

an 8:5 mixture of unreacted **41a** (29%) and thioanisole (nmr analysis). Increasing the benzene concentration gave 0.23 g (71%) of benzoate **41b** as a yellow oil which solidified upon standing, mp 82–85°; ir (film) 1720 and 1580 cm^{-1} ; nmr 8.15–7.90 (m, 2 H), 7.70–7.05 (m, 8 H), 3.98 (AB quartet, $J = 12.5$ Hz, $\Delta\nu = 28.3$ Hz), 1.29 (s, 3 H), 1.18 (s, 3 H), and 0.90 (d, 3 H, $J = 6.5$ Hz).

(±)-Zizaene. To a refluxing solution of lithium (0.04 g, 5.8 mmol) in liquid ammonia (15 ml) under nitrogen was added a solution of benzoate **41b** (0.23 g, 0.54 mmol) in 5 ml of ether over a 30-min period. After an additional 30 min at reflux with stirring, the blue color was quenched by addition of ammonium chloride in small portions. The ammonia was then slowly evaporated. Pentane was added in small portions during the evaporation. The re-

sulting mixture was added to water, the layers were separated, and the aqueous portion was extracted with additional pentane. The pentane extracts were washed with 1 *N* NaOH and water, dried (MgSO_4), and evaporated. The pale yellow liquid (0.11 g) obtained was chromatographed on 10 g of silica gel. Elution with pentane gave 0.07 g (64%) of (±)-zizaene ((±)-**1**) as a clear, colorless liquid. This material has the same glpc retention time (column A at 118°) and ir and nmr spectra as zizaene (see above).

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Synthesis of 8 α -Histidyl-3,8-dimethyl-10-ethylisoalloxazine and Related Isoalloxazines¹

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Abstract: The new 10-ethylisoalloxazines, 6-hydroxymethyl-, 7-methyl-, 8-hydroxymethyl-3-methyl-, 9-hydroxymethyl-, and 8 α -histidyl-3,8-dimethyl-10-ethylisoalloxazine, have been synthesized, the 8 α -histidyl-3,8-dimethyl-10-ethylisoalloxazine being a model for 8 α -histidylriboflavin, the succinate dehydrogenase flavin. Synthesis was achieved, except for the histidyl compound, by condensation of the appropriately substituted 2-amino-*N*-ethylaniline with alloxan or *N*-methylalloxan, the substituted diaminobenzenes being obtained from carboxy-substituted 2-nitroacetanilides by consecutive reduction with diborane and Raney nickel-hydrogen. Conversion of 8-hydroxymethyl-3-methyl-10-ethylisoalloxazine to the corresponding bromomethyl derivative and condensation of the bromo derivative with *N* α -benzoylhistidine followed by hydrolysis yielded the 8 α -histidyl-3,8-dimethyl-10-ethylisoalloxazine. The new isoalloxazines exhibit, as expected, the spectral properties and fluorescence behavior of flavins and, in particular, 8 α -histidyl-3,8-dimethyl-10-ethylisoalloxazine closely resembles 8 α -histidylriboflavin. Visible and near uv absorbance band shifts of flavins resulting from protonation and from substituents on the benzene ring are interpreted on the basis of resonance interactions, visible maxima being assigned to resonance forms involving N¹⁰ lone electron pair interaction while near uv maxima are assigned to resonance forms involving electron release from the benzene ring.

In connection with the structure and mode of attachment of riboflavin covalently linked to the peptide chain of succinate dehydrogenase,^{2,3} this laboratory several years ago undertook the synthesis of flavin models substituted at the 8 α -methyl position with a nucleophilic function appropriate to an amino acid side chain, the choice of 8 α -substituted flavins as models for the succinate dehydrogenase flavin being made on the basis of the following considerations. The ribityl side chain and the pyrimidine ring having been ruled out by previous investigations^{2,3} as the flavin site of attachment, chemical considerations,^{4–6} in particular the ease of substitution of 8 α -methyl hydrogen by deuterium under mild conditions,⁵ and biosynthetic possibilities suggested the 8 α -methyl position as the site of attachment to the protein, the biosynthetic possibilities suggesting that ionization from the 8 α -methyl group provides a pair of electrons for

oxidation to the oxidation level requisite for substitution by a nucleophilic function of an amino acid residue such as the ϵ amino of a lysine residue or a nitrogen atom of the imidazole ring of a histidine residue, etc. Subsequent to our undertaking the synthesis of 8 α -methyl-substituted flavins, the 8 α position was identified⁷ as the site of attachment, the 8-methyl group being substituted^{8,9} by a ring nitrogen of a histidine residue.

To provide 8 α -methyl-substituted flavins models, as well as other methyl-substituted flavins, a general synthetic approach to *N*¹⁰-alkylisoalloxazines substituted in the benzene ring with hydroxymethyl groups was utilized (Scheme I) and in this paper we report on the synthesis of 3-methyl-8-hydroxymethyl-10-ethylisoalloxazine, 6- and 9-hydroxymethyl-10-ethylisoalloxazines, 7-methyl-10-ethylisoalloxazine, and 8 α -histidyl-3,8-dimethyl-10-ethylisoalloxazine.

Synthesis of the above compounds starts (Scheme I) with the corresponding carboxy-substituted *o*-nitroanilines (the four acids, Ia, Ib, Ic, and Id, being syn-

(1) Abstracted in part from the Ph.D. Thesis of P. Johnson, Sept 1971.

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(3) T. Y. Wang, C. L. Tsou, and Y. L. Wang, *Sci. Sinica*, **14**, 1193 (1965).

(4) P. Hemmerich, B. Prijs, and H. Erlenmeyer, *Helv. Chim. Acta*, **42**, 2164 (1959).

(5) E. Y. Bullock and O. Jardetzky, *J. Org. Chem.*, **30**, 2056 (1965).

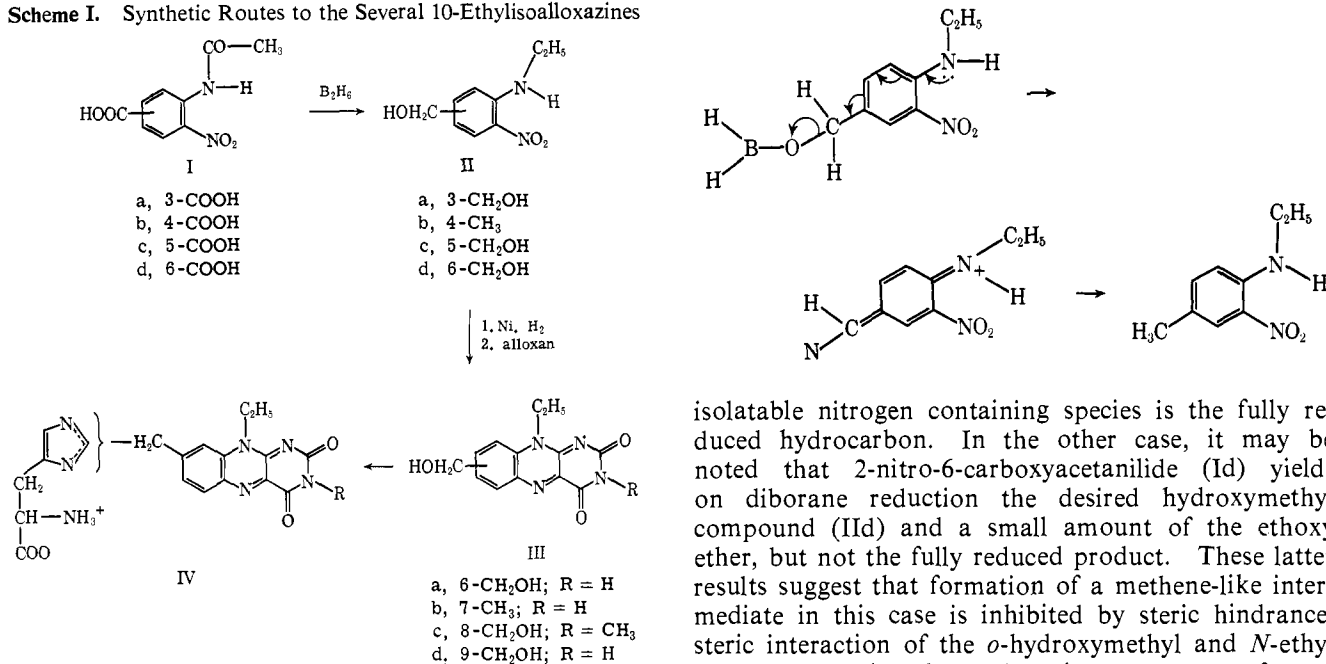
(6) F. Muller and P. Hemmerich, *Helv. Chim. Acta*, **49**, 2352 (1966).

(7) P. Hemmerich, A. Ehrenberg, W. H. Walker, L. E. G. Eriksson, J. Salach, P. Bader, and T. P. Singer, *FEBS (Fed. Eur. Biochem. Soc.) Lett.*, **3**, 37 (1969).

(8) H. W. Walker and T. P. Singer, *J. Biol. Chem.*, **245**, 4224 (1970).

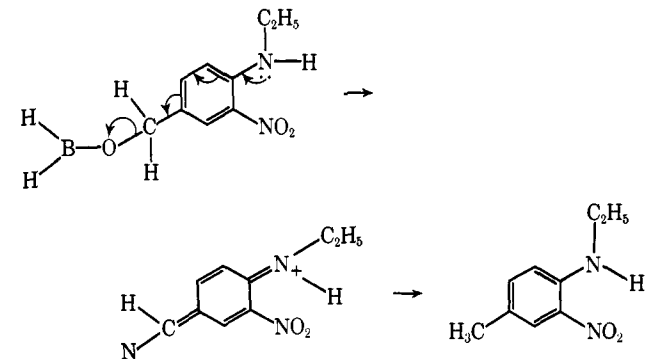
(9) S. Ghisla, U. Hartmann, and P. Hemmerich, *Angew. Chem., Int. Ed. Engl.*, **9**, 642 (1970).

Scheme I. Synthetic Routes to the Several 10-Ethylisoalloxazines



thetically available) and proceeds *via* acetylation to the acetanilide, simultaneous reduction by diborane¹⁰ to the hydroxymethyl-*o*-nitroethylaniline, Raney nickel catalyzed reduction of the nitro group, and condensation with alloxan to yield the isoalloxazine. For the synthesis of the histidylisoalloxazine, 3-methyl-8-hydroxymethyl-10-ethylisoalloxazine was converted by treatment with phosphorus tribromide to the 8-bromomethyl compound and the bromomethyl compound was then condensed with *N*^α-benzoylhistidine, the procedure of Ghisla, *et al.*,⁹ being used for the condensation and subsequent work-up.

The route to the several isoalloxazines depicted in Scheme I is straightforward, all of the synthetic steps being known and proceeding with little or no complication except for the diborane reductions where in one instance the desired product was not obtained and in other instances yields were low. *N*-Ethyl-4-hydroxymethyl-2-nitroaniline, expected from the diborane reduction of 2-nitro-4-carboxyacetanilide (Ib), was not obtained but, instead, a small amount of the further reduction product, *N*-ethyl-4-methyl-2-nitroaniline (IIb), and a nonnitrogenous product were obtained. With respect to this reduction of the carboxyl group of 2-nitro-4-carboxyacetanilide to its lowest reduction state, similar results have been obtained¹¹ with aromatic aldehydes and ketones bearing electron-releasing substituents, the diborane reductions proceeding to the hydrocarbon level, an intermediate methene-like species being suggested. Extending this suggestion to the present example of reduction to the hydrocarbon, generation of a methene-like intermediate would be expected to occur after reduction of both the acetanilide to the *N*-alkylaniline and the carboxyl group to the alcohol and then only with the alkylamino function ortho and para to the alcohol function. The behavior of the para-substituted acid, Ib, is clearly consistent with this mechanism and, indeed, in this case, the only



isolatable nitrogen containing species is the fully reduced hydrocarbon. In the other case, it may be noted that 2-nitro-6-carboxyacetanilide (Id) yields on diborane reduction the desired hydroxymethyl compound (IIId) and a small amount of the ethoxy ether, but not the fully reduced product. These latter results suggest that formation of a methene-like intermediate in this case is inhibited by steric hindrance, steric interaction of the *o*-hydroxymethyl and *N*-ethyl groups preventing the coplanarity necessary to formation of an *o*-methene. The small amount of ethoxy ether may then be accounted for by the action of the quenching reagents, acid and alcohol, subsequent to the reduction.

Absorption maxima for the several isoalloxazines synthesized in this study and for several related compounds are presented in Table I. It is apparent from the data that the new isoalloxazines show maxima typical of the flavins; one band in the 440-nm region, one in the 350-nm region, and two in the uv region are seen in neutral solution and on protonation the long wavelength maxima disappears while the next band is shifted to longer wavelength.¹² Further, comparison of the spectral characteristics of 8 α -histidyl-3,8-dimethyl-10-ethylisoalloxazine with those for natural⁸ and synthetic⁹ 8 α -histidylriboflavin shows that with the exception of the neutral flavin bands centered around 350 nm, the spectra are almost identical, the cationic forms, in particular, showing a shoulder at 405 nm and only small differences with respect to the other two bands. This similarity in spectra, of course, further corroborates the assignment of the 8 α -histidylriboflavin structure to the succinate dehydrogenase flavin.

With respect to the other flavins of Table I, it may be noted that the hydroxymethylisoalloxazines and the 8 α -substituted riboflavins are of particular interest in connection with correlation of flavin structure and spectra. Substitution of riboflavin's 8 α hydrogen by histidyl or hydroxyl has no effect on the 445-nm band but shifts the 368-nm band to shorter wavelengths while removal of the 8-hydroxymethyl group of the 10-ethylisoalloxazine (IIId) to the 6 and 9 positions (IIIa and IIIc, respectively) has an opposite effect. The long wavelength band (446 nm) of the 8-hydroxymethyl compound shifts to shorter wavelengths and the near uv band (348 nm) shifts to longer wavelengths. These spectral observations and the red shift on protonation may be correlated in a qualitative and limited manner on the basis of resonance considerations simi-

(10) L. F. Fieser and M. Fieser, "Reagents for Organic Synthesis," Wiley, New York, N. Y., 1967, p 199.

(11) K. M. Bixwas, L. E. Houghton, and S. H. Jackson, *Tetrahedron, Suppl.*, 7, 261 (1966).

(12) This red shift is typical of the flavins.

Table I. Visible and UV Maxima^a

Isoalloxazine	Neutral ^b	Cationic ^c
6-Hydroxymethyl-10-ethyl	430, 357, 263, 217 10,040, 8,300, 29,300, 20,400	365, 263 15,250
9-Hydroxymethyl-10-ethyl	432, 352, 263, 222 9,010, 8,150, 28,400, 22,800	371, 265 14,200, 21,700
6,8-Dimethyl-10-L-arabityl ^d	435 (s), 392, 380 (s), 360, 325, 266, 222	
7-Methyl-10-ethyl	442, 350, 265, 218 10,100, 7,700, 35,300, 26,400	367, 262 14,400, 23,100
7-Methyl-10-D-ribityl ^f	440, 356, 267, 226	
8 α -Histidyl-3,8-dimethyl-10-ethyl	443, 344, 263, 217 11,100, 9,430, 35,000, 28,700	405 (s), 371, 262 20,000, 29,900
8-Hydroxymethyl-3-methyl-10-ethyl	446, 348, 264, 217 10,480, 8,210, 37,700, 31,100	372, 265 18,100, 30,100
Riboflavin ^g	445, 368	395
8 α -Hydroxyriboflavin ^g	445, 359	378
8 α -Histidylriboflavin ^h	445, 355, 268, 219 12,000, 9,500, 37,000, 46,000	405 (s), 373, 264 10,000, 14,400, 34,500
6,7-Dimethyl-10-D-ribityl ⁱ	\approx 450, \approx 385	
7,8-Dimethyl-10-L-arabityl ^d	450, 372, 325 (s), 270, 222	

^a s shoulder, molar absorbance below wavelength in nm. ^b pH 7.0, 0.03 M phosphate. ^c 6 N hydrochloric acid. ^d R. Kuhn, P. Desnuelle, and F. Weygand, *Chem. Ber.*, **70**, 1293 (1937). ^e R. Kuhn and F. Weygand, *ibid.*, **67**, 1409 (1934). ^f R. Kuhn, H. Vetter, and H. W. Rzeppa, *ibid.*, **70**, 1302 (1937). ^g Reference 7. ^h References 8 and 9. The synthetic 8 α -histidylriboflavin is presumed to be a mixture of the N¹ and N³ histidyl isomers. ⁱ M. Tishler, K. Pfister, R. D. Babson, K. Ladenburg, and A. J. Fleming, *J. Amer. Chem. Soc.*, **69**, 1488 (1947); the 450-nm peak is flat with molar extinction 6500.

lar to those applied in explanation of the spectra of diphenylmethanes and tetracyclones.¹³

These considerations are based on the resonance forms depicted in Scheme II. Form a represents the neutral ground state structure, b and c represent the monoprotonated¹⁴⁻¹⁶ ground state structure, and the other forms represent the excited state structures arising from resonance interactions involving the N¹⁰ lone-pair electrons and from resonance interactions giving rise on the benzene ring to positive charge on carbons 6 and 8 and relievable by hyperconjugation with methyl substituents in these positions.

Assuming N¹⁰ polarization as energetically more favorable than polarization at carbons 6 and 8, then the longest wavelength band (440-nm region) may be assigned to the quinoidal diimino resonance forms d and e and the near uv band (350-nm region) may be assigned to resonance forms f and g.¹⁷ With these assignments in mind and considering protonation effects first, it may be noted that the ground state of the monocation (forms b and c) with positive charge localized on N¹ and N¹⁰, respectively, is stabilized against excitation to the quinoidal diimino form e while excitation to resonance form g, involving positive charge on the benzene ring, is favored. A similar situation does not prevail in the neutral ground state, form a. Accordingly, on protonation a hypsochromic shift of the \sim 440-nm band and a bathochromic shift of the

(13) These compounds exhibit two maxima and each wavelength maximum is assigned to a particular set of resonance forms, the sets differing in axes of conjugation; L. N. Ferguson, "Modern Structural Theory of Organic Chemistry," Prentice-Hall, Englewood Cliffs, N. J., 1963, p 506. Quantum mechanical calculations indicate polarization along different axes for the flavin maxima in question; P. S. Song, "Flavins and Flavoproteins," Henry Kamin, Ed., University Park Press, Baltimore, Md., 1971, p 37.

(14) Assignment of protonation to N-1 is based on spectral evidence¹⁵ and on X-ray evidence.¹⁶

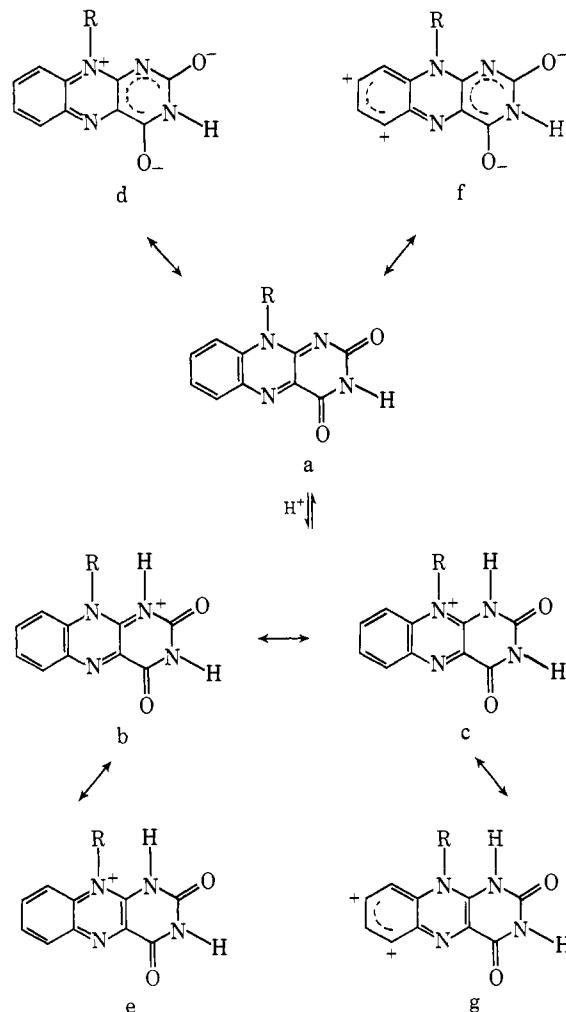
(15) K. H. Dudley, A. Ehrenberg, P. H. Hemmerich, and F. Muller, *Helv. Chim. Acta*, **47**, 1354 (1964).

(16) C. J. Fritchie, Jr., *Chem. Commun.*, 1486 (1968).

(17) In a preliminary suggestion, both these maxima have been assigned¹³ to resonance forms involving only N¹⁰ electron pair interactions.

(18) E. M. Kosower, "Molecular Biochemistry," McGraw-Hill, New York, N. Y., 1962, p 221.

Scheme II. Resonance Forms for Neutral and Monoprotonated Isoalloxazines



\sim 350-nm band are to be expected. These two effects of protonation are clearly operative for the histidyl derivatives presented in Table I while other flavins including those listed in Table I may only manifest the

bathochromic shift of the ~ 350 -nm band. For the histidyl derivatives, the 443-nm band of 8 α -histidyl-3,8-dimethyl-10-ethylisoalloxazine and the 445-nm band of 8 α -histidylriboflavin shift to a 405-nm shoulder, while the respective 344- and 355-nm bands shift to 371 and 373 nm. For the other flavins of Table I, the bathochromic shift of the ~ 350 -nm band is clearly evident while the hypsochromic shift of the ~ 440 -nm band is not. Presumably, the shift of the ~ 440 -nm band is masked by the now more intense near uv band.

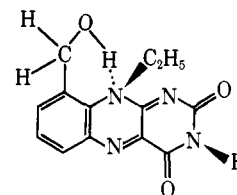
Assignment of the long wavelength band to quinoidal diimino resonance forms and assignment of the near uv band to resonance forms involving direct electron release from carbons 6 and 8 of the benzene ring or hyperconjugative electron release from methyl substituents in these positions also permit interpretation of the effects of substitution in the benzene ring and, in particular, substitution of methyl group hydrogen. With respect to this latter effect, substitution of an 8-methyl hydrogen by histidine or hydroxyl has no effect on the long wavelength of riboflavin, all three compounds (Table I), riboflavin, 8 α -histidylriboflavin and 8 α -hydroxyriboflavin, demonstrating a 445-nm maximum. On the other hand, substitution at 8-methyl does alter near uv maxima, this band shifting from 368 nm in riboflavin to 359 nm in 8 α -hydroxyriboflavin and to 355 nm in 8 α -histidylriboflavin. Similarly, the long wavelength bands of 8-hydroxymethyl-3-methyl-10-ethylisoalloxazine and 8 α -histidyl-3,8-dimethyl-10-ethylisoalloxazine are close to that of riboflavin (446 and 443 nm, respectively) while the near uv band is shifted to 348 and 344 nm, respectively.

The above effects of 8-methyl substitution, constancy of the long wavelength band and a hypsochromic shift of the near uv band, are, of course, in keeping with the wavelength assignments; resonance form d, assigned to the long wavelength band and involving N¹⁰ lone pair interactions, is not expected to be altered by 8-methyl substitution while resonance form f, assigned to the near uv band and involving hyperconjugation influenced electron release from the benzene ring, is expected to be altered by 8-methyl substitution. Since, in the cases mentioned above, the shifts of the near uv band are hypsochromic, it is clear that hyperconjugation is inhibited¹⁹ by hydroxyl and histidyl substitution at the 8-methyl position. Corollary to these findings, removal of the 8-methyl group results in a hypsochromic shift of the 368-nm band of riboflavin without substantially altering the 445-nm band, the corresponding bands for 7-methyl-10-ethylisoalloxazine and 7-methyl-10-D-ribitylisoalloxazine being 350 and 442 nm, and 356 and 440 nm, respectively. Of further interest in connection with these hyperconjugation effects, 6,8-dimethyl-10-L-arabitylisoalloxazine with both methyl groups capable of hyperconjugation shows several bands in the near uv, the most intense being at 392 nm, suggesting that this band is due to a bathochromic shift of the 368-nm band of riboflavin arising from the expected effects on resonance form f of hyperconjugative electron release from both methyl groups.

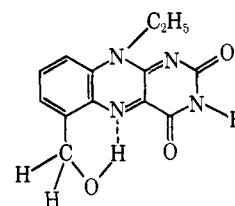
While the near uv band of the flavins of Table I is influenced considerably by substituents in the benzene ring, the long wavelength band is much less influenced

(19) Hemmerich, *et al.*,⁷ have used the term restricted hyperconjugation in connection with such effects.

and (with the exception of the long wavelength band of the first three flavins of Table I), as for the other flavins, is almost identical to the 445-nm band of riboflavin. These flavins and their long wavelength bands are 6-hydroxymethyl-10-ethylisoalloxazine (430 nm), 9-hydroxymethyl-10-ethylisoalloxazine (432 nm), and 6,8-dimethyl-10-L-arabitylisoalloxazine (435 nm). Considering 9-hydroxymethyl-10-ethylisoalloxazine first, it would seem that this hypsochromic shift of the long wavelength band is due to inhibition of N¹⁰ lone pair resonance interaction, the inhibition arising from H bonding between N¹⁰ and 9 α -hydroxyl and/or from



steric inhibition to the coplanar geometry required for N¹⁰ electron participation in resonance form d. While this latter possibility, steric inhibition of resonance, may be operative with 9-hydroxymethyl-10-ethylisoalloxazine, it is not operative with 7,9-dimethyl-10-L-arabitylisoalloxazine (long wavelength maximum of the latter compound at 450 nm). Steric inhibition of resonance is also not possible with 6-hydroxymethyl-10-ethylisoalloxazine (long wavelength maximum at 430 nm). For this flavin, H bonding between N⁵ and 6 α -hydroxyl is possible and such H bonding would stabilize ground states against excitation and, in this



connection, it is of interest to note that both the long wavelength maximum (430 nm) and the near uv maximum (357 nm) are at shorter wavelengths than 6,7-dimethyl-10-D-ribitylisoalloxazine (long wavelength maximum at 450 nm and near uv maximum at 385 nm).

Figure 1 presents the pH dependency of fluorescence for the several new isoalloxazines. As expected, 6-hydroxymethyl-10-ethylisoalloxazine, 7-methyl-10-ethylisoalloxazine, and 9-hydroxymethyl-10-ethylisoalloxazine show pH dependency curves similar to that of riboflavin mononucleotide (FMN) and of riboflavin,⁹ the curves being characterized by a broad maximum fluorescence over the approximate pH range 3.5–9.0.

3-Methyl-8-hydroxymethyl-10-ethylisoalloxazine and 8 α -histidyl-3,8-dimethyl-10-ethylisoalloxazine also exhibit similar pH dependencies, the acid limbs of the pH *vs.* fluorescence curves being the same as those for the other compounds and for riboflavin. However, the alkaline limb in the case of these two compounds is displaced toward more acid pH values. This displacement of the alkaline limb by 8 α substitution has been previously noted^{7–9} and the curve obtained herein for 8 α -histidyl-3,8-dimethyl-10-ethylisoalloxazine is similar to those⁹ for synthetic and natural 8 α -histidylriboflavin except for a displacement of the pK of the alkaline limbs

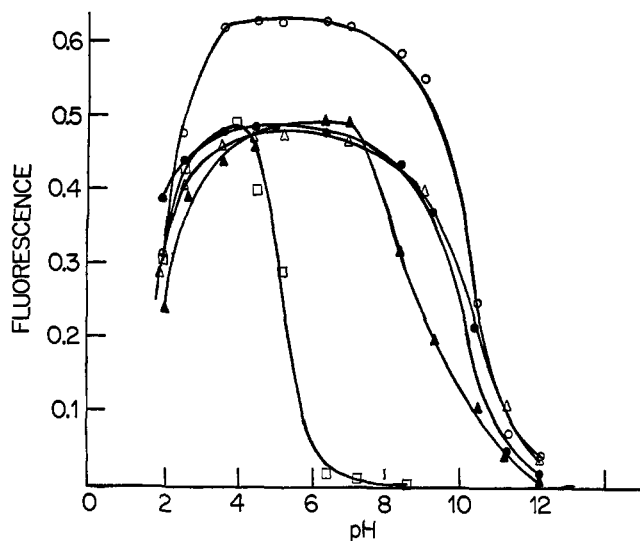


Figure 1. pH dependency of fluorescence for 7-methyl-10-ethylisoalloxazine (○), 6-hydroxymethyl-10-ethylisoalloxazine (●), 9-hydroxymethyl-10-ethylisoalloxazine (△), 8-hydroxymethyl-3-methyl-10-ethylisoalloxazine (▲), and 8 α -histidyl-3,8-dimethyl-10-ethylisoalloxazine (□). Ordinate represents instrument response relative to 1×10^{-7} M riboflavin 5-phosphate; excitation and emission wavelengths set at the respective maxima for the individual compounds.

to an approximate value of 5 (a pK of 4.4 being reported⁸ for natural 8 α -histidylriboflavin). Interpretation of fluorescence data cannot be attempted since this study and previous studies of the pH dependency of fluorescence have been carried out at only one excitation wavelength (usually the excitation maximum at pH 3.25) and excitation maxima and emission maxima as a function of pH not having been determined. However, the bell-shaped curves for isoalloxazines, such as 7-methyl-10-ethylisoalloxazine and 8-hydroxymethyl-3-methyl-10-ethylisoalloxazine (the former lacking 8 α ionizable hydrogen and the latter N³ ionizable hydrogen) suggest that the alkaline limb of such curves is due to either addition of hydroxide ion or addition of water followed by removal of a proton.

Experimental Section

Melting points were determined with the Fisher-Johns melting point apparatus and are corrected. Ultraviolet and visible spectra were obtained with the Cary 14 spectrophotometer, 1-cm quartz cells being employed. Infrared spectra of liquids (neat) and solids (potassium bromide pellet) were obtained with the Beckman IR-8 spectrophotometer, polystyrene being employed as a standard. Fluorescence spectra were obtained with a Farrand Model MK-1 spectrofluorometer equipped with a Heath Servo recorder. Slit widths were set at 1 mm, and 1-cm quartz cuvettes were employed. Mass spectra were obtained at 70 eV and an operating temperature of 250° with an LKB-9000 combined gas chromatograph-spectrometer. Microanalyses were performed by the laboratory of Dr. A. Bernhardt, Mulheim (Ruhr), Germany. Analytical thin layer chromatography (tlc) was performed on Eastman precoated silica gel sheets using the Eastman Chromagram apparatus. For tlc of isoalloxazines the upper layer of 4 parts of butanol (reagent grade), 1 part of glacial acetic acid (reagent grade), and 5 parts of water (distilled and deionized) was used as the eluting solvent. Preparative thin layer chromatography plates were prepared by coating 20×20 cm clean and dry glass plates with a 2-mm slurry of silica gel G in butanol-acetic acid water (4:1:5, upper layer) and then drying the plates overnight in an oven at 70°.

Materials. *m*-Aminobenzoic acid, *p*-aminobenzoic acid, and phosphorus tribromide (Aldrich Chemical Co.) were used without further purification. Alloxan monohydrate and 3-nitrophthalic anhydride (Eastman) were used without further purification.

Tetrahydrofuran (Quaker Oats Co.) was distilled from calcium hydride, bp 65°. Diborane, a 1 M solution in tetrahydrofuran, was supplied by Alfa Inorganics, Inc. Raney nickel was prepared from nickel-aluminum alloy (K & K Laboratories) according to the method of Pavlic and Adkins²⁰ and stored under absolute ethanol. Absolute ethanol (Commercial Solvents Corp.) was used without further purification. Riboflavin (Nutritional Biochemical Corp.) was recrystallized from acetic acid before use, mp 278–280° (lit.²¹ mp 278–280°). *N*-Benzoyl-L-histidine monohydrate (Cyclo Chemical Corp.) was used without further purification. Dimethylformamide (Fisher certified reagent grade) was stirred with dry potassium hydroxide pellets and decanted into an equal volume of dry benzene. The benzene was distilled at atmospheric pressure and then the dimethylformamide was distilled *in vacuo*, bp 82° (5 mm), and used within 24 hr. *N*-Methylalloxan monohydrate was prepared by the method of Billmann and Berg,²² mp 154–156° (lit. mp 156°). Neutral, 80–200 mesh alumina (Fisher Scientific Co.) was used for column chromatography. Silica gel G for thin layer chromatography was purchased from Kenco Chemical Co. Silica gel, 60–200 mesh, grade 950, used for column chromatography, was purchased from Fisher Scientific Co.

Preparation of Acetamidonitrobenzoic Acids. 3-Acetamido-2-nitrobenzoic acid and 3-acetamido-4-nitrobenzoic acid were prepared according to the method of Kaiser²³ by nitration of *m*-acetylaminobenzoic acid with fuming nitric acid followed by separation of isomers: 3-acetamido-2-nitrobenzoic acid, mp 242–243.5° (lit.²³ mp 240–241°); 3-acetamido-4-nitrobenzoic acid, mp 205.5–206° (lit.²³ 205–206°). 4-Acetamido-3-nitrobenzoic acid was prepared by nitrating *p*-acetylaminobenzoic acid with fuming nitric acid according to the method of Kaiser,²³ mp 221–222.5° (lit.²³ mp 220–221°). 2-Acetamido-3-nitrobenzoic acid was prepared by the method of Chapman and Stephen,²⁴ 3-nitrophthalic acid being treated with ammonia to give 2-amido-3-nitrophthalic acid, conversion to 3-nitroanthranilic acid *via* Hofmann degradation, and acetylation to the acetamido compound, mp 183–184° (lit. mp 180–181°).

Diborane Reduction of Acetamidonitrobenzoic Acids. *N*-Ethyl-3-hydroxymethyl-2-nitroaniline. A three-necked, round-bottom flask equipped with a magnetic stirrer, condenser, pressure equalizing dropping funnel, and nitrogen inlet was charged under nitrogen with 75 ml (0.075 mol) of 1 M diborane in tetrahydrofuran. After cooling to 0°, 3.36 g (0.015 mol) of 3-acetamido-2-nitrobenzoic acid in 50 ml of tetrahydrofuran was added through the dropping funnel over a period of 1.5 hr, temperature being maintained at 0–5°. After the addition, the reaction mixture, which had turned dark, was refluxed for 2.0 hr. The mixture was then cooled, and 10 ml of absolute ethanol followed by 10 ml of 6 N hydrochloric acid was slowly added. The resulting orange colored solution was refluxed for 1.5 hr, made alkaline with saturated sodium hydroxide solution, and extracted with 500 ml of ether. The ether layer was separated, and after drying over anhydrous sodium sulfate, was concentrated *in vacuo*. The resulting residue, a black oily mixture, was dissolved in dry benzene and chromatographed on an alumina column. The column (12 \times 70 mm) was prepared by slowly pouring alumina (30 g) into a glass column filled with high boiling petroleum ether. After settling, the alumina column was washed with petroleum ether and then with 100 ml of benzene. The benzene solution of the reaction mixture was placed on the column and elution was initiated with benzene, 25-ml fractions being collected. After the forerun (tubes 1–3), a purple colored band appeared (tubes 4–5), followed by a broad, dark, brown band (tubes 6–24). Remaining material was then eluted with absolute ethanol (tubes 25–31). Tubes 6–24, after pooling and solvent removal, yielded a brown oil soluble in hot water and on cooling yielding bright yellow needles, 0.10 g (0.00052 mol), of *N*-ethyl-3-hydroxymethyl-2-nitroaniline, 3.4% yield, mp 104–106°. Recrystallization from hot water gave a product of higher purity, mp 105–105.5°. Tlc in benzene-methanol (9:1) gave only one spot, R_f 0.58; ir (KBr) 770 (m), 1048 (m), 1280 (s), 1350 (s), 1400 (w), 1505 (m), 1610 (m), 3270 (m), and 3415 cm^{-1} (w); uv_{max} (95% ethanol) 234 (ϵ 16,000), 285 (3140), 278 (shoulder, 2980), and 295 $\text{m}\mu$ (shoulder, 1650);

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visible_{max} (95% ethanol) 428 m μ (ϵ 3710); mass spectrum *m/e* 196 (M⁺, 95), 181 (100), 118 (71), 91 (59), 90 (48), and 77 (71).

Anal. Calcd for C₉H₁₂N₂O₃: C, 55.10; H, 6.12; N, 14.28. Found: C, 54.87; H, 6.09; N, 14.42.

Other fractions yielded only intractable tars.

***N*-Ethyl-5-hydroxymethyl-2-nitroaniline.** A solution of 4.48 g (0.02 mol) of 3-acetamido-4-nitrobenzoic acid in tetrahydrofuran was reduced as described above with 100 ml (0.10 mol) of 1 *M* diborane.

Concentration of the dry ether extract yielded a black oil and product was isolated as described above from a 50-g alumina column, tubes 5–42 (benzene) giving a brown oil which crystallized from hot water after charcoal treatment to give 3.0 g (0.015 mol) of *N*-ethyl-5-hydroxymethyl-2-nitroaniline, 77% yield, orange needles, mp 95–96°. Tlc in benzene–methanol (9:1) gave an *R*_f 0.43; ir (KBr) 750 (m), 1050 (m), 1220 (s), 1280 (m), 1350 (w), 1390 (m), 1495 (s), 1575 (s), 1625 (s), and 3500 cm⁻¹ (s); uv_{max} (95% ethanol) 234 (ϵ 20,000) and 287 m μ (5700); visible_{max} (95% ethanol) 422 m μ (ϵ 6300); mass spectrum *m/e* 196 (M⁺, 100), 181 (100), 118 (67), 91 (30), 90 (27), and 77 (46).

Anal. Calcd for C₉H₁₂N₂O₃: C, 55.10; H, 6.12; N, 14.28. Found: C, 55.03; H, 6.24; N, 14.43.

Evaporation of fractions 43–49 yielded an orange colored oil which crystallized from benzene to give yellow needles, 0.057 g (0.00034 mol) of 5-hydroxymethyl-2-nitroaniline, 1.7% yield, mp 139–140.5°. Tlc in benzene–methanol (9:1) gave one spot with an *R*_f 0.11; ir (KBr) 752 (w), 1040 (m), 1165 (s), 1220 (s), 1320 (s), 1410 (m), 1478 (s), 1560 (m), 1625 (s), 3395 (s), and 3505 cm⁻¹ (s); uv_{max} (absolute ethanol) 243 (ϵ 17,453) and 284 m μ (6321); visible_{max} (absolute ethanol) 403 m μ (ϵ 5556); mass spectrum *m/e* 168 (M⁺, 100), 122 (13), 77 (29), and 65 (19).

Anal. Calcd for C₇H₉N₂O₃: C, 50.00; H, 4.76; N, 16.66; O, 28.58. Found: C, 49.85; H, 4.90; N, 16.61; O, 28.39.

***N*-Ethyl-2-hydroxymethyl-6-nitroaniline.** A solution of 4.48 g (0.02 mol) of 2-acetamido-3-nitrobenzoic acid in tetrahydrofuran was reduced with 100 ml (0.10 mol) of 1 *M* diborane as previously described. Concentration of the dry ether extract yielded a brown oil which in this case was purified on a dry-packed column of 50 g of alumina. The first 50 ml of benzene eluted a reddish-yellow band which upon distillation furnished an orange liquid, 0.30 g (0.0013 mol), 6.7% yield, bp 135–140° (0.2 mm), identified as the ethyl ether of *N*-ethyl-2-hydroxymethyl-6-nitroaniline: *n*_D²⁴ 1.5780; ir (neat) 740 (s), 1165 (m), 1200 (m), 1350 (m), 1445 (m), 1530 (m), 1610 (s), 3000 (s), and 3398 cm⁻¹ (m); uv_{max} (95% ethanol) 242 (ϵ 18,610) and 280 m μ (shoulder, 3426); visible_{max} (95% ethanol) 410 m μ (ϵ 3695); mass spectrum *m/e* 224 (M⁺, 55), 161 (91), 145 (77), 117 (100), 91 (78), and 77 (55).

Anal. Calcd for C₁₁H₁₄N₂O₃: C, 58.93; H, 7.14; N, 12.50; O, 21.43. Found: C, 59.09; H, 6.89; N, 12.53; O, 21.57.

Further elution of the column with benzene yielded the desired product, 3.0 g (0.015 mol) of *N*-ethyl-2-hydroxymethyl-6-nitroaniline, 76.6% yield, bp 137–138° (0.1 mm). Tlc in benzene–methanol (9:1) gave one spot, *R*_f 0.92; *n*_D²⁵ 1.5635; ir (neat) 740 (w), 1050 (s), 1250 (w), 1380 (w), 1530 (m), 1610 (s), 3000 (s), and 3380 cm⁻¹ (s); uv_{max} (95% ethanol) 232 (ϵ 22,400) and 281 m μ (shoulder, 4580); visible_{max} (95% ethanol) 415 m μ (ϵ 4000); mass spectrum *m/e* 196 (M⁺, 18), 161 (70), 117 (79), 91 (100), and 65 (49).

Anal. Calcd for C₉H₁₂N₂O₃: C, 55.10; H, 6.12; N, 14.28. Found: C, 54.93; H, 6.31; N, 14.09.

***N*-Ethyl-4-methyl-2-nitroaniline.** A solution of 4.48 g (0.02 mol) of 4-acetamido-3-nitrobenzoic acid in tetrahydrofuran was reduced with 100 ml (0.01 mol) of 1 *M* diborane as previously described. After work-up, the crude black oil was purified by adsorption from benzene solution on a dry-packed column of alumina (50 g), followed by elution with benzene. The first fraction was yellow colored, and after evaporation of solvent, it was rechromatographed on another dry-packed column of alumina (30 g). Elution with high boiling petroleum ether furnished an orange red oil which crystallized from 80% aqueous ethanol, affording 0.4 g (0.0022 mol) of *N*-ethyl-4-methyl-2-nitroaniline, 11% yield, mp 53.54°. Tlc in benzene–methanol (9:1) gave one spot with an *R*_f 0.90; ir (KBr) 780 (m), 1075 (m), 1235 (s), 1280 (s), 1350 (s), 1480 (m), 1525 (s), 1575 (s), 1635 (s), and 3410 cm⁻¹ (s); uv_{max} (95% ethanol) 235 (ϵ 21,170) and 283 m μ (4250); visible_{max} (95% ethanol) 440 m μ (ϵ 5467); mass spectrum *m/e* 180 (M⁺, 100), 165 (95), 120 (41), 91 (34), and 65 (30).

Anal. Calcd for C₉H₁₂N₂O₂: C, 60.00; H, 6.67; N, 15.56; O, 17.77. Found: C, 60.09; H, 6.73; N, 15.43; O, 17.60.

10-Ethyl-6-hydroxymethylisalloxazine. A 500-ml pressure bottle was charged with a solution of 0.1 g (0.0005 mol) of *N*-ethyl-

3-hydroxymethyl-2-nitroaniline in 25 ml of absolute ethanol and 1.0 g of moist Raney nickel catalyst. The bottle was placed on the Parr, low-pressure, hydrogenation apparatus, charged with hydrogen gas at 32 lb/in.², and shaken for 12.0 hr, the yellow color of the solution disappearing during the course of the reaction. Catalyst was then removed by vacuum filtration and the colorless filtrate was concentrated to a residue by flash evaporation at 50°. The crude diamine was immediately dissolved in 10 ml of glacial acetic acid and the solution was added to a stirred mixture of 1 g of boric acid and 0.080 g (0.0006 mol) of alloxan in 18 ml of glacial acetic acid. The reaction mixture was then stirred at room temperature for 24.0 hr and then heated at 60° for 1 hr. The resulting orange colored solution was flash evaporated to a brown residue which on crystallization from dimethylformamide give yellow needles, 0.11 g (0.0004 mol), 79% yield, of 10-ethyl-6-hydroxymethylisalloxazine, mp 283–285°. Tlc gave only one spot with *R*_f 0.59; ir (KBr) 1180 (m), 1270 (s), 1410 (w), 1464 (w), 1580 (s), 1670 (s), 1715 (s), and 3480 cm⁻¹ (s); uv_{max} (phosphate buffer pH 7) 217 (ϵ 20,400) and 263 m μ (29,300); uv_{max} (6 *N* hydrochloric acid) 255 (shoulder, ϵ 26,100) and 263 m μ (27,625); visible_{max} (phosphate buffer pH 7) 357 (ϵ 8300) and 430 m μ (10,040); visible_{max} (6 *N* hydrochloric acid) 365 m μ (ϵ 15,250); fluorescence_{max} (glycine buffer pH 3.25) exc (exciting), 375 and anal (emission), 505 nm; mass spectrum *m/e* 272 (M⁺, 46), 228 (100), and 170 (32).

Anal. Calcd for C₁₃H₁₂N₄O₃: C, 57.35; H, 4.41; N, 20.58. Found: C, 57.27; H, 4.42; N, 20.74.

10-Ethyl-8-hydroxymethyl-3-methylisalloxazine. A solution of 4.0 g (0.02 mol) of *N*-ethyl-5-hydroxymethyl-4-nitroaniline in 25 ml of absolute ethanol was reduced to the crude diamine with 1.0 g of moist Raney nickel catalyst at an initial hydrogen pressure of 30 lb/in.². After removal of catalyst by filtration and flash evaporation, the crude diamine was dissolved in 25 ml of glacial acetic acid and added to a stirred mixture of 1.0 g of boric acid and 4.0 g (0.026 mol) of *N*-methylalloxan in 25 ml of glacial acetic acid. The mixture was stirred at room temperature for 24 hr and then refluxed for 1 hr. The solvent was flash evaporated (80°) and the resulting brown solid after dissolving in solvent used for elution was purified by chromatography on a 20 × 350 mm column of silica gel. The column was eluted with butanol–acetic acid–water (4:1:5 upper phase) and fractions of 25 ml were collected. After a void volume forerun, fractions 6–14 yielded upon pooling and evaporation 2.5 g (0.0088 mol) of 10-ethyl-8-hydroxymethyl-3-methylisalloxazine, 44.0% yield, mp 242–250° dec. Two recrystallizations from formic acid–water gave pure product, mp 245–248.5° dec. Tlc gave only one spot with *R*_f 0.70; ir (KBr) 1160 (w), 1195 (s), 1265 (s), 1410 (s), 1430 (s), 1550 (s), 1660 (s), 1725 (vs), and 3485 cm⁻¹ (s); uv_{max} (phosphate buffer pH 7) 217 (ϵ 31,110) and 264 m μ (37,710); uv_{max} (6 *N* hydrochloric acid) 218 (shoulder, ϵ 24,090) and 265 m μ (30,120); visible_{max} (phosphate buffer pH 7) 348 (ϵ 8,211) and 446 m μ (10,480); visible_{max} (6 *N* hydrochloric acid) 372 (ϵ 18,110); fluorescence_{max} (glycine buffer pH 3.25) exc, 362 and anal, 520 m μ ; mass spectrum *m/e* 286 (M⁺, 24), 270 (100), 268 (63), 242 (85), and 172 (75).

Anal. Calcd for C₁₄H₁₄N₄O₃: C, 58.74; H, 4.89; N, 19.58; O, 16.79. Found: C, 58.55; H, 5.04; N, 19.50; O, 16.96.

10-Ethyl-9-hydroxymethylisalloxazine. A solution of 0.98 g (0.005 mol) of *N*-ethyl-2-hydroxymethyl-6-nitroaniline in 15 ml of absolute ethanol was reduced to the crude diamine with 1.0 g of moist Raney nickel catalyst as described previously. After removal of solvent and catalyst, the residue was dissolved in 10 ml of glacial acetic acid and added to a mixture of 1.0 g of boric acid and 0.8 g (0.0056 mol) of alloxan in glacial acetic acid. This mixture was stirred at room temperature for 12 hr and warmed to 60° for 5 min, and the solvent removed by distillation under reduced pressure. The resulting brown residue was stirred with 5 ml of water and filtered; the solid was washed with cold water, and finally dried *in vacuo* at room temperature. The crude residue was then purified by preparative thin layer chromatography in silica gel. After development of the chromatogram with butanol–acetic acid–water, the yellow-green fluorescing band was scraped from the plate and extracted with hot glacial acetic acid. Upon cooling 0.93 g (0.0034 mol), 68% yield, of 10-ethyl-9-hydroxymethylisalloxazine, mp 283–284°, crystallized. Tlc gave one spot with an *R*_f 0.62; ir (KBr) 770 (m), 1195 (s), 1280 (vs), 1420 (s), 1475 (s), 1544 (s), 1665 (s), 1730 (s), and 3470 cm⁻¹ (s); uv_{max} (phosphate buffer pH 7) 222 (ϵ 22,811) and 263 m μ (28,410); uv_{max} (6 *N* hydrochloric acid) 265 m μ (ϵ 21,680); visible_{max} (phosphate buffer pH 7) 353 (ϵ 8160) and 432 nm (9010); visible_{max} (6 *N* hydrochloric acid) 371 nm (ϵ 14,230); fluorescence_{max} (glycine buffer pH 3.25) exc, 370 and anal, 510 nm; mass spectrum *m/e* 272 (M⁺, 14), 243 (100), 215 (26), and 172 (36).

Anal. Calcd for $C_{13}H_{12}N_4O_3$: C, 57.35; H, 4.41; N, 20.58; O, 17.66. Found: C, 57.25; H, 4.53; N, 20.47; O, 17.68.

10-Ethyl-7-methylisoalloxazine. The crude diamine from 0.30 g (0.0015 mol) of *N*-ethyl-4-methyl-2-nitroaniline was converted to the isoalloxazine with 1.0 g of boric acid and 0.24 g (0.0016 mol) of alloxan as previously described. Evaporation of solvent yielded a brown oil which crystallized (yellow needles) from dimethylformamide to give 0.20 g (0.0008 mol) of 10-ethyl-7-methylisoalloxazine, 51.1% yield, mp 313–315° dec. Tlc gave one spot with an R_f 0.65; ir (KBr) 820 (s), 1190 (s), 1290 (s), 1410 (s), 1430 (m), 1480 (m), 1590 (m), 1660 (s), 1735 (s), 2870 (m), and 3490 cm^{-1} (s); uv_{max} (phosphate buffer pH 7) 218 (ϵ 26,450) and 265 nm (35,300); uv_{max} (6 *N* hydrochloric acid) 262 nm (ϵ 23,110); $visible_{max}$ (phosphate buffer pH 7) 350 (ϵ 7722) and 442 nm (10,160); $visible_{max}$ (6 *N* hydrochloric acid) 367 nm (ϵ 14,960); $fluorescence_{max}$ (glycine buffer pH 3.25) exc, 365 and anal, 515 nm; mass spectrum m/e 256 (M^+ , 75), 228 (100), 157 (76), and 116 (15).

Anal. Calcd for $C_{13}H_{12}N_4O_2$: C, 60.95; H, 4.68; N, 21.87; O, 12.50. Found: C, 60.87; H, 4.77; N, 21.79; O, 12.51.

3,8-Dimethyl-10-ethyl-8 α -histidylisoalloxazine. A three-necked, round-bottom flask equipped with a magnetic stirrer, reflux condenser protected with a calcium chloride drying tube, and dropping funnel was charged with 4.0 g (0.014 mol) of 10-ethyl-8-hydroxy-methyl-3-methylisoalloxazine dissolved in 100 ml of dioxane and 10 ml of pyridine; 1.40 g (0.005 mol) of phosphorus tribromide was then added with stirring over a period of 2.0 hr. The reaction mixture was then stirred at room temperature for 3.0 hr, refluxed for 2.0 hr, cooled, and flash evaporated to a volume of 10 ml. The liquid residue was poured into 250 ml of chloroform and the chloroform solution was then washed twice with 250-ml portions of 1 *N* hydrochloric acid, and then twice with 250-ml portions of water. After drying over sodium sulfate, the chloroform was removed by flash evaporation (50°). Crude 8-bromomethyl-10-ethyl-3-methylisoalloxazine, 3.2 g (0.009 mol), 67.0% yield, mp 281–302°, was obtained. Tlc showed three yellow-green fluorescing spots (R_f 0.31, 0.44 and 0.68) and two orange spots (R_f 0.12 and 0.81) when visualized under ultraviolet light. All attempts to obtain pure 8-bromomethyl derivative were unsuccessful.

The crude 8-bromomethyl-10-ethyl-3-methylisoalloxazine was then converted to 3,8-dimethyl-10-ethyl-8 α -histidylisoalloxazine by a modification of the method of Ghisla and coworkers,⁹ 4.2 g (0.012 mol) of crude 8-bromomethyl-10-ethyl-3-methylisoalloxazine in 100 ml of dimethylformamide being treated at 90° for 24.0 hr with 7.75 g (0.03 mol) of *N* α -benzoylhistidine. The reaction mix-

ture was then flash evaporated (80°) to a brown oil and the product was isolated by chromatography on a 20 \times 350 mm silica gel column, employing the upper phase of the butanol-acetic acid-water mixture and collecting 50-ml fractions. On evaporation, fractions 2–5 gave *N* α -benzoylhistidine, 3.9 g (0.015 mol), mp 246–249° (lit.²⁶ mp 250°). The remaining fractions were pooled, and after the solvent was removed by flash evaporation, the brown residue was refluxed for 2.0 hr with 6 *N* hydrochloric acid. The solvent was removed *in vacuo* and the oily residue crystallized from formic acid-water to give 1.7 g (0.004 mol) of 3,8-dimethyl-10-ethyl-8 α -histidylisoalloxazine, 33.5% yield, mp 337–344° dec. Several recrystallizations from formic acid-water yielded orange-brown needles, mp 338–344° dec. Tlc gave two close yellow-green fluorescing spots, R_f 0.36 and 0.38; (KBr) 820 (m), 1150 (m), 1420 (m), 1440 (w), 1500 (m), 1555 (w), 1620 (m), 1715 (m), 3050 (s), and 3450 cm^{-1} (s); uv_{max} (phosphate buffer pH 7) 217 (ϵ 28,690) and 263 nm (35,120); uv_{max} (6 *N* hydrochloric acid) 262 nm (ϵ 29,910); $visible_{max}$ (phosphate buffer pH 7) 344 (ϵ 9430) and 443 nm (11,100); $visible_{max}$ (6 *N* hydrochloric acid) 371 (ϵ 20,030) and 405 nm (shoulder, 10,800); $fluorescence$ (glycine buffer pH 3.25) exc, 355 and anal, 522 nm; mass spectrum m/e 423 (M^+ , 83), 395 (100), 310 (70), 296 (35), and 81 (25).

Anal. Calcd for $C_{20}H_{21}N_7O_4$: C, 56.74; H, 4.97; N, 23.17. Found: C, 56.96; H, 5.12; N, 22.97.

pH-Dependent Fluorescence. Variation of fluorescence with pH was determined for 1.00×10^{-7} *M* solutions of the isoalloxazines. The following buffer solutions were employed: pH 2.00–3.65, glycine-hydrochloric acid; pH 3.90–5.30, sodium acetate-acetic acid; pH 5.80–7.00, potassium dihydrogen phosphate-potassium hydroxide; pH 8.20–9.20, potassium monohydrogen phosphate-potassium hydroxide; pH 10.20 and greater, sodium carbonate-sodium hydroxide. Fisher reagent grade chemicals were used in all cases, and all buffers were stored at 5° prior to use.

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Kinetics of Reactions in Solutions under Pressure. XXVI. Fragmentation of Chloroacetylhydrazide¹

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Abstract: The reaction of chloroacetylhydrazide with aqueous hydroxide ion to give chloride ion and products derived from ketene and diazene (diimide) is first order in each reagent up to a pH of 12. The activation volume for the reaction is negative. *N,N'*-Dimethyl substitution leads to a very much (10^7 - 8) slower rate of chloride production, whereas *N,N*-dimethyl substitution retards the reaction only moderately (10 times). A yellow, transient intermediate is observed in the reaction of the parent compound; stopped-flow experiments show that its formation rate becomes independent of base at a pH of 12. The uv-visible spectrum of the intermediate has been determined; the disappearance is first order and independent of base. Neither ketene nor diazene can account for it. These observations are interpreted in terms of rate controlling α -lactam formation rather than of a concerted fragmentation as described in the recent literature; the color is tentatively attributed to an anion of acetyldiazene.

Fragmentations are reactions of the general type $A-B-C-D-X \rightarrow A-B + C=D + :X$. A wide

variety of examples has been reported² and the general mechanism has been discussed.³ One of the more

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