heated at reflux temperature for 3 hr. The solution was allowed to cool at room temperature, and Dowex 50W-X4 was added (*ca.* 10 ml of washed resin) with stirring until the pH of the solution was *ca.* 7. The resin was removed by filtration and washed with 40 ml of hot H_2O. The filtrate and washings were combined and evaporated to dryness, EtOH (50 ml) was added, and again evaporated to dryness. The resulting solid was dissolved in hot H_2O (30 ml), all insoluble material was removed by filtration, and the solution was allowed to stand at 5° for 18 hr. The solid was collected by filtration and dried at 110° *in vacuo* to yield 0.38 g of **8**, mp 303–304°.

Anal. Calcd for $C_7H_8N_4O$: C, 51.22; H, 4.88; N, 34.15. Found: C, 51.19; H, 4.89; N, 33.99.

2-Methylformycin (12) and 1-Methylformycin (10). Formycin (**9**, 4.0 g) and sodium (0.44 g) were added to EtOH (100 ml) and the mixture was stirred to effect a clear solution. Methyl iodide (1 ml) was then added and the solution was stirred at room temperature. An additional quantity of methyl iodide (1 ml) was added at the end of the first and the second hour and the solution was then stirred for an additional 16 hr. The solid was removed by filtration, recystallized from isopropyl alcohol, and dried to yield 1.13 g (24.4%) of 2-methylformycin (**12**). An analytical sample was prepared by two additional recrystallizations from isopropyl alcohol and dried *in vacuo* at 110°, mp 205–206°.

Anal. Calcd for $C_{11}H_{15}N_5O_4$: C, 46.98; H, 5.37; N, 24.91. Found: C, 47.01; H, 5.51; N, 25.16.

The filtrate, after removal of the 2-methylformycin (**12**), was evaporated to dryness following the addition of silica gel[31] (*3 g*). The residue was applied to the top of a dry column (silica gel, 1.5 × 24 in.) and eluted with the upper phase of an ethyl acetate–1-propanol–water (4:1:3) mixture. The fractions were monitored with tlc on SilicAR 7GF in the same solvent system, fractions containing only the compound of R_f 0.48 were collected and combined, and the solvent was removed *in vacuo*. The solid was recrystallized twice from EtOAc–MeOH and dried *in vacuo* at 110° to yield 0.16 g (3.8%) of 1-methylformycin[32] (**10**), mp foams 170–173°, dec >200°.

Anal. Calcd for $C_{11}H_{15}N_5O_4$: C, 46.98; H, 5.37; N, 24.91. Found: C, 46.79; H, 5.69; N, 25.03.

2-Methyl-3-(β-D-ribofuranosyl)pyrazolo[4,3-*d*]pyrimidin-7-one (11) (2-Methylformycin B). 2-Methylformycin (**12**, 0.4 g) was added to aqueous 1 *N* sodium hydroxide (10 ml) and the solution was heated at reflux temperature for 3 hr. The solution was cooled to room temperature, Dowex 50W-X2 (H^+, 10 ml, previously washed with 100 ml of H_2O) was added, and the mixture was stirred until the pH was adjusted to *ca.* 4. The resin was removed by filtration and washed with boiling H_2O (50 ml). The combined filtrate and washing was evaporated to dryness, and EtOH (50 ml) was added and removed *in vacuo*. This process was repeated again and the resulting solid was recrystallized twice from a mixture of MeOH–EtOAc to yield 0.064 g (16%) of **11**, mp 213–215°.

Anal. Calcd for $C_{11}H_{14}N_4O_5$: C, 46.85; H, 5.00; N, 19.87. Found: C, 46.82; H, 5.04; N, 20.18.

Acknowledgment. The authors wish to thank Dr. R. P. Panzica for several helpful suggestions.

Registry No.—**1**, 28668-15-7; **2**, 51222-23-2; **3**, 32183-13-4; **4**, 51222-24-3; **5**, 32183-14-5; **6**, 51222-25-4; **7**, 51222-26-5; **8**, 51222-27-6; **9**, 6742-12-7; **10**, 51222-28-7; **11**, 51481-59-5; **12**, 42204-46-6.

References and Notes

(1) This research was supported by Research Contract No. PH-43-65-1041, No. C-72-3710 with the Division of Cancer Treatment, National Cancer Institute, National Institutes of Health, Public Health Services, and Research Grant No. GM-12862-08, National Institutes of Health.
(2) To whom correspondence should be addressed.
(3) M. Hori, E. Ito, T. Takita, G. Koyama and H. Umezawa, *J. Antibiot., Ser. A*, **17**, 96 (1964).
(4) G. Koyama and H. Umezawa, *J. Antibiot., Ser. A*, **18**, 175 (1965).
(5) R. K. Robins, L. B. Townsend, F. C. Cassidy, J. F. Gerster, A. F. Lewis, and R. L. Miller, *J. Heterocycl. Chem.*, **3**, 110 (1966).
(6) G. Koyama, K. Maeda, H. Umezawa, and Y. Itake, *Tetrahedron Lett.*, 597 (1966).
(7) K. R. Darnall, L. B. Townsend, and R. K. Robins, *Proc. Nat. Acad. Sci. U. S.*, **57**, 548 (1967).
(8) F. F. Davis and F. W. Allen, *J. Biol. Chem.*, **227**, 907 (1957).
(9) F. Streightoff, J. D. Nelson, J. E. Cline, K. Gerzon, R. H. Williams, and D. C. DeLong, Abstracts, Ninth Interscience Conference on Antimicrobial Agents and Chemotherapy, Oct 1969, Paper No. 18.
(10) T. Sawa, Y. Fukagawa, I. Homma, T. Takeuchi, and H. Umezawa, *J. Antibiot., Ser. A*, **20**, 317 (1967).
(11) T. Sawa, Y. Fukagawa, Y. Shimauchi, K. Ito, M. Hamada, T. Takeuchi, and H. Umezawa, *J. Antibiot., Ser. A*, **18**, 259 (1965).
(12) T. Takeuchi, J. Iwanaga, T. Aoyagi, M. Murase, T. Sawa, and H. Umezawa, *J. Antibiot., Ser. A*, **20**, 297 (1967).
(13) D. C. Ward, A. Cerami, E. Reich, G. Acs, and L. Altwerger, *J. Biol. Chem.*, **244**, 3243 (1969).
(14) M. Ikehara, K. Murao, F. Harada, and S. Nishimura, *Biochem. Biophys. Acta*, **174**, 696 (1968).
(15) P. Prusiner, T. Brennen, and M. Sundaralingham, *Biochemistry*, **12**, 1196 (1973).
(16) M.-T. Chenon, R. J. Pugmire, D. M. Grant, R. P. Panzica, and L. B. Townsend, *J. Heterocycl. Chem.*, **10**, 427 (1973).
(17) A preliminary report has been published: R. A. Long, L. B. Townsend, D. W. Miles, H. Eyring, and R. K. Robins, Abstracts of Papers, 161st National Meeting of the American Chemical Society, Los Angeles, Calif., March 28–April 2, 1971, ORGN 112.
(18) J. W. Jones and R. K. Robins, *J. Amer. Chem. Soc.*, **85**, 193 (1963).

(19) L. B. Townsend and R. K. Robins, *J. Amer. Chem. Soc.*, **85**, 242 (1963).
(20) R. K. Robins, L. B. Holum, and F. W. Furcht, *J. Org. Chem.*, **21**, 833 (1956).
(21) R. A. Long, J. F. Gerster, and L. B. Townsend, *J. Heterocycl. Chem.*, **7**, 863 (1970).
(22) A. O. Geiszler, U. S. Patent 3,121,092 (Feb 11, 1964); *Chem. Abstr.*, **60**, 12020 (1964).
(23) L. B. Townsend, D. W. Miles, S. J. Manning, and H. Eyring, *J. Heterocycl. Chem.*, **10**, 419 (1973).
(24) D. W. Miles, W. H. Inskeep, L. B. Townsend, and H. Eyring, *Biopolymers*, **11**, 1181 (1972).
(25) The structure of **12** has now been corroborated by an X-ray study: J. E. Abola, M. J. Sims, D. J. Abraham, A. F. Lewis, and L. B. Townsend, *J. Med. Chem.*, **17**, 62 (1974).
(26) M.-T. Chenon, R. J. Pugmire, D. M. Grant, R. P. Panzica, and L. B. Townsend, *J. Heterocycl. Chem.*, **10**, 431 (1973).
(27) T. R. Krugh, *J. Amer. Chem. Soc.*, **95**, 4761 (1973).
(28) L. B. Townsend and R. K. Robins, *J. Amer. Chem. Soc.*, **84**, 3008 (1962).
(29) Melting points were determined with a Thomas-Hoover capillary apparatus and are uncorrected. Ultraviolet spectra were recorded on a Beckman DK-2 spectrophotometer and evaporations were performed under diminished pressure at 40° with a Rotoevaporator unless otherwise stated. The MCD spectra were determined on the Cary Model 60 with MCD attachment.
(30) U. S. Patent 3,121,092; *Chem. Abstr.*, **60**, 2030 (1964).
(31) Baker silica gel, chromatographic quality, deactivated with 10% H_2O and containing 0.5% by weight Du Pont 609 Phosphor: B Leov and M. Goodman, *Chem. Ind. (London)*, 2026 (1967).
(32) The synthesis of **10** has been reported (J. Zemlicka, Abstracts, 4th International Congress on Heterocyclic Chemistry, Salt Lake City, Utah, July 9–13, 1973, p 15) as the major product using different reaction conditions.

Cyano Adducts of 1-Substituted Pyridinium Salts[1a]

Robert H. Reuss,[1b] Nelson G. Smith, and Lawrence J. Winters*[1c]

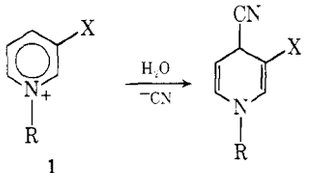
Department of Chemistry, Drexel University, Philadelphia, Pennsylvania 19104, and Department of Chemistry, Virginia Commonwealth University, Richmond, Virginia 23284

Received October 18, 1973

The isolation and characterization of several cyano adducts **11a–e** of 1-substituted pyridinium salts **10** is described. These represent the first examples of this type of compound. In addition, the first Reissert-like compound (**12a**) from pyridine is reported. Contrary to earlier suggestions, the title compounds are relatively stable. An explanation of the stability on the basis of interaction of the N substituent with the reactive dihydropyridine ring is presented.

The synthesis of stable, simple dihydropyridines has received increased attention recently.[2] Such compounds are of interest theoretically[2,3] and as precursors in synthetic applications.[2,4]

It has been shown that cyanide reacts with 1,3-disubstituted pyridinium salts **1** to afford the corresponding 4-cyano adducts **2**.[2] Only salts related to **1** yield isolable

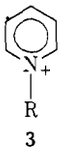


1 **2**

R = alkyl
X = $CONH_2$, CO_2Me, CN, COMe, COPh

products.[2] That adducts of other salts were not observed was assumed to be due both to the low electrophilicity of the salt and the lack of resonance stabilization of the corresponding cyano adduct which is only possible in species such as **2**.[5]

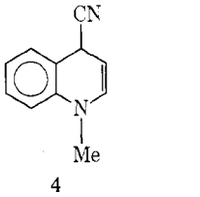
While 3-unsubstituted pyridinium salts **3** had been found to be unreactive with cyanide,[5] related reactions are



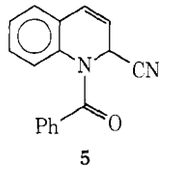
3

R = alkyl, aryl, acyl

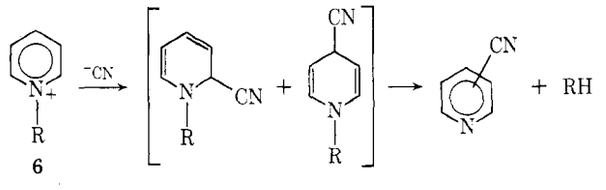
known. Thus, the corresponding quinolinium adducts (**4**, **5**) have been known for many years,[6,7] while 1-alkoxy- and 1-amidopyridinium salts **6** yield transient cyano adducts which decompose with loss of an alcohol or amide to



4



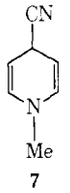
5



6

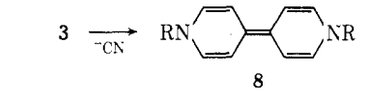
R = OR, RNCOR'

produce cyanopyridines.[8] In contrast to Gauthier's results, cyano adduct **7** was found to be stable in DMSO.[9]



7

Within the last several years it has been reported that **3** (R = alkyl or aryl) reacts with cyanide to afford dihydrobipyridine **8** or its oxidized derivatives.[10,11] Although



8