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9-Halo-derivatives of Flavins (1a)

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Some progress has been made toward understanding biological flavin-dependent oxidations in terms of molecular structure (2). To circumscribe more completely the specificity of biochemical systems for flavins, it seemed desirable to synthesize 7,8-dimethylisoalloxazines with different substituents in position 9. The conditions for halogenation in this position, heretofore not reported, also should provide an indication of the relative negative polarizability of the flavin molecule at this site. Under conditions where protonation of N-1 occurs, it would be expected that sufficient electron withdrawal would occur from the benzenoid carbon *ortho* to position 6 that an electrophile would preferably attack position 9:
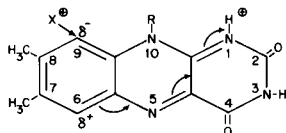
The present paper describes the bromination and chlorination of flavins specifically in position 9. Moreover, spectra of the analogs so formed reflect the inductive effect plus some loss of coplanarity in their flavoquinone states.

Lumiflavin (3) is converted to a monobromo analog in good yield by N-bromosuccinimide in trifluoroacetic acid with catalytic quantities of dibenzoyl peroxide. Similar treatment with N-chlorosuccinimide results in a lower yield of the chlorolumiflavin. Such halogenations of 2',3',4',5'-tetraacetylriboflavin (4) followed by acid hydrolysis to remove the acetyl groups, lead to the monohaloriboflavins. These latter can be photolyzed in neutral to acid solutions to the corresponding lumichrome analogs.

That the halogen function must be in position 9 is proven by comparisons of the NMR spectra of the lumiflavin analogs whose principle characteristics are summarized by the data in Table I.

Whereas lumiflavin shows two hydrogen peaks at δ values of 8.42 and 8.24, the 9-deutero analog has only the low-field hydrogen peak at 8.41, and the 6,9-dideutero analog lacks both. Since the bromolumiflavin, like 9-deuterolumiflavin, shows only a low-field peak at 8.40 characteristic of the remaining aryl hydrogen at position 6, the bromo substituent must be at position 9 from which the high-field aryl hydrogen was displaced. The total assignments

TABLE I

Characteristics of NMR Spectra of
Lumiflavin and Analogs (a)

Substance	Aryl-H 6	9	N-CH$_3$ 10	Aryl-CH$_3$ 8	7
Lumiflavin	8.42	8.24	4.67	2.87	2.73
9-Deuterolumiflavin	8.41	–	4.66	2.86	2.72
6,9-Dideuterolumiflavin	–	–	4.66	2.86	2.72
9-Bromolumiflavin	8.40	–	4.84	2.97	2.80

(a) Chemical splitting is given in δ values (ppm) with CF_3COOD as solvent and tetramethylsilane as reference.
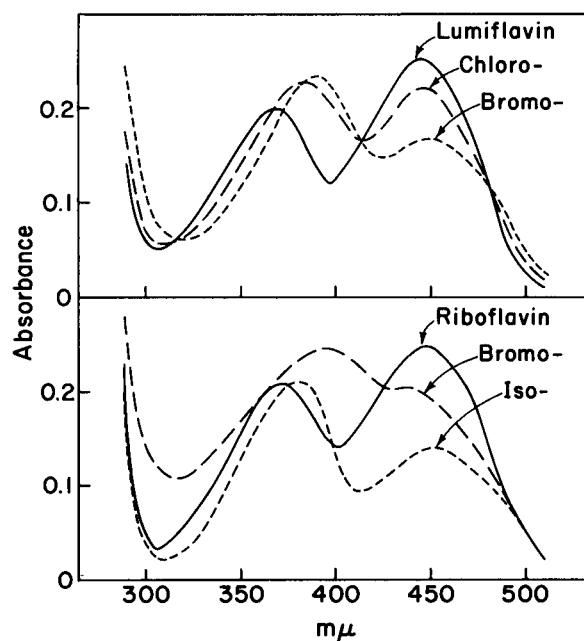

Figure 1. Absorption Spectra of Lumiflavin and its 9-Chloro- and 9-Bromo- Analogs (top) and of Riboflavin and its 9-Bromo- and Iso- Analogs (bottom). Flavins were 2×10^{-5} M in $2 M$ acetic acid at room temperature.

given in the table agree with those reported earlier for the 7,8-dimethylisoalloxazine nucleus (5).

As seen from the absorption spectra of lumiflavin and its 9-chloro and 9-bromo analogs given in the top of Figure 1, the presence of a halo function in position 9 causes a decrease in absorption and slight red shift at 442 mμ and increase with red shift from 370 to 390 mμ. The somewhat similar differences in absorption spectra of 9-bromoriboflavin and isoriboflavin compared to riboflavin are shown in the bottom of Figure 1. Spectra reported for 7,9- and 6,8-dimethyl-9-(1'-L-arabityl)isoalloxazines are generally more like each other than that for the 7,8-dimethyl compound (6). Moreover, the spectrum for the 7,9-dimethyl analog resembles that for isoriboflavin which bears methyls at positions 6 and 7. Since either a methyl or halo substituent in position 9 elicits a similar spectrum, an effect in addition to an inductive one may operate. Also as spectra of 6-methyl analogs and even slightly the 7,8-tri- and tetramethylene flavins (7) bear general resemblance to spectra of 9-substituted flavins, a contribution to the decrease around 450 mμ with increase near 375 mμ may be due to some deformation of the coplanarity in the flavoquinone structure. This explanation appears likely when consideration is given to the spacial relations recently determined in 1,3,10-trimethylisoalloxazine by X-ray analysis (8).

A further and very sensitive measure of the effect of halogenation of flavins at position 9 is shown by the data on fluorescence give in Table II. The order for decrease in

TABLE II

Relative Fluorescence of Lumiflavin,
Riboflavin, and Their Analogs (a)

Substance	Relative fluorescence (%)
Lumiflavin	100
9-Chlorolumiflavin	23
9-Bromolumiflavin	1
Riboflavin	100
Isoriboflavin	20
9-Bromoriboflavin	11

(a) Light for activation was set at the λ max for each flavin from 442 to 452 mμ; that from emission was then measured at the optimum near 520 mμ with 10^{-5} M flavin in 0.1 M sodium phosphate buffer, pH 7.

fluorescence near 520 mμ of bromoflavin > chloroflavin and isoriboflavin > lumiflavin and riboflavin follows the decrease in absorbance near 450 mμ. Thus, part of the

reason for decreased fluorescence is due to excitation of fewer flavin molecules to singlet species. The greater absolute decrease in fluorescence compared to absorbance of halogenated flavins further points to an intramolecular quenching attributable to the halo substituent where bromo is more effective than chloro. It has been noted that 7,8-dichlororiboflavin has essentially the same molar absorbance as riboflavin, but the former is considerably less fluorescent (9).

Changes in the absorption spectrum of a 9-halogenated flavin as influenced by its ionic state are illustrated in Figure 2 with spectra of 9-bromolumiflavin. The flavin
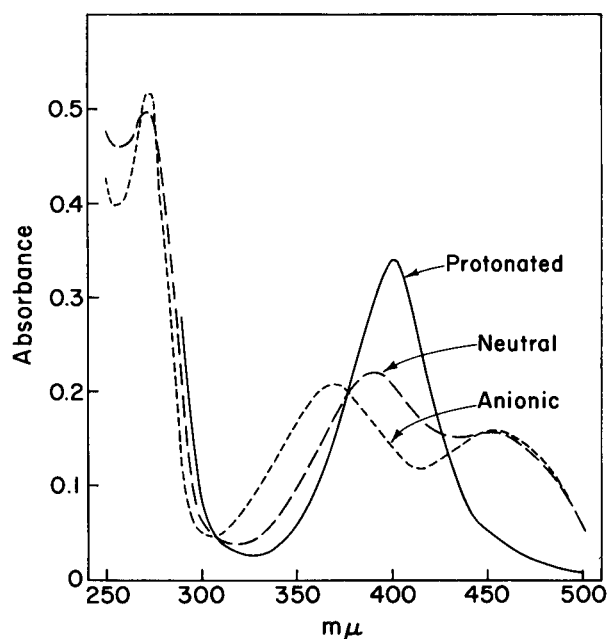

Figure 2. Absorption Spectra of 9-Bromolumiflavin as a Function of Hydrogen Ion Concentration. Flavin was 2×10^{-5} M in 6 N hydrochloric acid (protonated), 0.1 M sodium phosphate at pH 7 (neutral), and 0.1 M sodium hydroxide (anionic) all at room temperature.

anion exhibits optima at 370 and 452 mμ. The neutral quinone shows a shift from 370 to 392 mμ with slight intensification at this maximum. Protonation results in a large increase at 420 mμ similar to that seen at 390 mμ for protonated flavins with hydrogen at position 9 (10).

Overall, the production of 9-halogenated flavins may offer other examples whereby groups in a position of the benzenoid moiety of isoalloxazine near N-10 can perturb the structure and function of the vitamin and its coenzyme forms.

EXPERIMENTAL

flavins were supplied by F. Muller of Basel University. The NMR spectra were run by K. Aegeter of Basel University; fluorescence assays were by J. A. Roth of Cornell University.

Thin-Layer Chromatography.

MN-Kieselgel S (Firma Macherey, Magel and Co., Germany) was used as the stationary phase with n-butyl alcohol/acetic acid/water (2/1/1) as solvent. Compounds were located on developed plates by examination under incandescent and ultraviolet lamps.

Spectra.

Light absorption spectra were determined with a Cary 16 recording spectrophotometer. Infra red spectra were run on potassium bromide pellets with a Beckman 8 IR apparatus. Maxima upon fluorescence emission were measured in an Aminco-Bowman spectrophotofluorometer.

Syntheses.

In general, 2 mmoles each of the appropriate flavin and N-halosuccinimide were dissolved in 25 ml. of trifluoroacetic acid, 25 mg. of dibenzoyl peroxide was added, and the solution refluxed for 6 hours. The solution was evaporated to near dryness with warming under reduced pressure, the residue taken up in 25 ml. of water, and the suspension heated for 15 minutes at 50° to destroy any unreacted N-halosuccinimide. The crude halogenated flavin was filtered off or the supernatant solution decanted, the material was rinsed with water and dried.

9-Bromolumiflavin was enriched by partially dissolving the crude flavin in 25 ml. of N,N-dimethylformamide at 50°, allowing the suspension to cool to room temperature, and filtering off much of the less-soluble unreacted lumiflavin. The filtrate was evaporated to dryness under reduced pressure, the residue dissolved in 5 ml. of concentrated formic acid, and precipitation initiated with diisopropyl ether. The reddish crystals were filtered off, rinsed with ether, and dried. Final purification was obtained by barely dissolving the crystals in 0.2 N sodium hydroxide neutralizing with 0.2 N hydrochloric acid and filtering off the precipitate which was rinsed with water, ethanol, and diethyl ether for about a 50% overall yield. Thin-layer chromatography showed only one spot at R_f = 0.45. The melting point was 285-290°. The IR spectrum indicated substitution in the benzenoid portion. When dried for several hours at 50° *in vacuo*, the compound gave analysis calculated $C_{13}H_{11}BrN_4O_2$: C, 46.6; H, 3.3; N, 16.7; Br, 23.8. Found: C, 46.3; H, 3.3; N, 16.4; Br, 23.9.

9-Chlorolumiflavin was similarly enriched by partially dissolving the crude flavin in dimethylformamide. Aliquots of the filtrate

were streaked on thin-layer plates which were developed in the acid-butanol solvent. Chlorolumiflavin at R_f = 0.45 separated as a band above lumiflavin at R_f = 0.40. The plate material was scraped off, the flavin eluted from silica gel with 2 M acetic acid, and the suspension filtered to obtain solution for spectra.

9-Bromoriboflavin was prepared from 9-bromo-2',3',4',5'-tetraacetylriboflavin (R_f = 0.60) obtained by bromination of the tetraacetylriboflavin (R_f = 0.55). For this, the crude bromotetraacetylriboflavin was hydrolyzed by refluxing in 6 N hydrochloric acid for 1 hour. The solution was evaporated to dryness and the residue rinsed with ether and dissolved in the acid-butanol solvent for application to thin-layer plates. Bromoriboflavin at R_f = 0.40 separated from riboflavin at R_f = 0.35 and was eluted as above with 2 M acetic acid to obtain solution for spectra.

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