

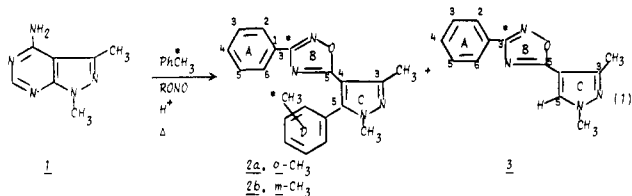
A Remarkable Fragmentation Reaction of a Pyrimidyl Radical

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Our laboratories have recently been investigating aryl substituted triazolo-, pyrazolo-, and imidazo-fused 5,6-heterobicyclic systems as potentially useful CNS agents.¹ During the course of these synthetic studies, we recently attempted the Gomberg-Bachmann-Hey arylation² of 4-amino-1,3-dimethylpyrazolo-[3,4-*d*]pyrimidine (**1**). When amine **1** was diazotized in toluene solution by using conditions recently reported for related purinyl systems³ (8 equiv of isoamyl nitrite added portionwise over 12–18 h, 10 mol % toluenesulfonic acid, 120 °C), little of the desired 4-aryl derivative was obtained. The major products, isolated by silica dry column chromatography using 35% ethyl acetate and 1% ammonia in hexanes as eluant, were the two C₂₀H₁₈N₄O isomers **2** (12%) and a third component (**3**, 7%, mp 132–134 °C) having molecular formula C₁₃H₁₂N₄O (eq 1).



Microanalytical,^{4a} mass spectral,^{4b,c} and ¹H NMR^{4d} analyses of **2a** confirmed the molecular formula and the surprising incorporation of two aromatic solvent molecules. The presence of 12 new aromatic carbon atoms was further established by ¹³C NMR (Table I). The fact that the pyrazolopyrimidine ring system was no longer intact was apparent by the striking absence of the pyrimidyl C-4 and C-7 in the ¹³C NMR and the hydrogen at C-7 in the ¹H NMR. The structure of **2a** was established by X-ray analysis⁵ (Figure 1) as a 1,2,4-oxadiazole with an *o*-tolyl substituent (eq 1).

Isomer **2b** was therefore established as a 1,2,4-oxadiazole with an *m*-tolyl substituent.⁶ Examination of the ¹H NMR of isomeric mixture **2** reveals two signals for each of the three methyl groups of **2** in the ratio of 2:1. Since the ortho isomer **2a** was determined to be the major component,^{4b,5} proton signals may be assigned

Table I. ¹³C NMR Chemical Shifts for **2** and **3**^a

ring carbon no. ^b	2 ^c	3
A-1	127.1	127.1
A-2	128.7	128.8
A-3	124.4	127.5
A-4	130.9	131.0
A-5	127.4	127.5
A-6	128.4	128.8
B-3	167.9* ^d	168.3*
B-5	171.7, 171.8	171.5
C-3	149.3	149.6
C-4	105.2, 104.7	106.1
C-5	145.1, 145.7	132.8
C-CCH ₃	13.9	13.4
C-NCH ₃	36.6, 37.0	39.1
D-CCH ₃	19.6*, 21.5*	

^a Chemical shifts are in δ downfield from internal Me₄Si. ^b Carbon signals of ring D were not assigned. ^c Major isomer **2a** signals are italicized. ^d Signals enhanced by ¹³C enrichment designated by an asterisk.

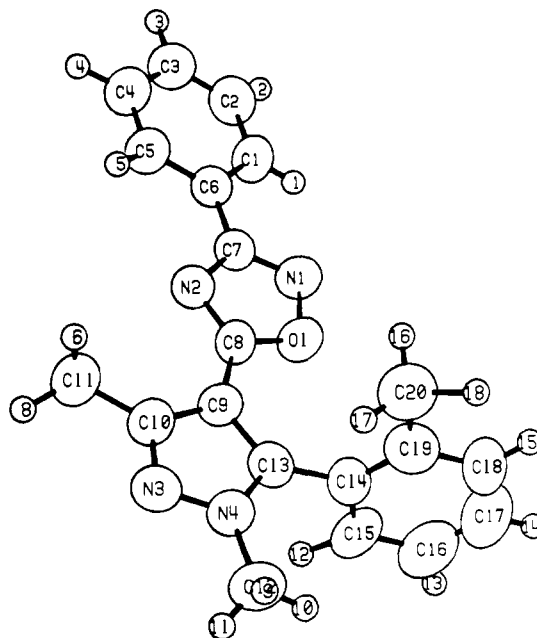


Figure 1. ORTEP drawing of molecular structure of major product isomer **2a**.

based upon the theory that the ortho analogue (**2a**) will create a twisted nonconjugated conformation which will cause an up-field methyl shift. Thus **2a** displays a methyl absorption at δ 2.15 whereas **2b** absorption occurs at a more typical δ 0.30 upfield shift). A similar upfield shift is observed in the ¹³C NMR (δ 19.6 and 21.5 for **2a** and **2b**, respectively) (Table I). The structure of **3** was assigned on the basis of similar mass spectral,^{7a} ultraviolet,^{7c} and ¹H^{7b} and ¹³C NMR analyses (Table I) as compared to **2**. NMR analysis of **3** was simplified by the absence of ring D.

The remarkable formation of **2** and **3** as the major products of diazotization of **1** may be rationalized as depicted in eq 2. Pyrimidyl radical **4** forms from **1** analogous to the purinyl system³ but collapses with loss of HCN to yield radical **5**. Reaction of **5** with aromatic solvent gives arylation product **6** (ortho substitution is the expected major process⁸) or hydrogen abstraction

(7) 5-(1,3-Dimethyl-1*H*-pyrazol-4-yl)-3-phenyl-1,2,4-oxadiazole (**3**): (a) *m/e* calcd for C₁₃H₁₂N₄O, 240.1011, found, 240.1017; (b) ¹H NMR (CDCl₃) δ 8.10 (m, 3 H, *o*-phenyl and pyrazolyl H), 7.50 (m, 3 H, *m*-, *p*-phenyl H), 3.96 (s, 3 H, NCH₃), 2.66 (s, 3 H, CCH₃); (c) UV (MeOH) 241, 264 (sh) nm.

(8) For discussions of isomer distribution as a result of radical attack on aromatic systems see: (a) Fleming, I. "Frontier Orbitals and Organic Chemical Reactions"; Wiley: New York, 1976: pp 191–194. (b) Allgood, D. R.; Williams, G. H. *Chem. Rev.* **1957**, *57*, 123–190.

(1) See for example: Albright, J. D.; Moran, D. B.; Wright, W. B., Jr.; Collins, J. B.; Beer, B.; Lipka, A. B.; Greenblatt, E. N. *J. Med. Chem.* **1981**, *24*, 592–600.

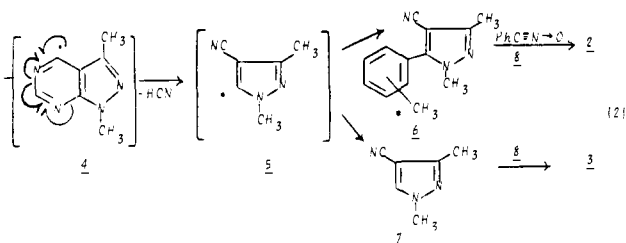
(2) For a recent review of aryl-aryl bond-forming reactions see: Sainsbury, M. *Tetrahedron* **1980**, *36*, 3327–3359.

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(4) 5-[1,3-Dimethyl-5-(2-methylphenyl)-1*H*-pyrazol-4-yl]-3-phenyl-1,2,4-oxadiazole (**2a**). (a) Anal. Calcd for C₂₀H₁₈N₄O: C, 72.71; H, 5.49; N, 16.96. Found: C, 72.34; H, 5.37; N, 16.91. (b) Isomer separation on GLC with 3% SE-30 at 250 °C (5 min) isothermal and 5 °C/min to 280 °C (hold); (c) *m/e* calcd for C₂₀H₁₈N₄O, 330.1481, found, 330.1478; (d) ¹H NMR (CDCl₃) δ 8.05 (m, 2 H, *o*-phenyl H), 7.40 (m, 7 H, *m*-, *p*-phenyl H and tolyl H), 3.80 (s, 3 H, NCH₃), 2.75 (s, 3 H, CCH₃), 2.15 (s, 3 H, tolyl CH₃); (e) UV (MeOH) 230, 240 nm.

(5) X-ray analysis was performed by Molecular Structure Corp., College Station, TX. Crystals of **2a** grown by sublimation at 60 °C were monoclinic space group *P2₁/C*, with *a* = 13.562 (2) Å, *b* = 11.875 (2) Å, *c* = 10.831 (1) Å, β = 93.22 (1)°, and *Z* = 4. Room-temperature data collection gave 3782 independent reflections with 1839 having *I* ≥ 3σ(*I*). The structure was solved by direct methods and refined anisotropically; hydrogen atoms were located in difference synthesis and refined isotropically with the exception of those in ring D, which were included at calculated positions with arbitrary isotropic thermal parameters. The final reliability index *R* = Σ||*F*_o − *F*_c||/Σ|*F*_o| was 0.067.

(6) **2b**: (a) *m/e* calcd for C₂₀H₁₈N₄O, 330.1481, found, 330.1481; (b) ¹H NMR (CDCl₃) δ 8.05 (m, 2 H, *o*-phenyl H), 7.40 (m, 7 H, *m*-, *p*-phenyl and tolyl H), 3.70 (s, 3 H, NCH₃), 2.70 (s, 3 H, CCH₃), 2.46 (s, 3 H, CH₃).



product **7**. Toluene in the presence of nitric oxide for extended periods of time may nitrosate or nitrate on the methyl group.⁹ Subsequent oximation and thermal elimination of nitrous acid would form benzonitrile *N*-oxide (**8**). Electrocyclic addition of *N*-oxide **8** to nitriles **6** or **7** forms 1,2,4-oxadiazoles **2** or **3**.¹⁰

Support for the proposed origin of **2** and **3** was obtained by using 20% ¹³C₃-enriched toluene as the reaction solvent. ¹³C enrichment (indicated by an asterisk in eq 1 and 2) occurred at ring B carbon 3 as well as the C-methyl of ring D as predicted by the above mechanism (Table I).

The reasons that **4** collapses to **5** (and thus to products) rather than reacting to form the desired arylation product are not clear. Further work to explore the generality of this reaction and to prepare the desired 4-arylated pyrazolo[3,4-*d*]pyrimidines is the subject of a future report from these laboratories.¹¹

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Registry No. **1**, 5346-58-7; **2a**, 82044-26-6; **2b**, 82044-27-7; **3**, 82044-28-8.

Supplementary Material Available: Tables of atomic coordinates and thermal parameters for **2a** (2 pages). Ordering information is given on any current masthead page.

(9) For a discussion of aralkyl side-chain oxidations and nitrations under these conditions see: (a) Sosnovsky, G. "Free Radical Reactions in Preparative Organic Chemistry"; MacMillan: New York, 1964; pp 217-227. (b) Touster, O. *Org. React.* **1953**, *7*, 327-377.

(10) Chang, M. S.; Lowe, J. U., Jr. *J. Org. Chem.* **1967**, *32*, 1577-1579.

(11) Press, J. B.; Eudy, N. H., to be submitted for publication.

Identity of Cosynthetic Factor 1 of *Streptomyces aureofaciens* and Fragment FO from Coenzyme F420 of *Methanobacterium* Species

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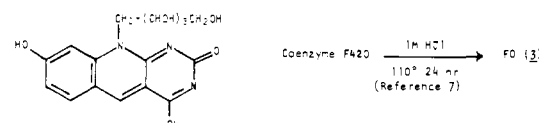
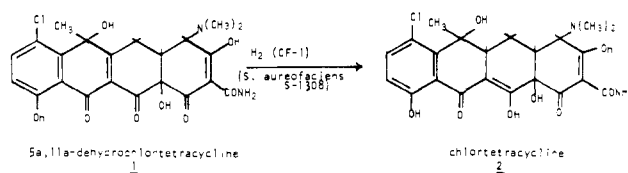
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In the early part of our work on the biosynthesis of the tetracyclines, we isolated from *Streptomyces aureofaciens* W-5 a substance designated cosynthetic factor 1 (CF-1), which was involved as a catalyst in the biological reduction of 5a,11a-dehydrochlortetracycline (**1**) to chlortetracycline (**2**).¹ At that time, the available quantity of CF-1 was insufficient to permit a complete structure determination. We were able, however, to postulate a close relationship to the pteridines and flavins and noted that the properties of CF-1 did not correspond exactly to any of the then known members of those families.¹

The identification of CF-1 as 7,8-didemethyl-8-hydroxy-5-deazariboflavin (**3**) has come about by our recent application of nuclear magnetic resonance methods to the study of the CF-1 structure. Carbon-13 NMR of CF-1 in dimethyl sulfoxide² clearly showed the presence of a ribityl side chain like that in riboflavin²

(1) Philip A. Miller, Newell O. Sjolander, Stephen Nalesnyk, Nancy Arnold, Sylvia Johnson, Albert P. Doerschuk, and J. R. D. McCormick, *J. Am. Chem. Soc.*, **82**, 5002 (1960).



Cosynthetic Factor 1,
FO fragment of F420,
7,8-didemethyl-8-hydroxy-5-deazariboflavin
3

and indicated a total of 16 carbon atoms. Line broadening indicated that only four of the nonribityl carbons carried protons. Proton NMR clearly showed the presence of a 1,2,4-trisubstituted benzene ring fragment,³ an additional isolated vinyl proton, two hydroxyl or imino protons, and eleven additional protons that could be accounted for by the ribityl side chain. Thus, 17 protons were seen, and this C₁₆H₁₇ partial formula, when taken with our originally reported microanalysis, suggested the molecular formula: C₁₆H₁₇N₃O₇·H₂O.⁴ Confirmation of this composition was found in the chemical ionization negative-ion mass spectrum of CF-1, which showed a strong molecular ion, M⁻ 363, and the fragment ions M⁻ 345 (loss of H₂O), M⁻ 320 (loss of CONH), and M⁻ 229 (loss of C₅H₁₀O₄).

At the time of our original work, vigorous acidic hydrolysis had yielded microgram quantities of a product having ultraviolet absorption spectra reminiscent of a hydroxycarbostryl,⁵ and this, with our more recent data, above, suggested a possible deazariboflavin structure. A literature search revealed the intervening work on coenzyme F420. This coenzyme has been implicated both in hydrogen transfer in the production of methane by several species of *Methanobacterium*^{6,7} and also as a cofactor in a photoreactivating system of *Streptomyces griseus*.⁸ From the very characteristic absorption spectra⁹ of CF-1 and coenzyme F420, it was evident that we were dealing with a closely related compound. It appeared likely that CF-1 was identical with the FO fragment derived hydrolytically from F420, and the very close agreement between some proton magnetic resonance shifts³ of CF-1 and synthetic FO left little doubt that the identification was correct. The exact correspondence of the infrared spectra¹⁰ of CF-1 and a sample of synthetic FO and the agreement of optical rotations of the two materials¹¹ confirmed the identity of the compounds.

(2) ¹³C NMR of CF-1 (20 MHz, Me₂SO-*d*₆) δ 164.1, 162.3, 157.9, 156.4, 143.9, 141.3, 133.6, 115.2 (2 C), 110.5, 102.0, 73.7, 72.6, 69.4, 63.2, 47.8. ¹³C NMR of the ribityl carbons of riboflavin (Me₂SO-*d*₆) δ 73.5, 72.6, 68.7, 63.2, 47.1.

(3) ¹H NMR of CF-1 (80 MHz, Me₂SO-*d*₆) (partial spectrum) δ 11.23, 10.98, 8.94, 8.05 (d, J = 10 Hz), 7.41, 7.06 (d, J = 10 Hz). ¹H NMR of FO (ref 12) (60 MHz, Me₂SO-*d*₆) (partial spectrum) δ 11.2 (br), 11.01, 8.89, 8.01 (d, J = 9 Hz), 7.40, 7.04 (d, J = 9 Hz).

(4) In ref 1 we reported the composition as C₁₉H₂₂N₄O₇, based on an erroneous neutral equivalent of 446.

(5) Unpublished work with E. R. Jensen of these laboratories.

(6) P. Cheeseman, A. Toms-Wood, and R. S. Wolfe, *J. Bacteriol.*, **112**, 527 (1972).

(7) L. Dudley Eirich, Godfried D. Vogels, and Ralph S. Wolfe, *Biochemistry*, **17**, 4583 (1978).

(8) A. P. M. Eker, A. Pol, P. vanden Meyden, and G. D. Vogels, *FEMS Microbiol. Lett.*, **8**, 161 (1980).

(9) See Figure 1 in ref 6 and Figure 2 in ref 1.

(10) The infrared spectra of our material and that of the sample supplied by Professor Walsh were initially similar but not identical. After converting each sample to its ammonium salt and back to its free acid form, the major features of both were identical: IR (max) (KBr) 3400, 3160, 3050, 2790, 1735, 1710, 1625, 1585, 1495, 1450, 1395, 1340, 1275, 1225, 1150, 1115, 1090, 1065, 1030, 910, 845, 815, 795, 750, 680, 580, 550, 500, 425 cm⁻¹.

(11) Optical rotations, CF-1: [α]_D²⁵ +40 ± 2° (0.5% in 0.1 N NaOH); FO (ref 12): [α]_D²⁵ +38° (0.5% in 0.1 N NaOH).