hydrochlorides, the amino acids were dissolved in hot chloroform, and dry HCl was bubbled through the solution for 20 min. When the solution cooled, dry ether was added, and the precipitate was collected.

The nucleophilic attack of the sulfur-containing amino acids on the double bond of the maleic esters generates an asymmetric C atom. Because cysteine and homocysteine are either pure optical antipodes or a racemic mixture, a mixture of diastereomers was obtained by this reaction. Therefore, the ^{1}H NMR spectra of the resulting compounds show a complicated coupling pattern of the amino acid and the succinic acid protons such that the exact assignment is not possible without decoupling experiments.

***S*-[1,2-Bis(octadecyloxycarbonyl)ethyl]cysteine (1)**: yield 85%; mp 130 °C; ^{1}H NMR ($CDCl_3$) δ 0.85 (t, 6 H, $-CH_3$), 1.25 (s, 60 H, CH_2), 1.58 (s, 4 H, CH_2CH_2OOC), 4.0-4.2, (2t, 4 H, CH_2OOC), 2.7-3.95 (m, 6 H, no assignment possible); IR (KBr) 1740 (C=O ester), 1720 (C=O, acid). Anal. Calcd for $C_{43}H_{81}NO_6S$: C, 69.78; H, 11.03; N, 1.89; S, 4.33. Found: C, 68.72; H, 10.86; N, 2.01; S, 4.05.

***S*-(1-Carboxy-2-[(*N*,*N*-dioctadecylamino)carbonyl]ethyl)cysteine (2)**: yield 68%; mp 148 °C; ^{1}H NMR ($CDCl_3$) δ 0.85 (t, 6 H, CH_3), 1.25 (s, 60 H, CH_2), 1.85 (s, 4 H, CH_2CH_2NCO), 2.0-3.6 (m, 10 H, no assignment possible); IR (KBr) 1715 (C=O acid), 1620 (C=O, amide). Anal. Calcd for $C_{43}H_{84}N_2O_5S$: C, 69.31; H, 11.36; N, 3.75; S, 4.30. Found: C, 67.82; H, 10.53; N, 3.90; S, 4.70.

***S*-[1-Carboxy-2-([2,3-bis(hexadecyloxy)propoxy]carbonyl)ethyl]cysteine (3)**: yield 67%; mp 190-195 °C; ^{1}H NMR ($CDCl_3$) δ 0.85 (t, 6 H, CH_3), 1.23 (s, 52 H, CH_2), 1.52 (s, 4 H, CH_2CH_2O), 2.1-3.9 (m, 13 H, no assignment possible), 4.0-4.3 (m, 2 H, CH_2OOC); IR (KBr) 3600-2400 (NH_3^+, OH), 3000-2800 (CH_2), 1720 (C=O, ester, acid), 1625, 1580 (NH_3^+). Anal. Calcd for $C_{42}H_{81}O_8NS$: C, 66.36; H, 10.74; N, 1.84; S, 4.22. Found: C, 64.46; H, 9.47; N, 1.90; S, 4.32.

***S*-[1-Carboxy-2-([2,3-bis(octadecyloxy)propoxy]carbonyl)ethyl]cysteine (4)**: yield 50%; mp 134 °C; ^{1}H NMR ($CDCl_3$) and IR (KBr) similar to **3**. Anal. Calcd for $C_{46}H_{89}O_8NS$: C, 68.36; H, 10.10; N, 1.73; S, 3.97. Found: C, 67.92; H, 10.51; N, 1.96; S, 3.82.

***S*-[1-Carboxy-2-([2,3-bis(hexadecyloxy)propoxy]carbonyl)ethyl]-homocysteine (5)**: yield 40%; mp 165 °C; ^{1}H NMR ($CDCl_3$) 0.85 (t, 6 H, CH_3), 1.23 (s, 52 H, CH_2), 1.52 (s, 4 H, CH_2CH_2O), 2.1-3.9 (m, 15 H, no assignment possible), 4.0-4.3 (m, 2 H, CH_2OOC); IR (KBr) similar to **3**. Anal. Calcd for $C_{43}H_{83}O_8NS$: C, 66.71; H, 10.81; N, 1.81; S, 4.14. Found: C, 65.70; H, 10.7; N, 1.70; S, 4.18.

***S*-[1-Carboxy-2-([bis(2,3-octadecyloxy)propoxy]carbonyl)ethyl]homocysteine (6)**: yield 54%; mp 164 °C; ^{1}H NMR ($CDCl_3$) similar to **5**; IR (KBr) similar to **3**. Anal. Calcd for $C_{47}H_{81}O_8NS$: C, 68.65; H, 10.17; N, 1.70. Found: C, 66.97; H, 10.93; N, 1.79.

Registry No. 1 (isomer 1), 99355-64-3; **1** (isomer 2), 99355-65-4; **2** (isomer 1), 99355-66-5; **2** (isomer 2), 99355-67-6; **3**, 99355-68-7; **4**, 99355-69-8; **5**, 99355-70-1; **6**, 99355-71-2; maleic anhydride, 108-31-6; octadecanol, 112-92-5; maleic acid dioctadecyl ester, 7516-70-3; maleic acid (2,3-*o*-dihexadecyloxy)glycerol monoester, 99355-72-3; malic acid (2,3-*o*-dioctadecyloxy)glycerol monoester, 99355-73-4; *N*,*N*-dioctadecylmaleamic acid, 82798-00-3; L-cysteine, 52-90-4; D,L-homocysteine, 454-29-5; 1,2-*o*-(dihexadecyloxy)glycerol, 6076-35-3; 1,2-*o*-(dioctadecyloxy)glycerol, 6076-38-6.

Evidence for a Competing Condensation Reaction in the Alloxan Synthesis of Flavins: Synthesis and Crystal and Molecular Structures of 7-Chloro-8-methylalloxazine and 7,10-Dimethyl-8-[(2-hydroxyethyl)thio]isoalloxazine

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Abstract: The reaction of 2-amino-5-chloro-*N*,4-dimethylaniline with alloxan monohydrate in boric acid-acetic acid mixtures has been shown to produce 7-chloro-8-methylalloxazine (III) in addition to the expected 8-chlorolumiflavin (I). The position of heteroatom substitution in III and I has been verified by optical and ^{1}H NMR studies and by single-crystal X-ray diffraction structure determinations of III·DMF and of a derivative of I, 8-[(2-hydroxyethyl)thio]lumiflavin, IV·H_2O: III·DMF, monoclinic, space group $P2_1/c$ with a = 8.764 (4) Å, b = 16.030 (8), c = 11.047 (6) Å, β = 95.00 (2)°, V = 1546 Å^3, Z = 4, 2480 reflections with $I > 2\sigma(I)$, R_F = 0.056, R_{wF} = 0.048; IV·H_2O, triclinic, space group $P\bar{1}$ with a = 9.194 (2) Å, b = 9.848 (3) Å, c = 11.821 (4) Å, α = 87.50 (2)°, β = 119.46 (3)°, γ = 58.16 (2)°, V = 722.6 Å^3, Z = 2, 1799 reflections with $I > 2\sigma(I)$, R_F = 0.047, R_{wF} = 0.050. Both structures were solved by direct methods. III·DMF is the first structurally characterized alloxazine and exhibits bond lengths and angles consistent with those expected for the alloxazine tautomer. Reaction of other substituted 2-amino-*N*-methylanilines with alloxan was examined, and formation of an alloxazine with reversed substitution compared to that of the isoalloxazine also obtained was observed in all cases. These results strongly suggest that substituted 2-amino-*N*-methylanilines can condense with alloxan in either of two orientations, only one of which produces the isoalloxazines. The other orientation produces an N(5)-alkylalloxazinium intermediate that spontaneously dealkylates to give the observed alloxazine. No evidence was obtained for formation of an alloxazine via N(10)-dealkylation of the isoalloxazine under the conditions examined.

Because of the presence of the isoalloxazine nucleus in riboflavin and its coenzyme derivatives FMN and FAD[2] and the importance of flavin-dependent enzymes in biology,[3] much effort has been devoted to chemical synthesis of riboflavin analogues as model systems for mechanistic studies and as biological agonists or antagonists of the natural coenzyme.[4-6] Reasonably facile syntheses of isoalloxazines have been reported: from 2-amino-*N*-alkylanilines and alloxan, alloxantin, isodialuric acid, or 5-halobarbituric acid;[4] from *N*-alkylanilines and violuric acid;[4] from 2-arylazoanilines and barbituric acid;[4] from N-substituted 5,6-diaminouracils and dimeric biacetyl;[4] from 5-amino-6-(alkylamino)pyrimidines and *o*-benzoquinones;[4] from 6-(*N*-alkyl-

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(2) Abbreviations: FMN, flavin mononucleotide or riboflavin-5-monophosphate; FAD, flavin adenine dinucleotide or riboflavin adenosine diphosphate.

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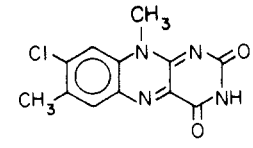
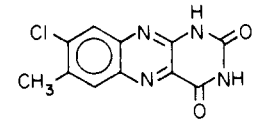
anilino)uracils by nitrosative and nitrative cyclization followed by reduction[7a-c] or by treatment with *N*-nitrosodimethylamine-$POCl_3$;[7d] from 5-nitro-6-(N-substituted-anilino)uracils by dehydrative cyclization with concentrated sulfuric acid and reduction;[8] from 5-anilino-6-(alkyl- or arylamino)uracils by heating in dimethylformamide under oxygen;[9] and from 6-(*N*-alkylanilino)-5-aminouracils by oxidative cyclization with diethyl azodicarboxylate or nitrosobenzene.[10] Of these methods, the first is by far the most widely employed and has been used to prepare a variety of riboflavin analogues;[4,5] it is, however, characterized by highly variable yields, depending strongly and unpredictably on the number, nature, and placement of substituents on the *o*-phenylenediamine nucleus.

In recent years, particular attention has been focused on the preparation of flavin derivatives functionalized at the 8-position. This is because the flavin coenzymes in a number of flavoenzymes are covalently attached to the polypeptide backbone via the 8-position[3b,11] and because a number of naturally occurring flavin derivatives contain hetero substituents at C(8) (e.g., 8-hydroxy-FAD from *Peptostreptococcus elsdenii*[12a] and *Megasphaera elsdenii*;[12b] roseoflavin, an 8-(dimethylamino)flavin from *Streptomyces davawensis*;[13] and F420,[14] an 8-hydroxy-7-demethyl-5-deazaflavin from methanogenic bacteria). Recent work has also shown that 8-chloro-[15] and 8-mercaptoflavins[16] can serve as sensitive active site probes of flavoproteins. Several synthetic routes to 8-substituted flavins (such as 8-chloroflavins[5,7a,17,18]) have been developed, and the ease of substitution of such derivatives at the 8-position has been demonstrated.[18b,19]

In preparing 8-chlorolumiflavin (I) via the alloxan route, we noted that in addition to the desired product, substantial amounts of another material were obtained. Electronic and ^1H NMR spectra were consistent with its initial formulation as the corresponding alloxazine derivative (II), presumably arising from the N(10)-dealkylation reactions that are well documented for flavins.[20-23] Misgivings about this structure assignment together



(I)



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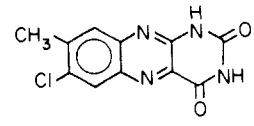

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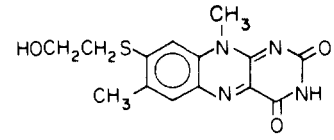

(IV)

with the availability of well-formed single crystals and the lack of any structurally characterized oxidized alloxazines prompted us to perform a single-crystal X-ray analysis, which showed that the compound was in fact the isomeric 7-chloro-8-methylalloxazine (III). A literature search revealed that no crystal structure had been reported on any synthetic 8-substituted flavin derivative, the structural assignments having been based on convincing but indirect evidence from a combination of spectroscopic studies.[12,13b,19c] Because our structural results on the alloxazine derivative might be construed as casting doubt on the correctness of the original structure assignments, we undertook to obtain unequivocal structural evidence for the substitution pattern of the purported 8-haloflavins obtained by the alloxan method. Although suitable crystals of the parent compound could not be obtained, we were successful in obtaining crystals of the product of the substitution reaction with 2-mercaptoethanol. The crystallographic results show that the material is the expected 8-[(2-hydroxyethyl)-thio]lumiflavin (IV) and establish unequivocally that the original lumiflavin was indeed the 8-chloro derivative. This indicates that the 7-chloro-8-methylalloxazine arises from a previously unsuspected competing condensation reaction, in which the N-alkyl-*o*-phenylenediamine condenses with alloxan in a reverse fashion, presumably giving rise to an N(5)-alkylalloxazinium intermediate that spontaneously dealkylates under the reaction conditions.

Experimental Section

Materials and Methods. 3-Chloro-4-methylaniline, 4-methyl-2-nitroaniline, alloxan monohydrate, and platinum oxide and palladium–charcoal catalysts were obtained from Aldrich Chemical Co. and were used

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without further purification. 2-Mercaptoethanol and triethylamine were from J.T. Baker Chemical Co. and E.M. Industries, Inc., respectively. All solvents were used as supplied, except for dimethylformamide, which was dried over BaO and distilled before use. Dinitrogen gas was purified and dried by passage over hot BASF catalyst R-3-11 and supported P_2O_5 (Aquasorb). 5-Chloro-4-methyl-2-nitroaniline was prepared by acetylation, nitration, and base hydrolysis of 3-chloro-4-methylaniline.[5a] N-Methylation of 4-methyl-2-nitroaniline and 5-chloro-4-methyl-2-nitroaniline was achieved by the method of Müller,[24] using formaldehyde and sulfuric acid. Authentic samples of 8-chloro-7,10-dimethylisoalloxazine, 7,10-dimethylisoalloxazine, and 8-methylalloxazine were prepared from the corresponding isoalloxazine 5-oxides and alloxazine 5-oxides by published procedures.[7c,18b] All operations during flavin synthesis were carried out under low-intensity red light.

Physical Measurements. Optical spectra were obtained on a Cary 219 or Cary 17D spectrophotometer. ^1H NMR spectra were recorded in CF_3CO_2H with $(CH_3)_4Si$ (TMS) as internal standard on a Varian EM-390 or Nicolet NTC-360 spectrometer. The positive ion electron-impact mass spectra were recorded by using a VG-7070 mass spectrometer operating at 70 eV.

Reaction between Alloxan Monohydrate and 2-Amino-5-chloro-*N*,4-dimethylaniline. 5-Chloro-*N*,4-dimethyl-2-nitroaniline (3.50 g; 17.5 mmol) in glacial acetic acid (80 mL) and water (20 mL) was hydrogenated over platinum oxide (200 mg) at room temperature and 60 psi pressure until the solution became colorless (~3–4 h). The resulting suspension was filtered under nitrogen directly into a mixture of alloxan monohydrate (3.7 g; 23 mmol) and boric acid (6.8 g; 110 mmol) in glacial acetic acid (200 mL), which had been thoroughly degassed and flushed with nitrogen. The reaction mixture was degassed again and heated to reflux for 5–10 min. After stirring for ~12 h at room temperature, the solution gave a yellow solid, which was collected by filtration in air and washed with water (~3 L), ethanol (~20 mL), and ether (~5 mL). The air-dried solid (shown by NMR to be a mixture of alloxazine (III) and isoalloxazine (I)) was fractionally crystallized from hot formic acid to afford pure 7-chloro-8-methylalloxazine (III, less soluble) and 8-chloro-7,10-dimethylisoalloxazine (I, more soluble).

8-Chloro-7,10-dimethylisoalloxazine (I): mp 315–23 °C dec; yield 2.6 g, 54%; ^1H NMR δ 2.78 (3 H, s, 7-CH_3), 4.64 (3 H, s, 10-CH_3), 8.45, 8.48 (2 H, 6-H and 9-H); optical spectrum λ_{max} (nm) (ϵ, M^{-1} cm^{-1}) (DMF) 272 (36 100), 335 (7860), 420 (sh), 443 (12 000), 470 (sh); mass spectrum, *m/e* 276 (M^+).

7-Chloro-8-methylalloxazine (III): mp 350 °C dec; yield 1.8 g, 39%; ^1H NMR δ 2.77 (3 H, s, 8-CH_3), 8.03 (1 H, s, 9-H), 8.37 (1 H, s, 6-H); optical spectrum λ_{max} (nm) (ϵ, M^{-1} cm^{-1}) (DMF) 324 (6350), 383 (10 000), 399 (sh); mass spectrum, *m/e* 262 (M^+).

Reaction between Alloxan Monohydrate and 2-Amino-*N*,4-dimethylaniline. 2-Nitro-*N*,4-dimethylaniline (1.5 g; 9.0 mmol) in glacial acetic acid (45 mL) and water (10 mL) was hydrogenated over Pd/C (200 mg) and reacted with alloxan monohydrate (2.1 g; 13.1 mmol) and boric acid (3.8 g; 62 mmol) in the same manner as described above. Workup of the reaction mixture and fractional crystallization from hot formic acid gave pure 7,10-dimethylisoalloxazine (V, more soluble) and 8-methylalloxazine (VI, less soluble).

7,10-Dimethylisoalloxazine (V): mp 320–22 °C dec; yield 0.9 g, 41%; ^1H NMR δ 2.77 (3 H, s, 7-CH_3), 4.63 (3 H, s, 10-CH_3), 8.33, 8.40 (3 H, 6-H, 7-H, and 9-H); optical spectrum λ_{max} (nm) (ϵ, M^{-1} cm^{-1}) (DMF) 269 (32 800), 330 (7120), 395 (sh), 420 (sh), 445 (9340), 476 (sh).

8-Methylalloxazine (VI): mp 335–45 °C dec; yield 0.2 g, 10%; ^1H NMR δ 2.77 (3 H, s, 8-CH_3), 7.96 (1 H, d, 7-H), 8.00 (1 H, s, 9-H), 8.32 (1 H, d, 6-H); optical spectrum λ_{max} (nm) (ϵ, M^{-1} cm^{-1}) (DMF) 335 (8270), 373 (9780), 392 (sh).

Preparation of 7,10-Dimethyl-8-[(2-hydroxyethyl)thio]isoalloxazine (IV). To a degassed suspension of 8-chloro-7,10-dimethylisoalloxazine (0.15 g, 0.54 mmol) in DMF (20 mL), triethylamine (2.3 mL) and 2-mercaptoethanol (0.09 g, 1.1 mmol) were added. The reaction mixture was heated at 100 °C for ~8 h under N_2 with constant stirring. After cooling, the resulting yellow–orange solid was filtered and washed with water (20 mL), ethanol (10 mL), and diethyl ether (15 mL) to give 0.14 g (82%) of the desired product: mp 205–12 °C dec; ^1H NMR δ 2.67 (3 H, s, 7-CH_3), 3.76 (2 H, t, SCH_2), 4.37 (2 H, t, OCH_2), 4.57 (3 H, s, 10-CH_3), 4.82 (1 H, t, OH), 8.00, 8.20 (2 H, 6-H and 9-H); optical spectrum λ_{max} (nm) (ϵ, M^{-1} cm^{-1}) (DMF) 272 (29 600), 388 (sh), 425 (sh), 453 (25 100), 477 (21 700); mass spectrum, *m/e* 318 (M^+). Derivative formate ester (prepared by heating in formic acid): mp 280–85 °C dec; ^1H NMR δ 2.67 (3 H, s, 7-CH_3), 3.74 (2 H, t, SCH_2), 4.69 (2 H, t, OCH_2), 4.60 (3 H, s, 10-CH_3), 8.12, 8.27, and 8.30 (3 H, 6-H, 9-H and HC(O)O-); optical spectrum λ_{max} (nm) (ϵ, M^{-1} cm^{-1}) (DMF) 272 (30 500), 390 (sh), 426 (sh), 452 (25 500), 476 (22 100); mass spectrum, *m/e* 346 (M^+).

(24) Müller, F. *Methods Enzymol.* **1980**, *66*, 265–267.

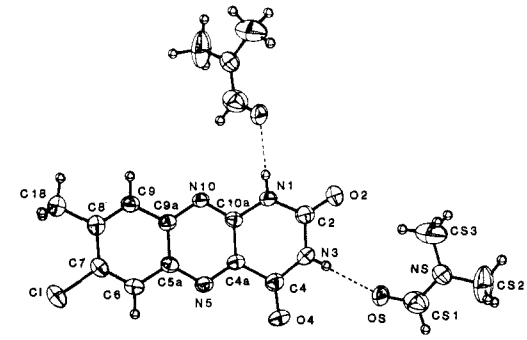

Figure 1. ORTEP drawing of the structure of III·DMF, showing the atomic labeling scheme.

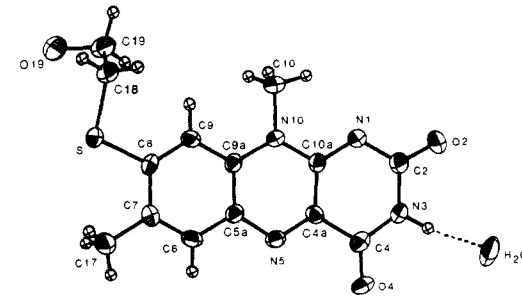

Figure 2. ORTEP drawing of the structure of IV·H_2O, showing the atomic labeling scheme.

X-ray Analysis. Crystallization of 7-Chloro-8-methylalloxazine (III). Yellow, rectangular prisms of III·DMF were obtained by slow evaporation of a DMF solution.

Crystallization of 7,10-Dimethyl-8-[(2-hydroxyethyl)thio]isoalloxazine (IV). The compound was dissolved in formic acid with mild heating and allowed to stand at room temperature in a closed container with ethanol. After several days, red, plate-like crystals of the formate ester of IV were obtained. Further diffusion of ethanol vapor into the filtered solution afforded yellow–orange crystals of IV·H_2O, which were collected and air-dried. The crystals of the formate ester were not suitable for X-ray study.

Crystal Data. The procedures used were similar for the two compounds. In each case, precession photographs were used to determine the crystal symmetry, space group, and preliminary cell dimensions. Precise unit cell dimensions were obtained by a least-squares fit to the observed angular settings for 15 strong general reflections for each compound, measured with Mo K_α radiation on a Nicolet P3m automated diffractometer. Crystal data and details of the data collections are listed in Table I. The intensities of two reference reflections, measured after every 50 scans, did not vary significantly during data collection.

Structure Solution and Refinement. Data reduction and calculations involved in structure determination were carried out on XDS Sigma 2 and CDC Cyber 172 computers at the University of Virginia. Both structures were solved by direct methods with the use of the MULTAN 80 program.[25] E-maps for the two compounds revealed the positions of the ring atoms and the chlorine atom in the case of 7-chloro-8-methylalloxazine (III). The remaining non-hydrogen atoms were located by Fourier syntheses phased by the observed atoms. The coordinates for the hydrogen atoms, except those of the methyl groups (obtained from difference Fourier maps), were calculated. The positions of one of the hydrogen atoms of the solvate water molecule and of the hydroxyl hydrogen in the (2-hydroxyethyl)thio side chain could not be located from difference electron density maps. The structures were refined (by block-diagonal matrix least-squares methods) by using anisotropic thermal parameters for the non-hydrogen atoms to the final unweighted and weighted residual indices given in Table I. All H atom positions were refined, except those of the DMF solvate atoms, which were held fixed. Difference Fourier maps, calculated at the end of the refinement, did not show any structurally significant features, the highest peak having a height of 0.3 e/Å^3.

(25) Main, P.; Fiske, S. J.; Hull, S. E.; Lessinger, L.; Germain, G.; Declercq, J. P.; Woolfson, M. M. "MULTAN 80: A System of Computer Programs for the Automatic Solution of Crystal Structures from X-ray Diffraction Data"; University of York: England, and Louvain, Belgium, 1984.

Table I. Crystal Data and X-ray Data Collection Procedures

	III·DMF	IV-H_2O
A. Crystal Parameters at 20 °C		
formula	$C_{14}H_{14}ClN_5O3$	$C_{14}H_{16}N_4O_4S$
fw, amu	335.8	336.4
space group	C_{2h}^5–$P2_1/c$ (No. 14)	C_i'–$P\bar{1}$ (No. 2)
a, Å	8.764 (4)	9.194 (3)
b, Å	16.030 (8)	9.848 (3)
c, Å	11.047 (6)	11.821 (4)
α, deg		87.50 (2)
β, deg	95.00 (2)	119.46 (3)
γ, deg		58.16 (2)
V, Å^3	1546	722.6
Z	4	2
ρ_{calcd}, g cm^{-3}	1.443	1.546
$F(000)$	696	352
μ, cm^{-1}	2.76	2.54
B. Measurement of Intensity Data		
radiation	graphite monochromated Mo Kα λ = 0.7107 Å	
scan speed, deg min^{-1}	2.93–19.3	
min max 2θ, deg	0–60	
scan range	1.2° below $2\theta\alpha_1$ to 1.2° above $2\theta\alpha_2$	
scan type	θ–2θ	
background count	at either end of scan range for a total time equal to that spent on the scan	
data collected	single quadrant	single hemisphere
reflections excluding systematic absences	3551	3385
reflections with $I > 2\sigma(I)$	2480	1799
R_F, R_{wF}(anisotropic)	0.056, 0.048	0.047, 0.050

Table II. Positional and Equivalent Isotropic Displacement Parameters for the Crystal Structure of 7-Chloro-8-methylalloxazine, DMF Solvate[a]

atom	x/a	y/b	z/c	B_{eq}
Cl	−807 (1)	6763 (1)	3394 (1)	4.02 (4)
N(1)	5267 (3)	3510 (1)	6371 (2)	2.9 (1)
C(2)	5730 (3)	2744 (2)	5990 (2)	3.2 (1)
O(2)	6645 (2)	2306 (1)	6581 (2)	4.4 (1)
N(3)	5106 (3)	2504 (1)	4843 (2)	3.4 (1)
C(4)	4034 (3)	2924 (2)	4080 (2)	3.0 (1)
C(4a)	3555 (3)	3738 (2)	4565 (2)	2.5 (1)
O(4)	3528 (2)	2632 (1)	3108 (2)	4.6 (1)
N(5)	2524 (2)	4183 (1)	3907 (2)	2.7 (1)
C(5a)	2129 (2)	4920 (2)	4390 (2)	2.6 (1)
C(6)	1010 (3)	5425 (2)	3738 (2)	3.0 (1)
C(7)	609 (3)	6170 (2)	4204 (2)	2.9 (1)
C(8)	1271 (3)	6481 (2)	5340 (2)	3.0 (1)
C(9)	2355 (3)	5987 (2)	5975 (2)	2.9 (1)
C(9a)	2802 (3)	5206 (2)	5533 (2)	2.5 (1)
N(10)	3873 (2)	4734 (1)	6207 (2)	2.5 (1)
C(10a)	4215 (3)	4019 (2)	5725 (2)	2.4 (1)
C(18)	821 (4)	7316 (2)	5818 (3)	4.4 (2)
O(s)	3876 (3)	−963 (1)	−3818 (2)	4.8 (1)
C(s1)	3454 (4)	−219 (2)	−3797 (3)	5.2 (2)
N(s)	2710 (3)	179 (2)	−4722 (2)	4.1 (1)
C(s2)	2225 (5)	1048 (2)	−4596 (5)	8.5 (3)
C(s3)	2307 (5)	−233 (3)	−5863 (4)	8.1 (3)

[a] Positional parameters are given as fractions (×10^4) of the unit cell edges, and B_{eq} is given in Å^2. Standard deviations, given in parentheses, are applicable to the least significant figures.

Scattering factors for neutral atoms, with allowance made for the real part of the anomalous dispersion correction, were taken from Cromer and Waber[26] and that for hydrogen from Stewart et al.[27] The weighting factor used in the least-squares refinement was $1/[\sigma^2(F) + gF^2]$ with g = 0.001. The molecular drawings were prepared by using ORTEP.[28]

(26) Cromer, D. T.; Waber, J. T. In "International Tables for X-ray Crystallography"; Kynoch Press: Birmingham, England, 1974; Vol. IV.
(27) Stewart, R. F.; Davidson, E. R.; Simpson, W. T. *J. Chem. Phys.* **1965**, *42*, 3175–3187.
(28) Johnson, C. K. "ORTEP-II: A Fortran Thermal-Ellipsoid Plot Program for Crystal Structure Illustrations"; Oak Ridge National Laboratory: Oak Ridge, TN, 1976; ORNL-5138.

Table III. Positional and Equivalent Isotropic Displacement Parameters for the Crystal Structure of 7,10-Dimethyl-8-[(2-hydroxyethyl)thio]isoalloxazine[a]

atom	x/a	y/b	z/c	B_{eq}
S	9506 (2)	1570 (2)	838 (1)	2.6 (1)
N(1)	4210 (6)	6920 (4)	3892 (4)	2.4 (3)
C(2)	2600 (7)	7666 (6)	4036 (4)	2.6 (4)
N(3)	1111 (5)	7364 (4)	3435 (4)	2.5 (3)
C(4)	1140 (7)	6293 (5)	2725 (5)	2.7 (4)
C(4a)	2872 (6)	5510 (5)	2561 (4)	2.0 (4)
N(5)	2993 (5)	4512 (4)	1860 (3)	2.3 (3)
C(5a)	4557 (7)	3837 (5)	1685 (4)	2.1 (4)
C(6)	4686 (7)	2800 (6)	889 (5)	2.5 (4)
C(7)	6189 (6)	2107 (5)	667 (4)	2.3 (4)
C(8)	7670 (6)	2458 (5)	1233 (4)	2.1 (4)
C(9)	7594 (7)	3455 (6)	2024 (4)	2.4 (4)
C(9a)	6057 (6)	4147 (5)	2256 (4)	2.1 (4)
N(10)	5925 (5)	5169 (4)	3043 (4)	2.1 (3)
C(10a)	4324 (6)	5905 (5)	3179 (4)	1.9 (4)
C(17)	6303 (8)	979 (6)	−173 (5)	3.2 (5)
C(18)	10927 (7)	2444 (6)	1525 (5)	3.0 (5)
C(19)	12856 (7)	1353 (6)	3091 (5)	3.3 (5)
C(10)	7555 (7)	5426 (6)	3729 (5)	2.7 (5)
O(2)	2406 (5)	8614 (4)	4683 (4)	3.9 (4)
O(4)	−164 (5)	6043 (4)	2273 (4)	3.9 (4)
O(19)	14477 (5)	9664 (4)	3314 (4)	4.3 (3)
O(s)	8219 (6)	8901 (5)	4090 (4)	5.0 (4)

[a] Positional parameters are given as fraction (×10^4) of the unit cell edges, and B_{eq} is given in Å^2. Standard deviations, given in parentheses, are applicable to the least significant figures.

Table IV. Interatomic Distances (Å) and Angles (deg) in 7-Chloro-8-methylalloxazine·DMF

Distances			
N(1)–C(2)	1.372 (3)	C(6)–C(7)	1.359 (2)
N(1)–C(10a)	1.381 (3)	C(7)–C(8)	1.426 (3)
C(2)–N(3)	1.390 (3)	C(8)–C(9)	1.381 (3)
C(2)–O(2)	1.212 (3)	C(9)–C(9a)	1.411 (2)
N(3)–C(4)	1.381 (3)	C(9a)–N(10)	1.374 (2)
C(4)–O(4)	1.218 (3)	N(10)–C(10a)	1.310 (3)
C(4)–C(4a)	1.486 (3)	C(7)–Cl	1.746 (2)
C(4a)–C(10a)	1.431 (3)	C(8)–C(18)	1.503 (3)
C(4a)–N(5)	1.318 (3)	O(s)–C(s1)	1.249 (4)
N(5)–C(5a)	1.353 (3)	C(s1)–N(s)	1.327 (4)
C(5a)–C(9a)	1.422 (3)	N(s)–C(s2)	1.467 (4)
C(5a)–C(6)	1.417 (3)	N(s)–C(s3)	1.438 (4)
Angles			
C(2)–N(1)–C(10a)	124.9 (2)	C(9)–C(9a)–C(5a)	119.2 (2)
N(1)–C(2)–N(3)	115.1 (2)	C(9)–C(9a)–N(10)	119.9 (2)
N(1)–C(2)–O(2)	123.6 (2)	C(5a)–C(9a)–N(10)	120.9 (2)
O(2)–C(2)–N(3)	121.3 (2)	C(9a)–N10)–C(10a)	115.6 (2)
C(2)–N(3)–C(4)	127.9 (2)	N(10)–C(10a)–N(1)	118.2 (2)
N(3)–C(4)–C(4a)	114.0 (2)	N(10)–C(10a)–C(4a)	123.4 (2)
N(3)–C(4)–O(4)	121.7 (3)	C(4a)–C(10a)–N(1)	118.4 (2)
O(4)–C(4)–C(4a)	124.3 (3)	O(s)–C(s1)–N(s)	124.8 (3)
C(4)–C(4a)–N(5)	118.6 (2)	C(s1)–N(s)–C(s2)	120.7 (3)
C(4)–C(4a)–C(10a)	119.6 (2)	C(s1)–N(s)–C(s3)	121.3 (3)
C(10a)–C(4a)–N(5)	121.8 (2)	C(s2)–N(s)–C(s3)	117.9 (3)
C(4a)–N(5)–C(5a)	116.3 (2)		
N(5)–C(5a)–C(6)	119.2 (2)		
N(5)–C(5a)–C(9a)	122.0 (2)		
C(9a)–C(5a)–C(6)	118.7 (2)		
C(5a)–C(6)–C(7)	120.0 (3)		
C(6)–C(7)–C(8)	122.8 (2)		
C(6)–C(7)–Cl	118.7 (2)		
Cl–C(7)–C(8)	118.5 (1)		
C(7)–C(8)–C(9)	117.1 (2)		
C(7)–C(8)–C(18)	121.4 (2)		
C(18)–C(8)–C(9)	121.5 (2)		
C(8)–C(9)–C(9a)	122.2 (2)		

Positional and equivalent isotropic displacement parameters and their estimated standard deviations for the two compounds are given in Tables II and III. The atomic labeling schemes are shown in Figures 1 and 2, and selected bond distances and angles are given in Tables IV and V. Tables of observed and calculated structure amplitudes, anisotropic displacement parameters, and positional and isotropic displacement pa-

Table V. Interatomic Distances (Å) and Angles (deg) in 7,10-Dimethyl-8-[(2-hydroxyethyl)thio]isoalloxazine·H_2O

	Distances		
N(1)–C(2)	1.368 (5)	C(6)–C(7)	1.349 (6)
N(1)–C(10a)	1.331 (5)	C(7)–C(8)	1.438 (5)
C(2)–N(3)	1.400 (5)	C(8)–C(9)	1.384 (5)
C(2)–O(2)	1.228 (5)	C(9)–C(9a)	1.385 (5)
N(3)–C(4)	1.374 (5)	C(9a)–N(10)	1.399 (5)
C(4)–O(4)	1.215 (5)	N(10)–C(10a)	1.355 (5)
C(4)–C(4a)	1.479 (6)	C(7)–C(17)	1.518 (6)
C(4a)–C(10a)	1.443 (5)	C(8)–S	1.756 (4)
C(4a)–N(5)	1.308 (5)	S–C(18)	1.820 (4)
N(5)–C(5a)	1.359 (5)	C(18)–C(19)	1.523 (7)
C(5a)–C(9a)	1.415 (5)	C(19)–O(19)	1.420 (6)
C(5a)–C(6)	1.417 (6)	N(10)–C(10)	1.480 (5)
	Angles		
C(2)–N(1)–C(10a)	118.5 (4)	C(17)–C(7)–C(6)	120.5 (4)
N(1)–C(2)–N(3)	120.1 (4)	C(7)–C(8)–C(9)	120.4 (4)
N(1)–C(2)–O(2)	121.8 (4)	C(7)–C(8)–S	115.7 (3)
O(2)–C(2)–N(3)	118.1 (4)	S–C(8)–C(9)	123.9 (3)
C(2)–N(3)–C(4)	125.7 (4)	C(8)–S–C(18)	104.8 (2)
N(3)–C(4)–C(4a)	113.7 (4)	S–C(18)–C(19)	113.3 (3)
N(3)–C(4)–O(4)	122.1 (4)	C(18)–C(19)–O(19)	111.3 (4)
O(4)–C(4)–C(4a)	124.2 (4)	C(8)–C(9)–C(9a)	120.3 (4)
C(4)–C(4a)–N(5)	118.4 (2)	C(9)–C(9a)–C(5a)	120.1 (4)
C(4)–C(4a)–C(10a)	117.8 (4)	C(9)–C(9a)–N(10)	121.8 (4)
C(10a)–C(4a)–N(5)	123.8 (4)	C(5a)–C(9a)–N(10)	118.1 (4)
C(4a)–N(5)–C(5a)	117.8 (4)	C(9a)–N(10)–C(10a)	120.0 (4)
N(5)–C(5a)–C(6)	119.3 (4)	C(9a)–N(10)–C(10)	119.4 (4)
N(5)–C(5a)–C(9a)	122.4 (4)	C(10)–N(10)–C(10a)	120.6 (3)
C(9a)–C(5a)–C(6)	118.3 (4)	N(10)–C(10a)–N(1)	118.1 (4)
C(5a)–C(6)–C(7)	122.1 (4)	N(10)–C(10a)–C(4a)	117.7 (4)
C(6)–C(7)–C(8)	118.9 (4)	C(4a)–C(10a)–N(1)	124.1 (4)
C(6)–C(7)–C(17)	120.7 (4)		

rameters for hydrogen atoms are available in the supplementary material.

Results and Discussion

Structure of III·DMF. From the point of view of the present work, the most striking structural feature is clearly the presence of the chlorine and methyl substituents at C(7) and C(8), respectively. This obviously precludes formation of the alloxazine having occurred via N(10)-dealkylation of the anticipated flavin precursor.

Because III is the first structurally characterized oxidized alloxazine, a brief summary of pertinent structural features is in order (Figure 1). The alloxazine nucleus consists of nearly planar benzenoid, pyrazinoid, and pyrimidinoid rings, with mean displacements of atoms from planes of 0.003, 0.005, and 0.009 Å, respectively; the three rings are not coplanar, however. (See the supplementary material for least-squares planes.) Using the plane of the pyrazinoid ring as a reference, C(4) and C(9) are displaced from it by −0.017 and −0.013 Å, respectively, whereas N(1) and C(6) are displaced by 0.031 and 0.028 Å, respectively. The overall conformation is thus that of a propellor. The Cl and C(18) atoms are displaced to opposite sides of the benzenoid ring plane by 0.051 and 0.025 Å, respectively. O(2) and O(4) are displaced to the same side of the pyrimidinoid plane as is Cl by 0.029 and 0.022 Å, respectively, but the four atom groupings centered on C(2) and C(4) are rigorously planar. Comparison of bond distances and angles of III with those of typical isoalloxazines reveals significant differences only in the N(10)–C(10a)–N(1) and, to a lesser extent, C(4a)–N(5)–C(5a) regions. As expected, based on the typical valence bond structure for an alloxazine, the N(1)–C(10a) distance is ca. 0.07 Å longer than the N(10)–C(10a) distance, consistent with partial double bond character in the latter. This is exactly the opposite of the behavior normally observed for isoalloxazines, where the N(1)–C(10a) bond is the shorter (e.g., 1.369 (5) vs. 1.319 (5) Å for lumiflavin).[29] Similarly, the C(4a)–C(10a)–N(1) angle is ca. 5° less than the N(10)–C(10a)–C(4a) angle, and the internal angle at N(10) is 9° less than that at N(1). Both are the reverse of the trends observed for typical flavins,[29,30] presumably reflecting the difference in electron distribution in the cis-conjugated alloxazine vs. the trans-conjugated isoalloxazine.

The crystal structure of III·DMF consists of essentially planar alloxazine molecules connected by hydrogen bonds to the oxygen atom of the solvent of crystallization. These hydrogen bonds involve the hydrogen atoms attached to both N(3) and N(1); the N···O(s) are 2.891 and 2.868 Å, respectively, typical values for hydrogen bonds to the pyrimidine portion of the flavin nucleus.[29,30] The closest intermolecular contact is 3.113 Å (O(4)···Cl′).

Structure of IV·H_2O. The structure of IV (Figure 2) confirms that the precursor was 8-chloro-7,10-dimethylisoalloxazine, I, which could not have given rise to III by a simple dealkylation reaction. Metrical features of the isoalloxazine ring of IV are unremarkable. The benzenoid and pyrimidinoid rings are rigorously planar, with average deviations of atoms from these planes of 0.004 and 0.009 Å, respectively; each of these planes includes N(10). (See the supplementary material for least-squares planes.) These two planes are folded at an angle of 176.7° to one another about the N(5)···N(10) axis. The S atom and C(17) are displaced to opposite sides of the benzenoid plane in the same way as the corresponding substituent atoms in the alloxazine, by 0.054 and 0.029 Å, but O(2) and O(4) are displaced by 0.021 and 0.040 Å, respectively, to the opposite side of the pyrimidinoid ring from S. Compound IV is the first structurally characterized sulfur-substituted flavin; the C(8)–S distance of 1.756 (4) Å is normal for aromatic sulfides.[32] The ethylene side chain exhibits the expected gauche conformation (torsion angle O(19)–C(19)–C(18)–S, 65.3°).

The crystal structure of IV·H_2O also consists of essentially planar molecules linked by hydrogen bonds to the solvent of crystallization, but in this case, the structure is more complex. The solvent water acts as both a hydrogen bond acceptor (to the H on N(3); N···O, 2.80 Å) and a hydrogen bond donor (to O(2); O···O, 2.84 Å), as well as being involved in a hydrogen bond to the hydroxy group of the side chain (O(19); O···O, 2.71 Å). As a result, each water molecule links three isoalloxazine units. In addition, there is apparently a weaker hydrogen bond between the side chain OH and N(1) of an adjacent molecule (O(19)···N(1)′, 2.92 Å). These distances are within ranges previously observed for similar hydrogen bonds.[29-31] The closest intermolecular distance is 3.247 Å (C(2)···C(10a)′).

Reaction of 2-Amino-*N*-methylanilines with Alloxan. Although N(10)-dealkylation reactions under acidic[20] or basic[21,22] conditions and in the presence of light[23] or metal ions[20d] are well-documented for flavin derivatives, our structural results clearly indicate that the alloxazine product III must be formed via another pathway. Careful reading of the literature reveals that although lumichromes (alloxazines) are commonly observed as byproducts in the synthesis of flavins from alloxan,[20d] in no case has the structure been independently confirmed where an asymmetrically substituted 2-amino-*N*-alkylaniline was used. Instead, it has been assumed that the alloxazine was formed by dealkylation of the desired flavin. For example, alloxazine-7-sulfonate has been claimed to be the only alloxazine formed in the reaction of 2-amino-*N*-methylaniline-4-sulfonate with alloxan, either directly in a fast reaction or more slowly via 10-methylisoalloxazine-7-sulfonate.[20d] If our results are general, then another pathway for formation of alloxazines must exist and at least some of the structures proposed in the literature for lumichrome derivatives may be incorrect. Consequently, we have reinvestigated the reaction of alloxan with substituted 2-amino-*N*-methylanilines.

Chlorination with $POCl_3$ in DMF[18b] of 7,10-dimethylisoalloxazine 5-oxide (prepared from 6-(*N*-methyl-*p*-toluidino)uracil

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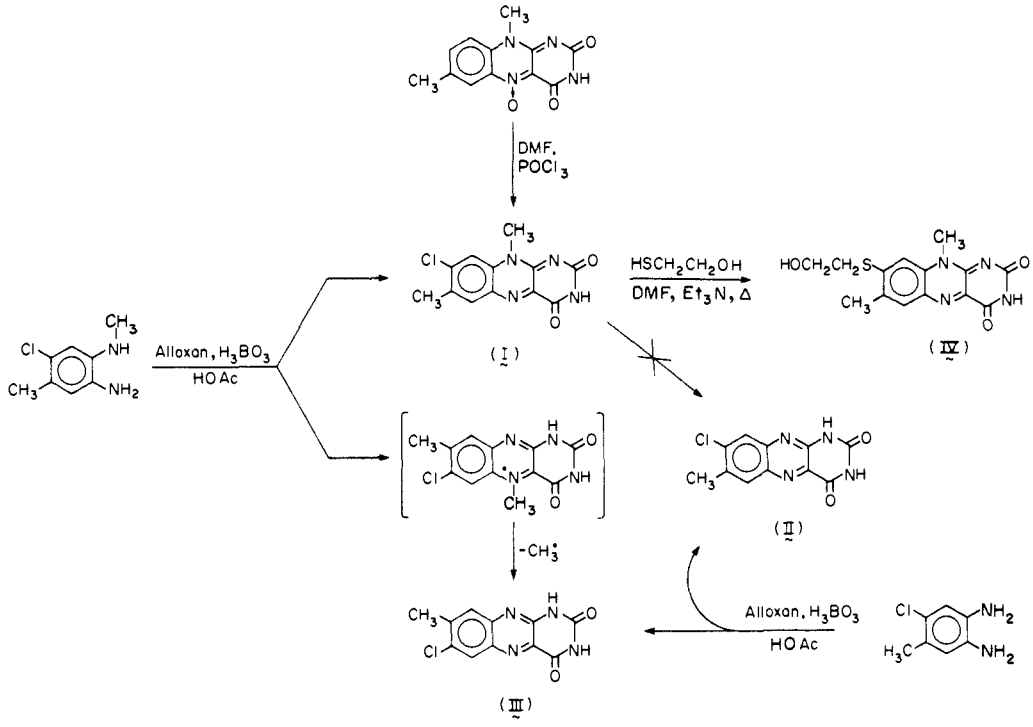
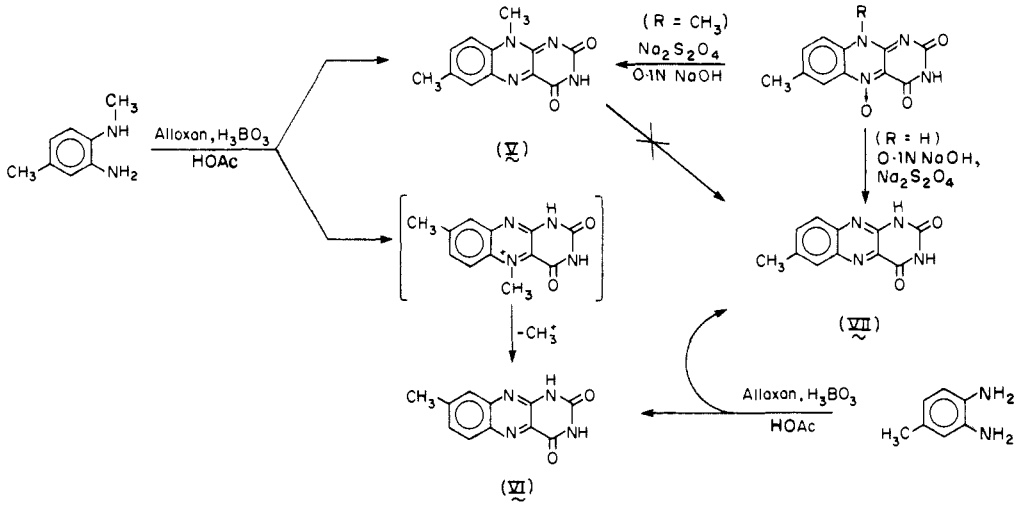
Scheme I



Scheme II



as described[7b,c]) gave 8-chloro-7,10-dimethylisoalloxazine in good yield and with unambiguous stereochemistry. The product was identical in all respects with compound I obtained from alloxan and hydrogenated 5-chloro-*N*,4-dimethyl-2-nitroaniline. We find that not only is I not converted to III upon standing or mild heating in acetic acid/boric acid mixtures, but it is not even readily dealkylated to 8-chloro-7-methylalloxazine (II) under these conditions in the absence of light.

In order to determine whether II and III can actually be distinguished readily by spectroscopic properties, we carried out the reaction of 4-chloro-5-methyl-*o*-phenylenediamine with alloxan, which would be expected to give II and III in approximately equal amounts. Although II and III could not be readily separated by crystallization or chromatography, proton NMR spectra of the crude reaction product in trifluoroacetic acid show the presence of two components in approximately equal amounts. One is identical to III, suggesting strongly that the other is indeed II. Comparison of the spectra with that of 8-methylalloxazines unambiguously established the peaks at 8.37 and 8.03 ppm in the spectrum of III as being due to the 6-H and 9-H aryl protons, respectively. This is consistent with the results observed by Müller and co-workers for a series of alloxazines and isoalloxazines.[33] These syntheses and the reactions discussed above for I–IV are summarized in Scheme I.

Although it was not our intent to perform an exhaustive survey of product distributions in flavin syntheses, the reaction of 2-amino-*N*,4-dimethylaniline with alloxan was explored to determine whether the observations reported above were indeed general. As indicated in Scheme II, the reaction gives both flavin (isoalloxazine), V, and lumichrome (alloxazine), VI, derivatives as products, in an approximately 4:1 ratio. The products were readily separated by fractional crystallization and identified by proton NMR spectra, but the stereochemistry of VI (viz., 7- or 8-methylalloxazine) could not be established from these data. Accordingly, pure 7-methylalloxazine VII was prepared with unambiguous stereochemistry and in good yield from the uracil route reported for flavins.[7c] The aromatic resonances at δ 8.15 (1 H, s, 6-H) and 8.08 (2 H, s, 7-H and 8-H) in the proton NMR of VII are distinct from those observed for VI (vide supra), suggesting that VI is indeed the "reversed" 8-methylalloxazine. In order to remove any ambiguity, the reaction of 4-methyl-*o*-

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phenylenediamine with alloxan was examined. Again, an approximately equimolar mixture of two alloxazine products was obtained. Proton NMR spectra clearly demonstrated that one was VII, and the other was identical with VI. These results are summarized in Scheme II.

Taken together, the results discussed above demonstrate that the reaction of alloxan with substituted 2-amino-*N*-alkylanilines produces an alloxazine with reversed stereochemistry in addition to the expected flavin. No evidence for the alloxazine produced by N(10) dealkylation of the flavin has been observed. The most reasonable explanation for the observed products is that alloxan can condense with 2-amino-*N*-alkylanilines in either of two possible orientations. One produces the N(10)-alkylisoalloxazine (flavin) directly, while the other yields an N(5)-alkylalloxazinium ion instead (Scheme I and II). Simple N(5)-dealkylation would yield an alloxazine with the observed stereochemistry. Although to our knowledge N(5)-alkylalloxazinium salts have not been prepared or detected directly, support for this hypothesis is afforded by a recent report on the chemistry of some closely related pterin derivatives.[34] Electrochemical oxidation of 5,6,6,7,7-pentamethyltetrahydropterin produces the N(5)-alkyldihydropteridinium ion, which rapidly demethylates in aqueous solution to give the 6,6,7,7-tetramethyldihydropterin[34] in a reaction parallel to that which we propose.

Inasmuch as a variety of subtle steric and electronic effects are likely to dictate which nitrogen of an *N*-alkyl-*o*-phenylenediamine attacks the central carbonyl of alloxan most rapidly, it is difficult at this time to generalize about factors favoring formation of the flavin over the "reversed" alloxazine. Nonetheless, the availability of a competing route in the condensation reaction with concomitant formation of an alloxazine with solubility and chromatographic behavior quite different from that of the isoalloxazine could well account for the highly variable yields encountered in classical flavin syntheses.[4] Conversely, a variety of substituted alloxazines could be available via this route by suitable choice of starting material.

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Supplementary Material Available: Tables of observed and calculated structure amplitudes, anisotropic displacement parameters, and positional and isotropic displacement parameters for hydrogen atoms (29 pages). Ordering information given on any current masthead page.

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Heteronuclear NMR Studies of Cobalamins. 4. α-Ribazole-3′-phosphate and the Nucleotide Loop of Base-on Cobalamins[1]

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Abstract: α-Ribazole-3′-phosphate (1-α-D-ribofuranosyl-5,6-dimethylbenzimidazole-3′-phosphate), the detached nucleotide of the nucleotide loop of cobalamins, has been prepared by sulfuric acid catalyzed hydrolysis of the phosphodiester of cyanocobalamin and characterized by ^{1}H, ^{13}C, and ^{31}P NMR of both the zwitterionic and dianionic forms. These NMR characteristics along with those of the cationic and neutral forms of α-ribazole have been used to analyze trends in the ^{13}C NMR resonances of the nucleotide loops of a series of 10 base-on cobalamins in which the apparent strength of benzimidazole coordination varies by more than 6 orders of magnitude. Trends in the benzimidazole carbon resonances are found to be dominated by electronic and dipolar shielding effects and a method is developed for estimating the magnetic anisotropy ($\Delta\chi$) of the cobalt atom dipole for cobalamins of known geometry. The ribose moiety chemical shifts are influenced by electronic and dipolar effects, and the ribose moiety appears to have a different conformation than that of the free nucleotide and nucleoside. Trends in the chemical shifts of the 2-hydroxypropylamine carbons and the trend previously reported for the phosphorus atom chemical shift are shown to be due primarily to regular changes in nucleotide loop conformation throughout the series of compounds. Two-bond and three-bond phosphorus–carbon coupling constants are consistant with this interpretation.

In recent publications,[2-4] we have investigated the ^{31}P NMR spectroscopy of cobalamins and showed that while all base-off cobalamins investigated have the same ^{31}P chemical shifts, the position of the ^{31}P resonance of base-on cobalamins varies directly with the apparent strength of coordination of the axial benzimidazole nucleotide. For this work, we have estimated the free energy of benzimidazole coordination ΔG_{Co}, from eq 1, where the

$$K_{\text{base-off}} = (1 + K_{Co})K_{Bz} \quad (1)$$

equilibrium constants are defined in eq 2-4, and pK_{Bz} is assumed to be equal to the pK_a of the detacted benzimidazolium nucleoside (α-ribazole, pK_a = 5.56 at 25 °C, ionic strength 1.0 M).[5] Thus, the change in ^{31}P chemical shift upon displacement of the axial base by protonation ($\Delta\delta_{31_P} = \delta_{31_P}^{\text{base-on}} - \delta_{31_P}^{\text{base-off}}$) is directly proportional to $-\Delta G_{Co}$,[2] for a series of 10 cobalamins in which K_{Co} (eq 4) varies over more than 6 orders of magnitude. In addition, for those cobalamins whose geometry is known from X-ray crystallography, the base-on ^{31}P chemical shift was shown to be directly related to the axial Co–N bond distance, the resonance moving downfield with decreasing Co–N bond distance (and increasing strength of coordination). These observations have been interpreted in terms of the work of Gorenstein and coworkers[6-8] who have shown that the ^{31}P chemical shift of phosphate

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