A Remarkable Fragmentation Reaction of a Pyrimidyl Radical

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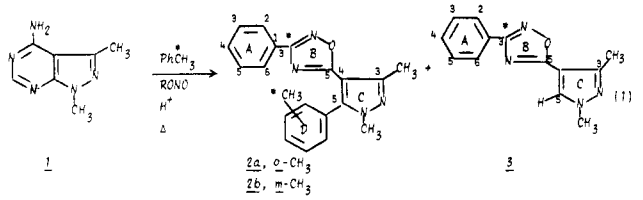
Our laboratories have recently been investigating aryl substituted triazolo-, pyrazolo-, and imidazo-fused 5,6-heterobicyclic systems as potentially useful CNS agents.[1] During the course of these synthetic studies, we recently attempted the Gomberg–Bachmann–Hey arylation[2] of 4-amino-1,3-dimethylpyrazolo-[3,4-*d*]pyrimidine (**1**). When amine **1** was diazotized in toluene solution by using conditions recently reported for related purinyl systems[3] (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 $C_{20}H_{18}N_4O$ isomers **2** (12%) and a third component (**3**, 7%, mp 132–134 °C) having molecular formula $C_{13}H_{12}N_4O$ (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 ^{13}C 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 ^{13}C NMR and the hydrogen at C-7 in the ^1H NMR. The structure of **2a** was established by X-ray analysis[5] (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 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 2.45 (δ 0.30 upfield shift). A similar upfield shift is observed in the ^{13}C 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 ^{13}C 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 system[3] 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 process[8]) or hydrogen abstraction

Table I. ^{13}C 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 Me_4Si. [b] Carbon signals of ring D were not assigned. [c] Major isomer **2a** signals are italicized. [d] Signals enhanced by ^{13}C enrichment designated by an asterisk.

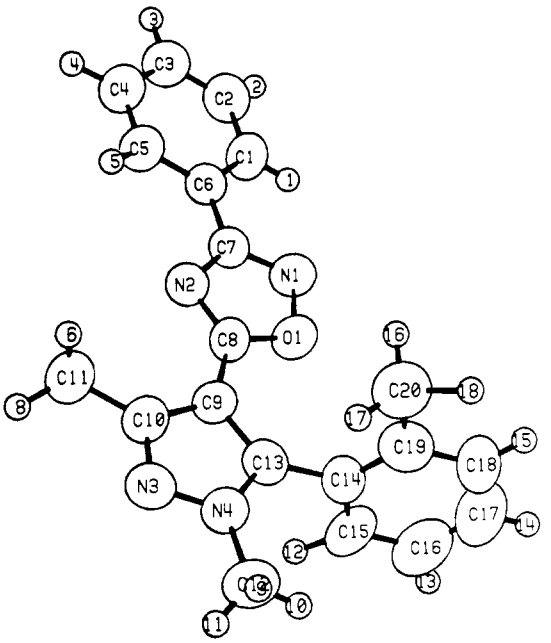


Figure 1. ORTEP drawing of molecular structure of major product isomer **2a**.

(1) See for example: Albright, J. D.; Moran, D. B.; Wright, W. B., Jr.; Collins, J. B.; Beer, B.; Lippa, 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.

(3) Nair, V.; Richardson, S. G. *J. Org. Chem.* **1980**, *45*, 3969–3974.

(4) 5-[1,3-Dimethyl-5-(2-methylphenyl)-1*H*-pyrazol-4-yl]-3-phenyl-1,2,4-oxadiazole (**2a**). (a) Anal. Calcd for $C_{20}H_{18}N_4O$: 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 $C_{20}H_{18}N_4O$, 330.1481, found, 330.1478; (d) ^1H NMR ($CDCl_3$) 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, NCH_3), 2.75 (s, 3 H, CCH_3), 2.15 (s, 3 H, tolyl CH_3); (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 $P2_1/C$, 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 $I \geq 3\sigma(I)$. 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 $R = \sum||F_o| - |F_c||/\sum|F_o|$ was 0.067.

(6) **2b**: (a) *m/e* calcd for $C_{20}H_{18}N_4O$, 330.1481, found, 330.1481; (b) ^1H NMR ($CDCL_3$) 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, NCH_3), 2.70 (s, 3 H, CCH_3), 2.46 (s, 3 H, tolyl CH_3).

(7) 5-(1,3-Dimethyl-1*H*-pyrazol-4-yl)-3-phenyl-1,2,4-oxadiazole (**3**): (a) *m/e* calcd for $C_{13}H_{12}N_4O$, 240.1011, found, 240.1017; (b) ^1H NMR ($CDCl_3$) 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, NCH_3), 2.66 (s, 3 H, CCH_3); (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.

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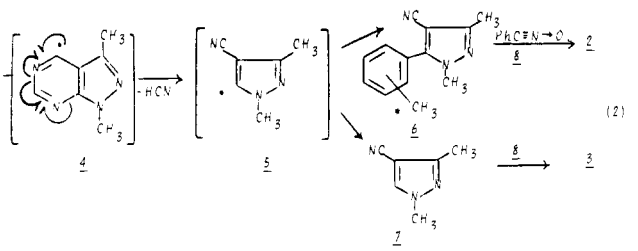

product **7**. Toluene in the presence of nitric oxide for extended periods of time may nitrosate or nitrate on the methyl group.[9] Subsequent oximation and thermal elimination of nitrous acid would form benzonitrile *N*-oxide (**8**). Electrocyclic addition of *N*-oxide **8** to nitriles **6** or **7**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

Support for the proposed origin of **2** and **3** was obtained by using 20% $^{13}CH_3$-enriched toluene as the reaction solvent. ^{13}C 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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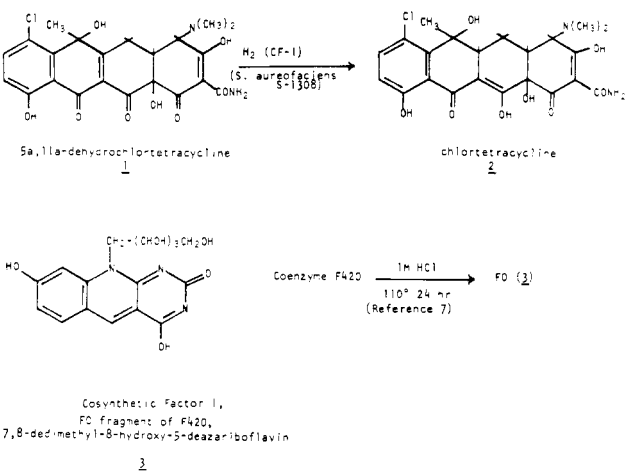
In the early part of our work on the biosynthesis of the tetracyclines, we isolated from *Streptomyces aureofaciens* W-5 a substance designated cosynthetic factor 1 (CF-1), which was involved as a catalyst in the biological reduction of 5a,11a-dehydrochlortetracycline (**1**) to chlortetracycline (**2**).[1] 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 sulfoxide[2] clearly showed the presence of a ribityl side chain like that in riboflavin[2]



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 $C_{16}H_{17}$ partial formula, when taken with our originally reported microanalysis, suggested the molecular formula: $C_{16}H_{17}N_3O_7 \cdot H_2O$.[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 H_2O), M^- 320 (loss of CONH), and M^- 229 (loss of $C_5H_{10}O_4$).

At the time of our orignial 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 spectra[9] 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 shifts[3] of CF-1 and synthetic FO left little doubt that the identification was correct. The exact correspondence of the infrared spectra[10] of CF-1 and a sample of synthetic FO and the agreement of optical rotations of the two materials[11] confirmed the identity of the compounds.

(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).

(2) ^{13}C NMR of CF-1 (20 MHz, Me_2SO-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. ^{13}C NMR of the ribityl carbons of riboflavin (Me_2SO-d_6) δ 73.5, 72.6, 68.7, 63.2, 47.1.

(3) ^1H NMR of CF-1 (80 MHz, Me_2SO-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, Me_2SO-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 $C_{19}H_{22}N_4O_7$, 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: $[\alpha]^{25}_D$ +40 ± 2° (0.5% in 0.1 N NaOH); FO (ref 12): $[\alpha]^{25}_D$ +38° (0.5% in 0.1 N NaOH).

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