Reactions of 4a-Peroxides and 4a-Pseudobases of N^{10}- and N^5-Phenethylflavins

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Abstract: A number of N^5,N^{10}-dialkylisoalloxazines have been synthesized in which either the N^5 or the N^{10} substituent is a meta-substituted phenethyl group. Some of these compounds have been subjected to experiments in order to determine whether an intramolecular transfer of oxygen can occur between the flavin and the phenyl group (a model for monooxygenase). When in the 1,5-dihydro reduced state, N^5-alkyl-substituted isoalloxazines react with molecular oxygen to give 4a-hydroperoxy derivatives. The hydroperoxides of the N^5-ethyl-N^{10}-phenethylflavins provide 4a-pseudobases on spontaneous decomposition. These in turn undergo ring contraction in base to yield 10a-spirohydantoins (Scheme V). The structure of a 10a-spirohydantoin (**28b**) is as established by X-ray crystallographic techniques. Spontaneous decomposition of 4a-hydroperoxides is not accompanied by intramolecular oxygen transfer to the phenethyl substituent groups at N^{10} or N^5 (eq 4). 10a-Spirohydantoins may also be obtained by base treatment of 4a-pseudobases that have been prepared separately from the oxidized isoalloxazine (i.e., flavinium cation). 4a-(Alkylperoxy)flavin derivatives, obtained by addition of alkyl peroxides to flavinium cations, undergo both spontaneous and photochemical conversion to 10a-spirohydantoin. These findings are discussed in terms of proposals which have been made for the mechanism of action of flavoenzyme monooxygenases.

Introduction

Monooxygenation of substrate by flavin monooxygenase enzymes occurs after combination of reduced enzyme with oxygen to provide an enzyme-bound dihydroflavin–oxygen adduct.[3] The synthesis of 4a-hydroperoxyflavins by direct combination of N^5-alky-1,5-dihydroflavins with O_2,[4,5] plus the establishment that the near-UV and visible spectra of the N^5-alkyl-4a-hydroperoxyflavins closely resemble[4] the reported spectra of the flavin monooxygenase–oxygen complexes (in the case of bacterial luciferase the spectra are superimposable), provides good evidence that 4a-hydroperoxyflavins are intermediates in the enzymatic reactions. The N^5-alkyl-4a-hydroperoxyflavins have been shown to be reasonable oxidizing agents. Thus, the second-order rate constants for the S-monooxidation of thioxane in the presence of N^5-alkyl-4a-hydroperoxyflavin has been found to be 10^5 times greater than the rate constant for the monooxygenation reaction by *tert*-butyl hydroperoxide.[6] Also, the N^5-alkyl-4a-hydroperoxyflavins have been shown to mimic the flavomonooxygenase reactions of N-monooxidation of amines[7] and the chemiluminescent oxidation of aldehydes.[4,6,8] The use of N^5-alkyl-4a-hydroperoxyflavins to carry out dioxygenase reactions is made possible by the finding that the peroxy moiety is transferred from the 4a-flavin peroxy anion to a number of phenolate ions.[9]

The insertion of oxygen into C–H bonds of aromatic ring structures is catalyzed both by cytochrome P-450 type heme enzymes[10] and by certain flavoprotein monooxygenases.[3] The aromatic substrates for the flavomonooxygenases are electron rich (phenolate anion etc.) so that the monooxygenation event may simply represent a nucleophilic displacement upon the 4a-hydroperoxy moiety (eq 1). On the other hand, a dioxygen transfer may be involved (eq 2)[9] or oxygen insertion may follow one or another of the postulated 4a-hydroperoxyflavin interconversions to generate an "oxene" intermediate[11] (eq 3 for the carbonyl oxide mechanism suggested by Hamilton[11a]).

The conversion of a biomolecular reaction into a unimolecular (intramolecular) reaction as a result of covalent linking of the reacting species is often accompanied by a dramatic increase in the rate constant.[12] The extent of this effect is dependent upon the conformational preference of the intramolecular reaction and the tightness of the transition state. The present study concerns the syntheses of and the decomposition of N^5- and N^{10}-phenethyl-4a-flavin peroxides. Our specific objectives have been (1) to ascertain if intramolecular oxygen transfer from the 4a-peroxy substituent to the phenethyl substituent occurs and (2) to determine the nature of the decomposition products of 4a-peroxy- and 4a-(alkylperoxy)flavins and the structurally related 4a-hydroxyflavins (i.e., 4a-flavin pseudobase).

Results and Discussion

The synthetic sequences employed in this investigation are provided in Schemes I–IV. Appropriate discussion of the reactions and pertinent observations concerning rates of transformations, spectra, etc. can be found in the Experimental Section.

Dihydroflavins **19c,d** (*t*-BuOH solvent) react nearly quantitatively with dissolved molecular oxygen to provide the 4a-hydroperoxyflavins (**31c,d**) (Scheme V). The rate for 4a-hydroperoxyflavin formation in dry *tert*-butyl alcohol is ca. 10^2 times slower than seen previously[5] in absolute methanol. With time the spectrum of the 4a-hydroperoxides changed to those of the 4a-pseudobases. The rate constant for the formation of 4a-hydroperoxide is about 10-fold greater than for its decomposition to form the 4a-pseudobase. Pseudobase formation appears to be nearly quantitative. Under mildly basic conditions the pseudobases rearrange to the 10a-spirohydantoins (**28c,d**). This formation of a 10a-spirohydantoin was proven by X-ray crystallographic studies (Figure 1). Thermal or photolytic decomposition of 4a-(alkylperoxy)flavins (alkyl = *n*-propyl or benzyl) also provides spirohydantoins.

(1) The University of California at Santa Barbara.
(2) Institute for Cancer Research, Fox Chase Cancer Center.
(3) Massey, V.; Hemmerich, P. *The Enzymes* **1976**, *12*, 191–252.
(4) Kemal, C.; Bruice, T. C. *Proc. Natl. Acad. Sci. U.S.* **1976**, *73*, 995.
(5) Kemal, C.; Chan, T. W.; Bruice, T. C. *J. Am. Chem. Soc.* **1977**, *99*, 7272.
(6) Kemal, C.; Chan, T. W.; Bruice, T. C. *Proc. Natl. Acad. Sci. U.S.* **1977**, *74*, 405.
(7) Ball, S.; Bruice, T. C. *J. Am. Chem. Soc.* **1979**, *101*, 4017.
(8) Kemal, C.; Bruice, T. C. *J. Am. Chem. Soc.* **1977**, *99*, 7064.
(9) (a) Kemal, C.; Bruice, T. C. *J. Am. Chem. Soc.* **1979**, *101*, 1635. (b) Muto, S.; Bruice, T. C., work in progress.
(10) Ullrich, V., Ed. "Microsomes and Drug Oxidations"; Pergammon Press: New York, 1977.
(11) (a) Hamilton, G. A. *Prog. Bioorg. Chem.* **1971** 83. (b) Keay, R. E.; Hamilton, G. A. *J. Am. Chem. Soc.* **1975**, *97*, 6875. (c) Org, W. H.; Dolphin, D. *Proc. Natl. Acad. Sci. U.S.A.* **1974**, *71*, 2646. (d) Dmitrienko, G. I.; Snieckus, V.; Viswanathor, T. *Bioorg. Chem.* **1977**, *6*, 421.
(12) (a) Bruice, T. C.; Benkovic, S. J. "Bioorganic Mechanisms"; W. A. Benjamin: New York, 1966; Vol. I, Chapter I. (b) Bruice, T. C. *The Enzymes* **1970**, *2*, Chapter 4. (c) Fife, T. H. *Adv. Phys. Org. Chem.* **1975**, *11*, 1.

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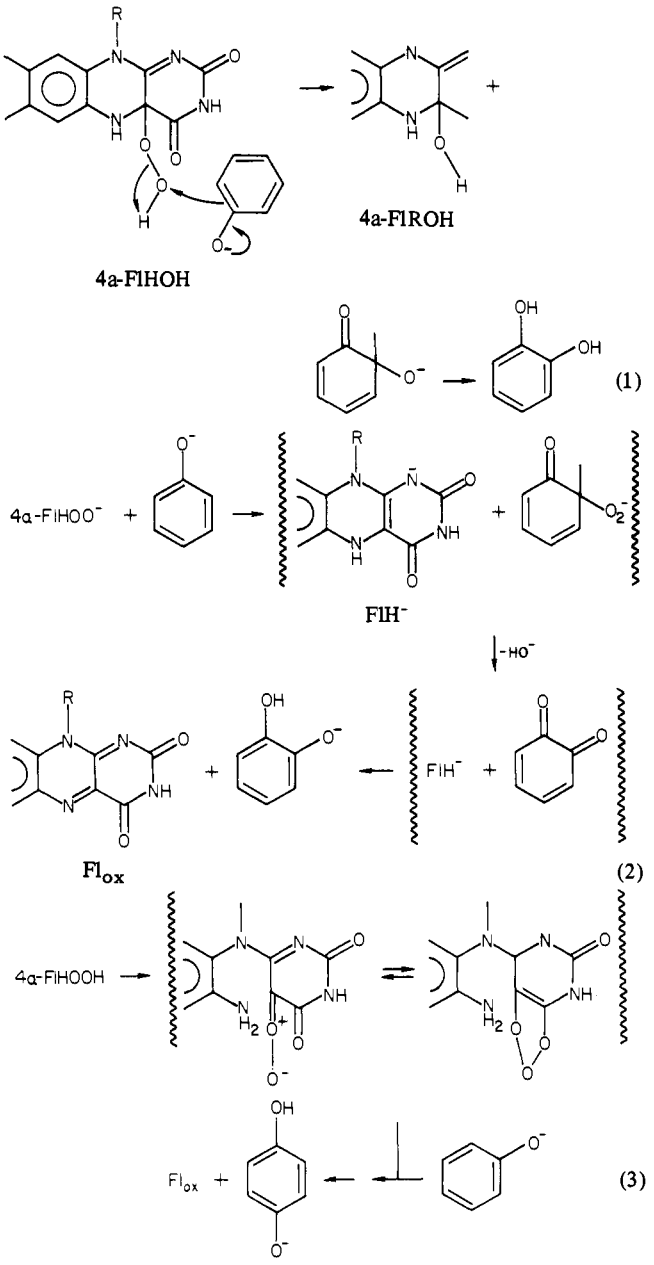
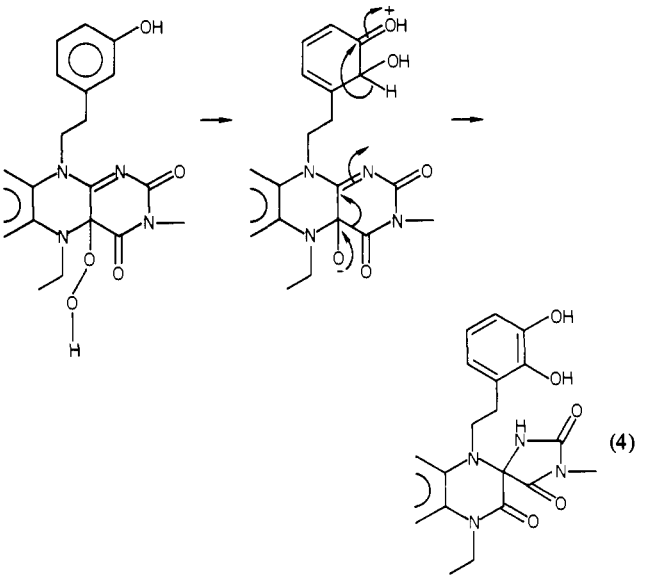


The 4a-hydroperoxyflavins **31c,d** possess a *m*-OH substituent upon the phenethyl side chain substituted on the N^{10} position of the isoalloxazine nuclei. One might anticipate an oxygen-transfer reaction from the 4a-hydroperoxy moiety to the hydroxyphenethyl substituent group to be possible if the phenolic substituent were to reside conformationally in the vicinity of the isoalloxazine ring. This could be so either if the 4a-hydroperoxide were to undergo a nucleophilic displacement by the π electrons of the phenyl substituent (as in eq 1) or if a rearrangement to a carbonyl oxide (eq 3) were involved. The mechanism of cleavage of the 4a-hydroperoxide O–O bond to form 4a-pseudobase is not well understood. The latter reaction must involve an oxygen transfer.[13] Any intramolecular oxygen transfer could be in competition with or accompany the spontaneous conversion of the 4a-hydroperoxyflavins to their respective 4a-pseudobases.

In order that we would not miss even traces of intramolecular oxygen-inserted products (eq 4) we carried out mass spectral analyses upon the residual material remaining after completion of the decomposition of the 4a-hydroperoxyflavins. The largest values of *m/e* corresponded to the mass of a spirohydantoin formed by loss of an oxygen from the 4a-hydroperoxyflavin (Scheme V).

(13) Conversion of 4a-hydroperoxyflavin to 4a-pseudobase in abosolute *tert*-butyl alcohol does not yield *tert*-butyl peroxide.



The structural assignments were made on the basis of high-resolution measurements (see Experimental Section). No evidence could be obtained for the reaction of eq 4. Similar results were obtained on examination of the mass spectra of the decomposition products obtained on reaction of oxygen with 1,5-reduced **23** (i.e., **22**). The absence of detectable oxygen-transfer products may be due to the entropic disadvantage in gaining the seven- to nine-membered cyclic transition states required for intramolecular oxygen transfer. On the other hand, ten-membered and larger cyclic transition states are required for the very rapid intramolecular hydride transfers obtained when the dihydropyridine nitrogen of dihydronicotinamide is bonded to the flavin N^{10} position by $(CH_2)_n$ bridges. In these instances there is an intramolecular complexing between flavin and dihydronicotinamide moieties.[14] It is known that flavins complex phenols[15] and an intramolecular interaction of phenol and flavin moities occurs in compounds **17a,b**. Thus, though the fluorescence emissions of the (*m*-methoxyphenethyl)flavins **15a,b** and **16** are normal, the (*m*-hydroxyphenethyl)flavins **17a,b** are only feebly fluorescent.

From the mass spectral data of this, and other studies contained herein, there can be discussed the major fragmentation patterns of the spirohydantoin products and these may be compared to those of related flavins. As examples, Figures 2 and 3 display the mass spectral patterns of the isoalloxazine **15b** and the related 10a-spirohydantoin **28b**. These patterns find interpretation in the fragmentation steps of Schemes VI and VII. For **15b** initial cleavage of the phenethyl group by hydrogen abstraction or bond migration (quasi-McLafferty rearrangement) (A) and single benzylic scission (B) compete to provide an almost equal relative intensity for *m/e* 321 and 308, accompanying the base peak at *m/e* 134. In contrast to the decomposition of the molecular ion of **15b**, the fragmentation of the molecular ion of **28b** appears to occur via the consecutive processes of benzylic cleavage followed by a quasi-McLafferty rearrangement involving the loss of ethylene (*m/e* 28) to provide a fragment ion *m/e* 339. The fragmentations of Scheme VII are characteristic for all the compounds assigned in this study as 10a-spirohydantoins. (Listings of the mass spectral analysis of flavins and 10a-spirohydantoins are available as supplementary material).

The pseudobases **25a–d**, as well as **26** and **27**, have been prepared directly from flavinium cations and shown to rearrange in *t*-BuOH with excess base to the corresponding spirohydantoins. Spectral characteristics of various 10a-spirohydantoins are available as supplementary material. The 10a-spirohydantoin **28b**

(14) Blankenhorn, G. *Eur. J. Biochem.* **1975**, *50*, 351.
(15) Fleischman, D.; Tollin, G. *Chem. Ber.* **1965**, *94*, 271; *Proc. Natl. Acad. Sci. U.S.A.* **1965**, *53*, 38. Harbury, H. A.; Foley, K. A. *Ibid.* **1958**, *44*, 662. Yagi, K.; Matsuoka, Y. *Biochem. Z.* **1956**, *238*, 138. Yeh, L.-S. L.; Ingraham, L. L. In "Flavins and Flavoproteins"; Singer, T. P., Ed.; Elsevier: Amsterdam, 1976; Chapter 85.

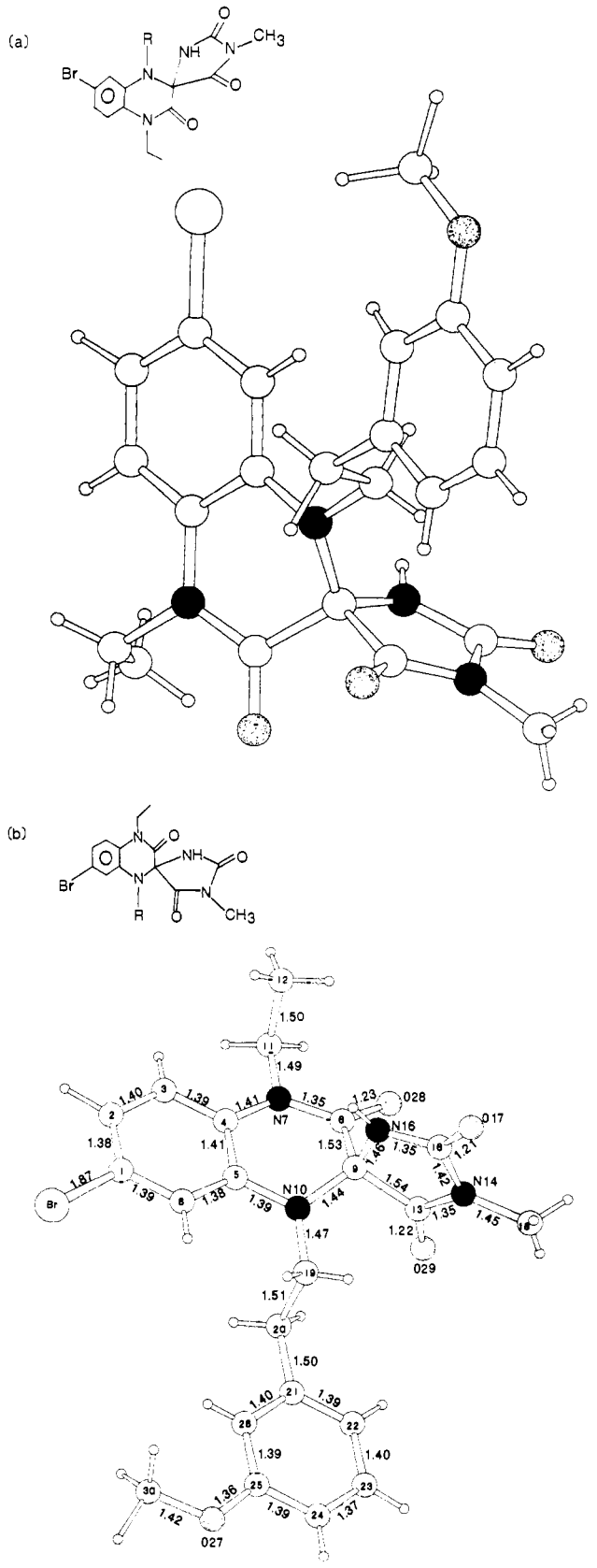


Figure 1. Two views of molecule **28b**: (a) Oriented as in formula. (b) Oriented to show connectivity most clearly. Bond distances are indicated with esd values of 0.004–0.006 Å.

received special attention (mass spectrum, NMR; see Figure 4 for the UV–vis spectrum) since this compound was chosen for X-ray diffraction studies. Two views of the structure of **28b**, determined by X-ray crystallography (Experimental Section), are given in Figure 1.

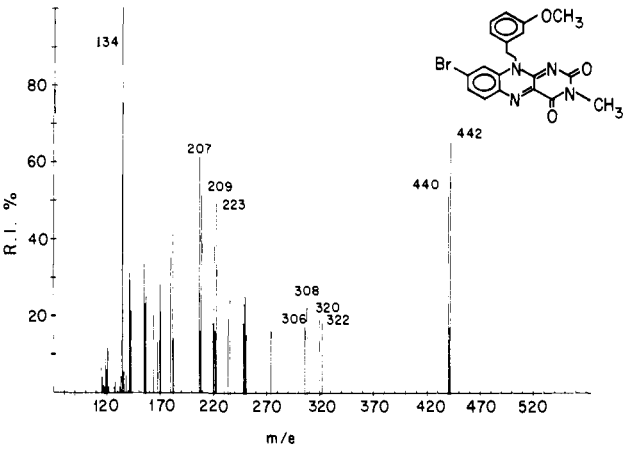


Figure 2. Mass spectral fragmentation pattern of the isoalloxazine **15b**. The relative intensities (RI %) have been increased by 10-fold at m/e greater than 140.

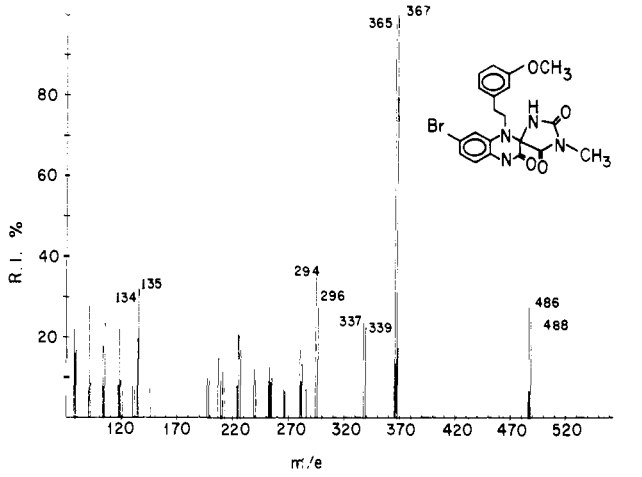


Figure 3. Mass spectral fragmentation pattern of the 10a-spirohydantoin **28b**.

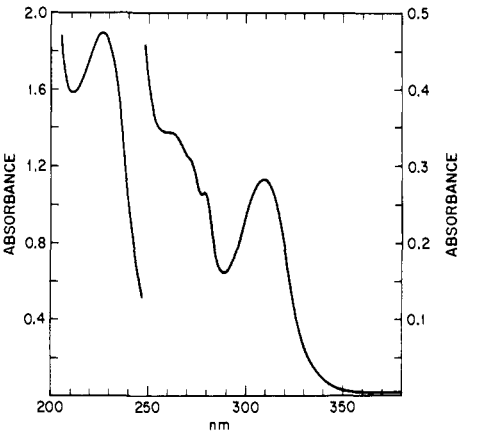
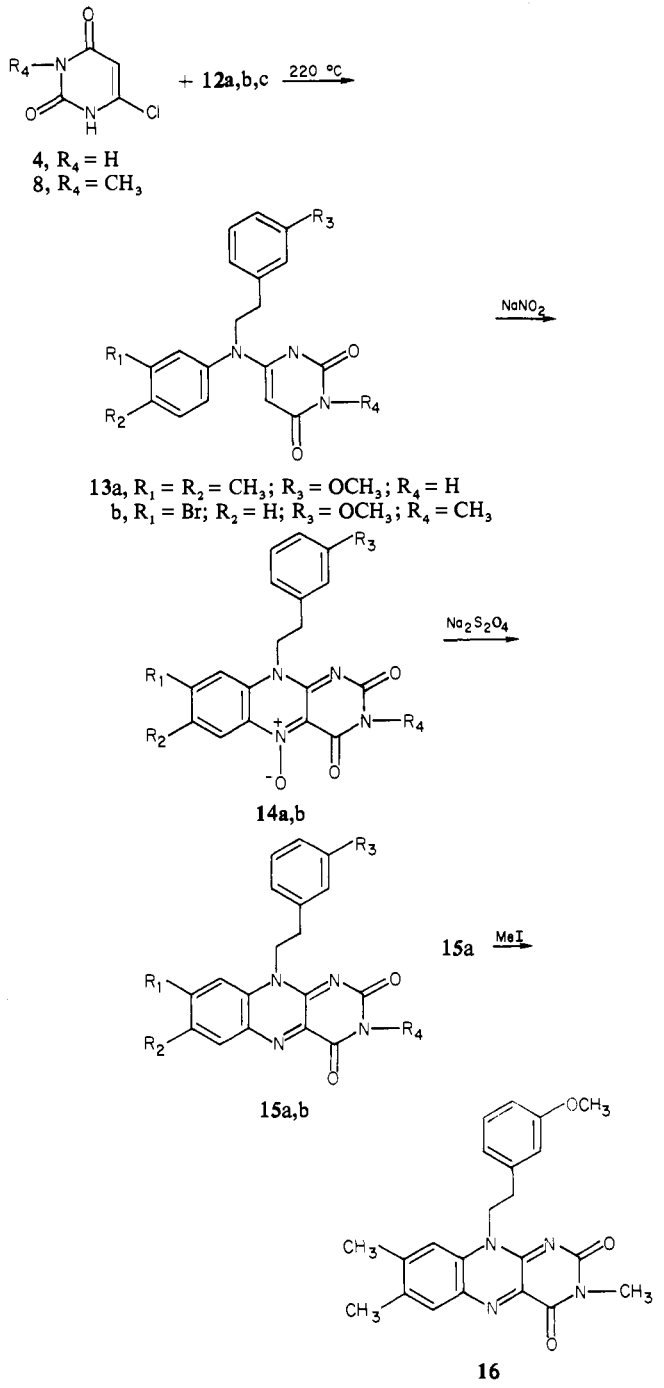


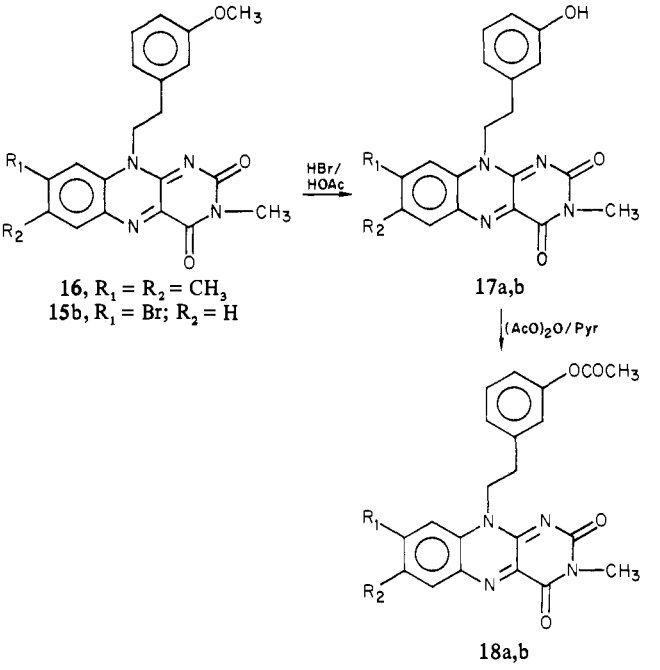
Figure 4. Absorption spectrum of the 10a-spirohydantoin **28b** in ethanol. The concentration of **28b** was 4.27×10^{-5} M. λ_{max}'s are at 228 (4.11×10^4 M^{-1} cm^{-1}), 263 (7.31×10^3), 272 (sh) (6.67×10^3), 279.5 (sh) (5.79×10^3), and 309 (6.68×10^3 M^{-1} cm^{-1}) nm. The spectra were taken of the crystalline material employed for the X-ray structure determination.

We have shown that 10a-spirohydantoins are major decomposition products of the 4a-hydroxy and 4a-peroxy adducts of the N^5-alkyl-N^{10}-phenethylisoalloxazines of this study. Thus, both **28a** and **28c** (exact mass, elemental analysis, NMR, UV–vis absorption) are formed upon photolysis or thermal decomposition of 4a-*n*-propylperoxy adducts of the appropriate N^5-ethylisoalloxazine, and **28c** arises on decomposition of the appropriate

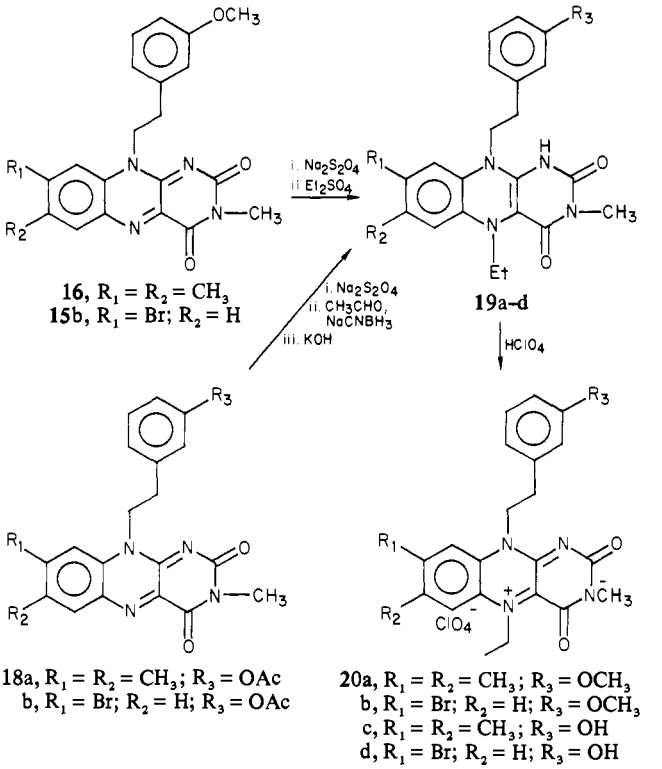
Scheme I



Scheme II



Scheme III

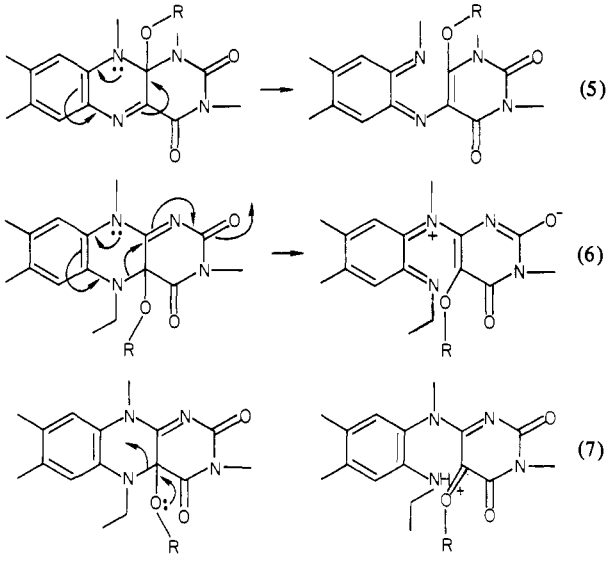


N^5-ethyl-4a-hydroperoxyisoalloxazine in base. The 10a-spirohydantoin **28b** (exact mass, X-ray structure, NMR, IR, UV–vis spectrum) is also formed upon photolysis of a 4a-*N*-propyl peroxide adduct of an N^5-alkylisoalloxazine and upon base-catalyzed rearrangement of a 4a-hydroxy adduct of this N^5-alkylisoalloxazine. Similar formations of other 10a-spirohydantoins have been established (UV–vis spectrophotometry, mass spectra) in this study to accompany the decomposition of other 4a-(*n*-alkylperoxy), 4a-hydroperoxy-, and 4a-hydroxy-N^5-alkylisoalloxazines. We have observed previously[8] that the absorbance of the spent reaction solution in the chemiluminescent decomposition of N^5-ethyl-4a-(alkylperoxy)flavins corresponded to that expected from the formation of a spirohydantoin. The present results substantiate that a 10a-spirohydantoin is a major product of these reactions.

"Intensely blue" intermediates have been observed, by Mager,[16] to be formed in processes he has investigated which involve both N^1- and N^5-alkyl 10a-substituted isoalloxazines. The formation of the blue intermediate from the N^1-alkyl 10a-oxygen substituted flavin could be shown, by trapping, to involve N^{10}–C^{10a} bond scission (eq 5). A similar rearrangement has been proposed to occur with N^5-alkyl isoalloxazine (eq 6). If the reaction of eq 6 were in effect for the 4a-hydroperoxyflavin, then protonation of the N^5 product would yield the carbanyl oxide intermediate proposed to be formed by Hamilton (see also ref 17) by the mechanism of eq 7. Mager proposes that spiro carbonyl oxides

(16) Mager, H. I. X. *Tetrahedron Lett.* **1979**, 2423; *Ibid.*, in press. Mager, H. I. X.; Addink, A. *Ibid.*, in press.

(17) Entsch, B.; Ballou, D. P.; Massey, J. *J. Biol. Chem.* **1976**, *251*, 2550.

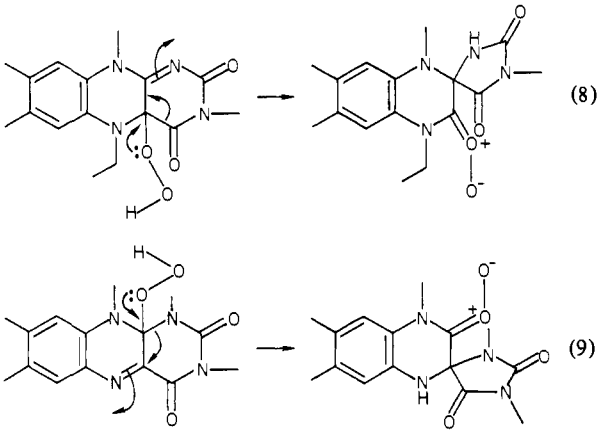


(5)

(6)

(7)

are formed as intermediates in spirohydantoin formation from 10a- and 4a-hydroperoxyflavins (eq 8 and 9). Such carbonyl oxides



(8)

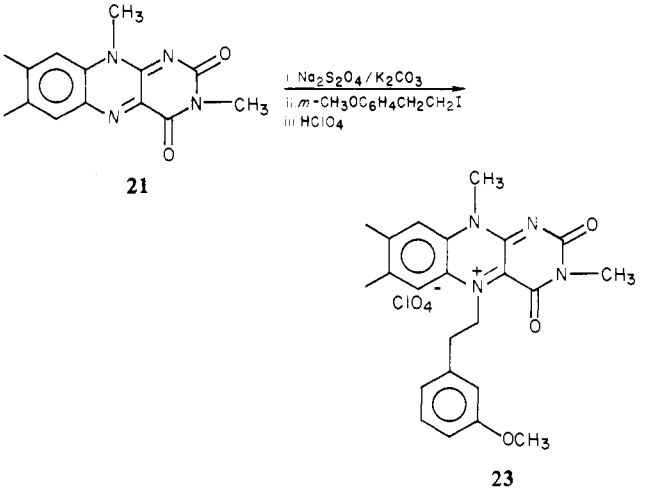
(9)

could not serve as the source of an "oxene intermediate" at the active site of a monooxygenase. Transfer of oxygen from such intermediates would yield a spirohydantoin. In contrast to the report of Yoneda and co-workers,[18] the 10a-spirohydantoin is not reconvertible to flavin at any pH. Indeed it is quite stable in 80% aqueous sulfuric acid. It is difficult to accept the concept of flavin and spirohydantoin as being interconvertible at the active site of an enzyme.

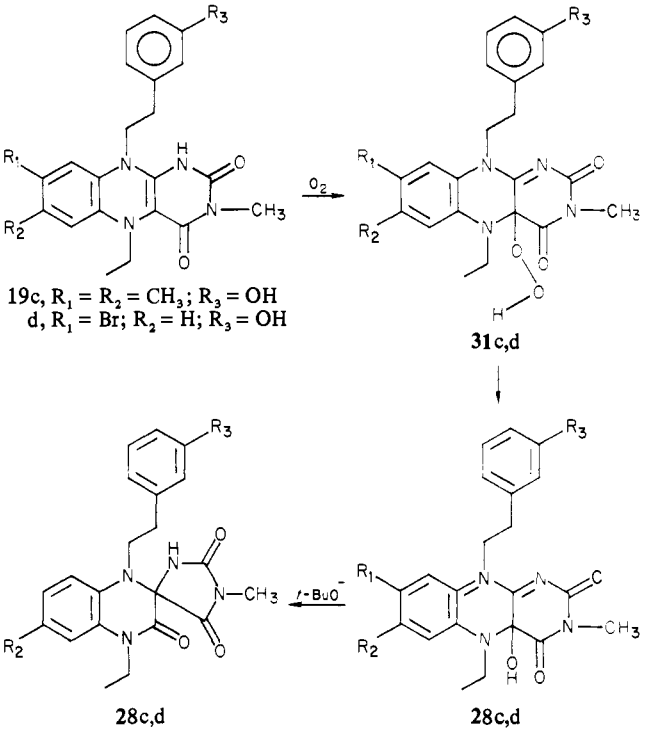
Experimental Section

Barbituric acid, 3-(dimethylamino)benzoic acid, 3,4-dimethylaniline, 3-bromoaniline, and (*m*-methoxyphenyl)acetic acid were obtained commercially. Solvents were purified as follows: reagent grade acetone (450 mL) was shaken intermittently with $KMnO_4$ crystals (500 mg) for 3 days; then it was distilled and the fraction boiling at 52 °C (400 mL) was dried over Drierite. Reagent grade *t*-BuOH (200 mL) was distilled from CaH_2 and the fraction boiling at 83 °C (180 mL) was stored under nitrogen. Chloroform was distilled once from Drierite after it was shaken with H_2O to remove ethanol. Acetonitrile was distilled from CaH_2. Dry benzene was prepared by distilling twice from phosphorus pentoxide. 2,6-Lutidine was purified by purging with HCl gas in benzene. The resulting colorless precipitate was collected by filtration, recrystallized from ethanol, and dissolved in 3 M NaOH solution. The organic phase was extracted with ether, which was dried over $MgSO_4$. The solvent was evaporated and the residue was refluxed with CaH_2 and fractionally distilled. *n*-Propyl peroxide was prepared according to Williams and Mosher,[19] and benzyl peroxide was prepared in a previous study.[8] For thin-layer chromatography either Eastman 1381 silica gel chromatogram sheets with a fluorescent indicator (no. 6060) or Merck no. 5539 pre-

Scheme IV



Scheme V



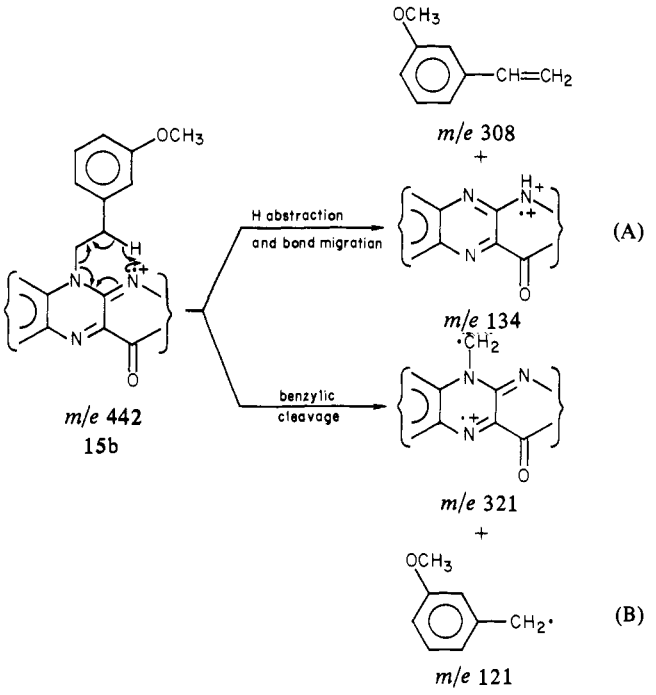
coated TLC silica gel 60-F-254 sheets were used. The deoxygenation of solvents was carried out by bubbling N_2 or argon through them for 4–6 h.

Apparatus. IR spectra were obtained with a Perkin-Elmer 137 spectrophotometer, UV measurements with a Cary 118C spectrophotometer (at 30 ± 1 °C), NMR spectra with a Varian T-60 instrument (Me_4Si internal standard), and fluorescence spectra with a Perkin-Elmer Model 512 fluorescence spectrophotometer. Elemental analyses were performed by Chemalytics Inc. High- and low-resolution mass spectra were measured by Professor G. J. Popják[20] and Dr. M. Uramoto.[21]

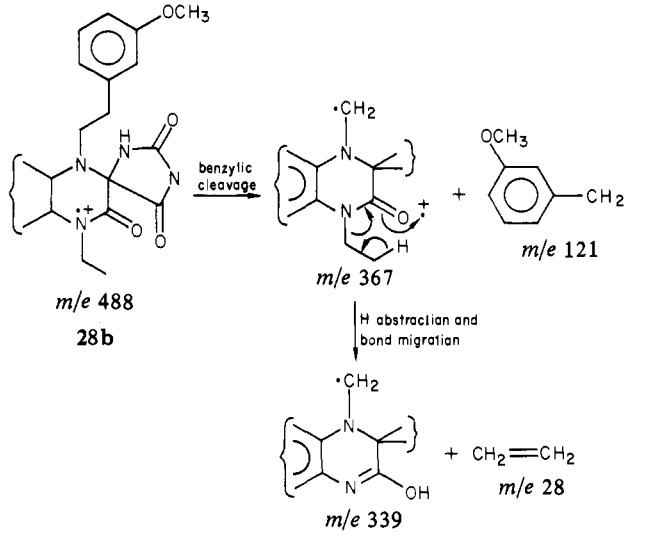
Synthetic Methods. 2,4,6-Trichloropyrimidine (2) was prepared (93% yield) from barbituric acid (**1**) by the method of Masuda.[22] **6-Chlorouracil (4)** was prepared from **2** via 2,4-dimethoxy-6-chloropyrimidine (**3**). Treatment of **2** with $NaOCH_3/CH_3OH$ provided **3**[23] [80%; colorless needles from petroleum ether, mp 76 °C (lit.[23a] 74–75, 73–74 °C)]. **3** was then converted to **4** by the method of Horwitz and Tomson[24] in 43%

(18) Yoneda, F.; Sakuma, Y.; Shinozuka, K. *J. Chem. Soc., Chem. Commun.* **1977**, 175.
(19) Williams, H. R.; Mosher, H. S. *J. Am. Chem. Soc.* **1954**, *76*, 2984.
(20) Courtesy of Professor G. J. Popják (UCLA).
(21) Courtesy of Dr. M. Uramoto (The Institute of Physical and Chemical Research, Japan).
(22) Masuda, T. *Pharm. Bull.* **1957**, *5*, 28.
(23) (a) Fisher, H. J.; Johnson, T. B. *J. Am. Chem. Soc.* **1932**, *54*, 727. (b) Pfleiderer, W.; Nubel, G. *Justus Liebigs Ann. Chem.* **1960**, *631*, 168.
(24) Horwitz, J. P.; Tomson, A. J. *J. Org. Chem.* **1961**, *26*, 3392.

Scheme VI



Scheme VII



yield and recrystallized from water: mp 295–298 °C dec; UV (H_2O) λ_{max} 261,[24] 264 nm.

3-Methyl-6-chlorouracil (8). 3-Methylbarbituric acid (7) was prepared by the method of Dickey and Gray.[25] 7, upon refluxing with a phosphoryl chloride–water mixture,[26] gave **8**: 38% yield; mp (recrystallized from H_2O) 294–296 °C dec; IR (KBr) $\bar{\nu}$ 3100, 2920, 2800, 1740, 1710, 1620, 1505, 1445, 990, 956, 855, and 760 cm^{-1}.

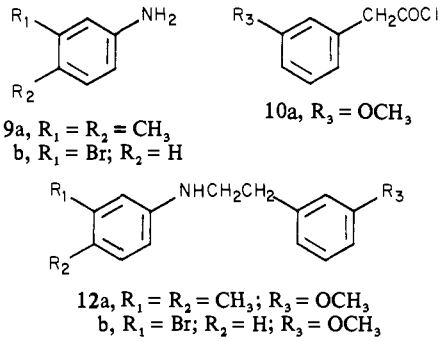
N-Substituted anilines 12a,b were prepared by a procedure similar to that described by Cope and Ciganek.[27] The anilines **9a,b** were allowed to react with the phenacetyl chloride (**10a**), and the resultant amides (**11a,b**) were reduced with $LiAlH_4$.

12a: 70% yield based on **11a**; bp 178–183 °C (0.4–0.35 mmHg); NMR ($CDCl_3$) δ 2.13 (6 H, s, C_3 and C_4 CH_3), 2.83 (2 H, t, CH_2CH_2N), 3.33 (2 H, t, CH_2CH_2N), 3.77 (3 H, s, OCH_3).

12b: purified by column chromatography; 77% yield based on **11b**; NMR ($CDCl_3$) δ 2.76 (2 H, t, J = 6.0, CH_2CH_2N), 3.25 (2 H, t, J = 6.0, CH_2CH_2N), 3.73 (3 H, s, OCH_3).

(25) Dickey, J. B.; Gray, A. R. "Organic Synthesis"; Wiley, New York, 1943, Collect. Vol. 2, p 60.
(26) Nubel, G.; Phleiderer, W. *Chem. Ber.* **1962**, *95*, 1605.
(27) Cope, A. C.; Ciganek, E. "Organic Syntheses"; Wiley, New York, 1963; Collect. Vol. 4, p 339.

Chart I



Synthesis of flavins 15a,b and 16 was accomplished by a modification of the general procedure by Yoneda and co-workers,[28] employing **12** and **4** or **8** as precursors (Scheme I). In a typical procedure, a mixture of **12a** (0.27 g, 1.06×10^{-3} mol) and **4** (0.14 g, 0.96×10^{-3} mol) was heated with stirring at ~220 °C for 10 min and then cooled; the solidified melt was triturated in ether and the crude **13a** (0.42 g) was collected by filtration. To a solution of **13a** (0.2 g) in 2 mL of acetic acid was added in one portion 0.08 g (1.16×10^{-3} mol) of $NaNO_2$ at room temperature. After vigorous stirring for 1 h, 10 mL of water was added and the red-brown precipitate of **14a** (0.17 g) was collected by filtration: R_f 0.6 (Eastman) (orange-yellow spot) (17:3 (v/v) C_6H_6–C_2H_5OH) with no fluorescence.

A mixture of 8.6 g of **14a**, 20 g of sodium dithionite, 80 mL of water, and 80 mL of ethanol was stirred at room temperature for 51 h and then reaction was terminated by addition of 7 mL of 30% hydrogen peroxide. An orange solid (**15a**) was obtained and collected by filtration: 5.72 g (69% based on **14a**); R_f 0.6 (Eastman) (yellow spot) (19:1 (v/v) $CHCl_3$–EtOH) with green-yellow fluorescence.

A solution of **15a** (5.72 g) in 150 mL of dimethylformamide containing 70 mL of methyl iodide and 35 g of potassium carbonate was stirred at room temperature for 23 h. The reaction was monitored by TLC (Eastman) (19:1 (v/v) ($CHCl_3$–EtOH). The resulting mixture was poured into water and the product (**16**) was extracted with chloroform. The organic layer was separated, dried over $MgSO_4$, and filtered, and the solvents were removed under reduced pressure. The resulting black crystals were suspended in benzene and collected by filtration. The orange solid was recrystallized from ethanol to provide fine orange crystals of **16** (2.75 g, 46% based on **15a**): mp 239–241 °C; R_f 0.9 (yellow spot) (19:1 (v/v) $CHCl_3$–EtOH) with intense green-yellow fluorescence: IR (KBr disk) $\bar{\nu}$ 1700, 1660, 1570, 1540, 1450, 1260 cm^{-1}; NMR ($CDCl_3$) δ 2.42 and 2.45 (3 H each, s, C_7 and C_8CH_3), 3.10 (2 H, t, $CH_2CH_2N^{10}$), 3.50 (3 H, s, N^3CH_3), 3.73 (3 H, s, OCH_3), 4.90 (2 H, t, $CH_2CH_2N^{10}$), 7.97 (1 H, s, C_9H); UV (MeOH) λ_{max} (ϵ) 269 (40 900), 352 (9150), 444 (12 500), 472 (sh) (9310) nm; UV (pH 5.0, 0.1 M acetate buffer) 269 (40 700), 372 (10 800), 449 (12 100), 472 (9620) nm.

Anal. Calcd for $C_{22}H_{22}O_3N_4$: C, 67.68; H, 5.68; N, 14.35. Found: C, 68.01; H, 5.81; N, 14.23. Fluorescence spectrum: λ_{em} (9:1 H_2O–MeOH) 523 nm (λ_{ex} 460 nm, $c = 2.203 \times 10^{-5}$ M) cf.[21] λ_{em} (9:1 H_2O–MeOH) 523 nm (λ_{ex} 467 nm, $c = 2.203 \times 10^{-5}$ M).

In the synthesis of **15b** from **8** and **12b**, a 1:2 mixture of $CHCl_3$ and ethanol was used as solvent in the reduction of **14b** to **15b**. Chromatography of the crude **15b** on a silica gel column (10:9.5:0.5 (v/v) ($CHCl_3$–$CH_3CO_2C_2H$–C_2H_5OH)) provided the product in 39% yield based on **13b**: R_f 0.55 (Merck) (bright yellow spot) (same solvent mixture as used for silica gel chromatography); recrystallized from $CHCl_3$ (minor)–C_2H_5OH (major), mp 265–266 °C dec (darkening at ~245 °C); IR (KBr disk) $\bar{\nu}$ 3400, 1710, 1660, 1570, 1530, 1270 cm^{-1}; NMR ($CDCl_3$) δ 3.10 (2 H, t, J = 7.5, CH_2CH_2Ar), 3.53 (3 H, s, N^3 CH_3), 3.77 (3 H, s, OCH_3), 4.83 (2 H, J = 7.5, $N^{10}CH_2CH_2$); UV (ethanol) λ_{max} (ϵ) 222.5 (27 830), 268.5 (3710), 341 (7460), 416 (sh) (9240), 435 (10 960), 460 (sh) (8160) nm.

Anal. Calcd for $C_{20}H_{17}O_3N_4Br$: C, 54.44; H, 3.88; N, 12.69; Br, 18.11. Found: C, 54.18; H, 3.64; N, 12.71; Br, 15.37. Exact mass measurement[20] calcd for $C_{20}H_{17}O_3N_4{}^{79}Br$, 440.04845; found, 440.04755. Calcd for $C_{20}H_{17}O_3N_4{}^{81}Br$, 442.04648; found, 442.04964.

3,7,8-Trimethyl-10-(3-hydroxyphenethyl)isoalloxazine (17a) and 3-methyl-8-bromo-10-(3-hydroxyphenethyl)isoalloxazine (17b) were obtained (Scheme II) by O-demethylation of **16** and **15b**, respectively. The procedure was applied to **16** as follows: 0.5 g of **16**, 24 mL of 48% HBr,

(28) Yoneda, F.; Sakuma, Y.; Ichiba, M.; Shimomura, K. *J. Am. Chem. Soc.* **1976**, *98*, 830.

and 25 mL of acetic acid were heated (110–120 °C) with stirring for 8 h. After cooling, the solution was poured onto ice and neutralized by addition of solid $NaHCO_3$ until cessation of CO_2 evolution. The resulting fine orange precipitate (0.52 g) was collected by filtration: R_f 0.7 (Eastman) (19:1 (v/v) ($CHCl_3$–C_2H_5OH) with no fluorescence; NMR (CF_3COOH) δ 2.68 (6 H, br s, C_7 and C_8 CH_3), 3.70 (3 H, s, N^3 CH_3).

17a,b were employed without further purification in the syntheses of **18a,b**, respectively.

3,7,8-Trimethyl-10-(3-acetoxyphenethyl)isoalloxazine (18a) and 3-Methyl-8-bromo-10-(3-acetoxyphenethyl)isoalloxazine (18b). The following is representative. To 0.2 g of **17a** suspended in 15 mL of pyridine was added 15 mL of acetic anhydride, and the mixture was stirred at room temperature for 1 h. The reaction was monitored by the appearance of an intense fluorescence on TLC (R_f 0.9 (Eastman), 19:1 (v/v), $CHCl_3$–C_2H_5OH). The reaction mixture was poured into ice water and stirred for 2 h. The resulting red-yellow solution mixed with a yellow-red solid was extracted with chloroform, and the extract separated, dried over $MgSO_4$, and filtered. The solvents were removed under reduced pressure. The residue was chromatographed on a silica gel column [19:(1–2) (v/v) $CHCl_3$–C_2H_5OH eluant] to give 0.24 g of **18a**: recrystallized from 1:1 (v/v) CH_3OH–H_2O; mp 235–237 °C dec; IR (KBr disk) $\bar{\nu}$ 1770, 1720, 1670, 1591, 1545, 1200 cm^{-1}; UV λ_{max} (MeOH) (ϵ) 269 (33 500), 350 (8100), 420 (sh) (8160), 444 (10 800), 472 (sh) (8370) nm; NMR ($CDCl_3$) δ 2.28 (3 H, s, $OCOCH_3$), 2.58 and 2.63 (3 H each, s, C_7 and C_8 CH_3), 3.17 (2 H, t, C CH_2CH_2N), 3.52 (3 H, s, N^3 CH_3), 4.92 (2 H, t, C $CH_2CH_2N^{10}$).

Anal. Calcd for $C_{23}H_{22}O_4N_4$: C, 66.01; H, 5.30; N, 13.39. Found: C, 66.21; H, 5.40; N, 13.70.

For the synthesis of **18b**, purification was carried out by silica gel chromatography (C_6H_6–$CH_3CO_2C_2H_5$ eluant (11:9, 1:1)) to give a bright yellow solid: IR (KBr disk) $\bar{\nu}$ 3400, 1760, 1710, 1660, 1580, 1540, 1200 cm^{-1}; NMR ($CDCl_3$) δ 2.94 (3 H, s, $OCOCH_3$), 3.52 (3 H, s, N^3 CH_3), 4.87 (2 H, t, $CH_2CH_2N^{10}$); mp >300 °C dec; exact mass measurement[21] calcd for $C_{21}H_{17}O_4N_4{}^{81}Br$, 470.04130; found, 470.04176; exact mass calcd for $C_{21}H_{17}O_4N_4{}^{79}Br$, 468.04327; found, 468.04701; mass spectrum,[13] *m/e* 469(13), 467 (14), 427 (21), 425 (22), 309 (53), 307 (55), 278 (12), 242 (11), 240 (11), 200 (99), 198 (100), 162 (19), etc.

Flavinium cation perchlorate salts 20a–d were prepared by alkylation of the appropriate 1,5-dihydroflavin at the N^5 position (Scheme III). Dithionite reduction and alkylation with diethyl sulfate[29] was employed for **16** and **15b**. **18a,b** were prepared by the reaction of the 1,5-dihydroflavin with acetaldehyde, followed by reduction of the resultant imine with $NaCNBH_3$.[6,30] The acetyl groups of **18a,b** were removed after their reductive alkylation by adjustment (with 1 N KOH) of the pH to 11 (anaerobic conditions). The N^5-ethyl-1,5-dihydroisoalloxazines (**19**) were isolated as pale yellow solids by acidification with degassed acetic acid under an N_2 atmosphere. These were immediately converted to the corresponding N^5-ethylisoalloxazinium perchlorates (**20**) by the method described by Ghisla et al.[29] and were isolated as deep purple solids. Regardless of the procedure employed, the various flavinium perchlorate salts were obtained in ca. 50% yield based on oxidized flavin and were characterized by the ratio of their absorbances at λ_{max} values in the vicinity of 430 and 550 nm ($A_{\sim 430}/A_{\sim 550}$ ranging from 1.30 to 1.46 in 1 N HCl).[31a] 3-Methyl-5-(3-methoxyphenyl)lumiflavin (**23**) was prepared by reaction of 1,5-dihydro-3-methylluminflavin **21** with 2-(3-methoxyphenyl)ethyl iodide[31b] (A_{432}/A_{554} = 1.30 in 1 N HCl) (Scheme IV).

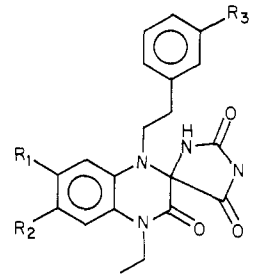
Products of Reaction of Flavinium Perchlorates 20a–c with *n*-Propyl Hydroperoxide Anion. The reaction mixture, composed of 200 mg (3.85 × 10^{-4} mol) of **20a**, 44.4 mg (3.95 × 10^{-4} mol) of potassium *tert*-butoxide, and 75 μL of 71% *n*-propyl hydroperoxide in methanol (3.85 × 10^{-4} mol), was dissolved in 100 mL of dry *tert*-butyl alcohol and stirred in the dark for 91 h. The course of the reaction was monitored by diluting 20-μL aliquots with 3 mL of dry *tert*-butyl alcohol and monitoring the chemiluminescent emission of the 4a-flavin propyl peroxide with a scintillation counter.[8] The reaction was stopped by addition of glacial acetic acid at the time when photoemission had decayed to a low level and no further decrease in emission was evident. The product formation was shown by TLC (Merck, 1:1 (v/v) ethyl acetate–benzene) not to be altered on addition of acetic acid.

The spent reaction mixture was evaporated to dryness and the residue dissolved in a small volume of chloroform; the mixture was placed on a silica gel (130 g) column and eluted with 1:1 (v/v) benzene–ethyl acetate. A major product (55 mg, R_f 0.7) was obtained along with three minor products (<5–10 mg), R_f 0.5, 0.4, and 0. Two of these were assignable to oxidized flavin (R_f 0.4) and the starting flavinium cation (R_f 0) on the basis of their fluorescence color and behavior on TLC. The product (R_f 0.7) was recrystallized from ethanol to give 50 mg (30% yield) of **28a** as fine colorless needles: mp 185.5–187 °C; mass spectrum, *m/e* 436 (26) (M^+), 315 (100) (base peak, $M^+ - C_8H_9O$ with m^* = 227.58), 287 (14) (*m/e* 315 – 28 with m^* = 261.5); UV (EtOH) λ_{max} (ϵ) 271 (sh) (6450), 278 (5610), 307 (6250) nm; IR (KBr disk) $\bar{\nu}_{C=O}$ 1790, 1740, and 1650, $\bar{\nu}_{OCH_3}$ 1260 cm^{-1}; NMR Me_2SO-d_6, δ 1.20 (3 H, t, CH_3 CH_2H), 2.28 (6 H, s, two aryl CH_3), 3.00 (3 H, s, NCH_3), 3.80 (3 H, s, OCH_3); exact mass measurement calcd for $C_{24}H_{28}O_4N_4$, 436.21104; found, 436.21736. Anal. Calcd for $C_{24}H_{28}O_4N_4$; C, 66.04; H, 6.47; N, 12.84. Found: C, 66.41; H, 6.54; N, 12.56.

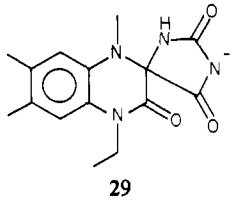
The reaction of **20c** (140 mg, 2.77 × 10^{-4} mol) with potassium *tert*-butoxide (63.3 mg, 5.64 × 10^{-4} mol) and *n*-propyl hydroperoxide (3.9 × 10^{-4} mol) in 100 mL of dry *tert*-butyl alcohol was carried out as described above for **20a**, providing a major product (60 mg, R_f 0.6) besides three minor products (<5–10 mg), R_f 0.3, 0.05, and 0. Two of these were assignable to oxidized flavin (**17a**) (R_f 0.05) and the starting flavinium cation (R_f 0) on the basis of their fluorescence color and behavior on TLC. The product (R_f 0.6) was recrystallized from 1:1 ethanol–water to give 55 mg (45% yield) of **28c** as colorless fine needles: mp 270–272 °C dec; mass spectrum, *m/e* 422 (2) (M^+), 315 (6) ($M^+ - C_7H_7O$), 287 (3) (*m/e* 315 – 28); UV (EtOH λ_{max} 264 (sh) (6760), 273 (sh) (6460), 281 (sh) (5420), 307 (6460) nm; IR (KBr disk) $\bar{\nu}_{C=O}$ 1780, 1730, 1725, and 1660, $\bar{\nu}_{OH}$ 3400, $\bar{\nu}_{NH}$ 3200 cm^{-1}; NMR ($CDCl_3$) δ 1.27 (3 H, t, NCH_2CH_3), 2.28 and 2.30 (3 H each, s, aryl CH_3), 3.03 (3 H, s, NCH_3), 4.0 (2 H, q, N CH_2CH_3), 5.62 (1 H, s, aryl OH); exact mass measurement calcd for $C_{23}H_{26}O_4N_4$, 422.19539; found, 422.19506. Anal. Calcd for $C_{23}H_{26}O_4N_4{\cdot}H_2O$; C, 62.71; H, 6.40; N, 12.72. Found: C, 63.69; H, 6.32; N, 12.68.

Photolysis of 4a-Flavin *n*-Propyl Peroxide Prepared by Addition of *n*-PrOOH to 20a–d. A 2,6-lutidine solution in dry benzene was prepared to be 0.991 × 10^{-5} mol/50 mL. *n*-Propyl hydroperoxide[32] (91% pure) was used in preparing a 1.014 × 10^{-5} mol/50 μL stock solution in dry benzene. In a 10-mL vial with a cap was mixed 3.96 × 10^{-6} mol of the flavinium perchlorate (**20a–d**) in 1–2 mL of dry benzene, 20 μL of the stock 2,6-lutidine (3.96 × 10^{-6} mol) solution, and 20 μL of the stock *n*-propyl hydroperoxide (4.06 × 10^{-6} mol) solution. When all the flavinium salt **20d** had dissolved (gentle agitation), the formation of the 4a-flavin *n*-propyl peroxide was monitored by TLC (Merck) (**20a**, R_f 0.6 in 10:9.5:0.5 (v/v) chloroform–ethyl acetate–ethanol; **20b**, R_f 0.8 in 1:1 (v/v) **20c**, benzene–ethyl acetate; R_f 0.52 in 1:1 (v/v) benzene–ethyl acetate; **20d**, R_f 0.84 in 1:1 (v/v) benzene–ethyl acetate; **23**, R_f 0.66 in 10.95:0.5 (v/v) chloroform–ethyl acetate–ethanol; **24**, R_f 0.48 in 1:1 (v/v) benzene–ethyl acetate, R_f 0.66 in 10:9.5:0.5 (v/v) chloform–ethy acetate–ethanol). When the reactions were complete, each vial was placed at a 20-cm distance from a 150 W, 120 V daylight W lamp and irradiated for an appropriate period of time determined by following the disappearance of the 4a-flavin *n*-propyl peroxide spot on TLC: **20a**, 120

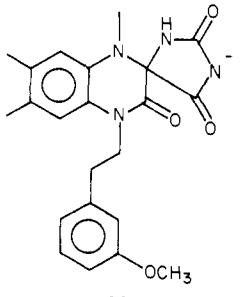
Chart II



28a, $R_1 = R_2 = CH_3$; $R_3 = OCH_3$
b, R_1 = Br; R_2 = H; $R_3 = OCH_3$
c, $R_1 = R_2 = CH_3$; R_3 = OH
d, R_1 = Br; R_2 = H; R_3 = OH



29



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(29) Ghisla, S.; Hartmann, U.; Hemmerich, P.; Muller, F. *Justus Liebigs Ann. Chem.* **1973**, 1388.

(30) Williams, R. F.; Bruice, T. C. *J. Am. Chem. Soc.* **1976**, *98*, 7752.

(31) (a) A_{431}/A_{546} = 1.32 (1 N HCl) for 1,5-dihydro-3-methyl-5-ethyllumiflavin perchlorate. (b) This compound [bp 77–82 °C (0.3–0.35 mmHg)] was prepared from 2-(3-methoxyphenyl)ethanol (Aldrich) by the method reported of: Landauer, S. R.; Rydon, H. N. *J. Chem. Soc.* **1953**, 2224.

(32) The purity was determined by iodometric titration (Mair, R. D.; Graupner, A. J. *Anal. Chem.* **1964**, *36*, 194) and NMR measurements.

Table I. Decomposition Rate Constant and λ_{max} Values for 4a-Hydroperoxyflavins

dihydroflavin employed	λ_{max} of hydroperoxyflavin, nm	$[t\text{-BuOK}]_0/[\text{dihydroflavin}]_0$	k_{obsd}, s^{-1}	λ_{max} of product, nm
19c	361	0.641	2.31×10^{-5}	308
		0.962	1.34×10^{-4}	
		1.924	7.52×10^{-4}	
19d	355	0	1.28×10^{-5}	282 sh, 350
		0.72	1.08×10^{-4}	
		1.08	3.24×10^{-4}	313
		2.16	6.18×10^{-4}	

min; **20b**, 120 min; **20c**, 195 min; **20d**, 60 min; **23**, 80 min, **24**, 120 min. After completion of reaction, solvent was removed under reduced pressure and mass spectral determinations[21] were carried out upon the residues (mass spectral results are available as supplementary material).

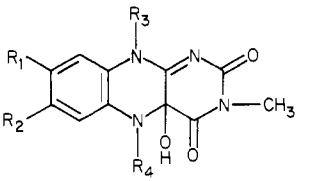
Reaction of Flavinium Perchlorate 24 with Benzyl Hydroperoxide Anion. A solution of **24** (0.752×10^{-4} mol, 30 mg), benzyl peroxide (20 μL), and *t*-BuOK (0.752×10^{-4} mol, 8.33 mg) in 20 mL of dry *t*-BuOH was stirred in the dark for 67 h. Aliquots (100 μL) of the reaction were withdrawn and diluted with 6 mL of *tert*-butyl alcohol, and the reaction was followed by repetitively scanning the absorption spectrum in the range of 300–450 nm. When no more spectral changes were observed, the reaction was quenched by the addition of 1 mL of acetic acid, and the solvent was removed under reduced pressure. TLC (Merck) of the residue with 1:1 (v/v) benzene–ethyl acetate showed a spot (R_f 0.2) corresponding to that of the 4a-pseudobase of **24**. This could be identified by the red color formed on spraying with a 1 M HCl solution. The residue was chromatographed on a silica gel (30 g) column, and its band turned black during elution wth benzene–ethyl acetate. The product isolated (7.5 mg) through chromatography exhibited no color test with 1 M HCl: R_f 0.6; UV (ethanol) λ_{max} 284, 305 nm; IR (KBr disk) $\bar{\nu}$ 3220, $\bar{\nu}_{C=O}$ 1790, 1735, and 1645, $\bar{\nu}_{C=C}$ 1601, 1510 cm^{-1}; mp 186 °C dec; NMR ($CDCl_3$) δ 1.22 (3 H, t, J = 7.2), 2.26 (6 H, s), 2.81 (3 H, s), 3.08 (3 H, s), 3.70 (2 H, q, J = 7.2), 6.62 (1 H, s), 6.80 (1 H, s). These spectral data compared to those data of **28a,c** strongly supported the end product being the corresponding spirohydantoin (**29**).

Oxidation of 1,5-Dihydroflavins (19c,d) to 4a-Hydroperoxides and the Spontaneous Decomposition of the Hydroperoxides (*tert*-Butyl Alcohol Solvent). The observations of the oxidations of **19d** are typical (Scheme V). A stock solution (3.86×10^{-5} M) of **19d** was prepared (dry N_2 atmosphere, glovebox) by dissolving solid dihydroflavin in anhydrous (distilled over CaH_2 under N_2) *t*-BuOH. A 3-mL aliquot of the solution was transferred under an inert atmosphere to a Thunberg cuvette which, after being sealed, was transferred to a thermostated spectrophotometer (30 °C). After temperature equilibration, a rapid stream of O_2 was passed through the solution for 1 min. Repetitive spectral scanning (UV–vis) established the formation of the characteristic[4-6,8] spectrum of a 4a-hydroperoxyflavin (λ_{max} 355 nm in *tert*-butyl alcohol). The first-order rate constant for formation of the 4a-hydroperoxyflavin **31d** was calculated to be 6.8×10^{-4} s^{-1}. From the A_{355} value 0.235 at completion of the reaction and the initial concentration of **19d**, an ε value of 6090 M^{-1} cm^{-1} was calculated for **31d**. This extinction coefficient may be compared to that of the 4a-hydroperoxy derivative of 1,3,5-trimethyllumiflavin in absolute methanol[3] (8000 M^{-1} cm^{-1}) and *tert*-butyl alcohol (9600 M^{-1} cm^{-1}). With time the spectrum of **31d** changed to that of the 4a-pseudobase (**25d**)—see pseudobase preparation. The conversion of **31d** → **25d** was associated with a first-order rate constant of 1.3×10^{-5} s^{-1}. The rate constant for decomposition of **31d** is ca. 10-fold less than that previously shown for the 4a-hydroperoxide of 5-ethyl-3-methyllumiflavin.[9a] From A_{313} at the completion of reaction and the determined value of ε for **25d** the conversion of 4a-hydroperoxide to 4a-pseudobase is quantitative based on the initial concentration of dihydroflavin employed.

Decomposition of 4a-Hydroperoxyflavins in the Presence of *t*-BuOK. The observations on the reactions of the 4a-hydroperoxide derived from **19d** (i.e., **31d**) are typical (Table I). The 4a-hydroperoxyflavin was generated exactly as detailed above, employing the same stock solution of the dihydroflavin. At completion of formation of **31d**, 0.5 mL of a solution of *t*-BuOK in *t*-BuOH was tipped in and the contents of the cuvette mixed. The final concentration of *t*-BuO^- equaled 0.72[**19d**]. The spectrum of the reaction mixture was scanned with time and in this manner the conversion of **31d** to the spirohydantoin (**28d**) was found to occur with a rate constant of 1.1×10^{-4} s^{-1}. This rate constant is ca. 10^2 less than the rate constant previously found for the decomposition of the 4a-peroxyanion of 5-ethyl-3-methyllumiflavin.[9a] At the completion of reaction the spectrum of the solution was found to be superimposable upon that of authentic **28d**. From the experimental A_{313} = 0.253 at completion and the ε = 6400 M^{-1} cm^{-1} for authentic **28d**, it was calculated that the yield of **28d** was quantitative based on the concentration of starting dihydroflavin.

On completion of the spectral studies the solutions were made slightly acidic by addition of acetic acid, solvent was removed under vacuum, and mass spectral analysis[21] was carried out upon the residue (mass spectral results are available as supplementary material).

Chart III



25a, $R_1 = R_2 = CH_3$; $R_3 = m\text{-}CH_3OC_6H_4CH_2CH_2$; R_4 = Et
b, R_1 = Br; R_2 = H; $R_3 = m\text{-}CH_3OC_6H_4CH_2CH_2$; R_4 = Et
c, $R_1 = R_2 = CH_3$; $R_3 = m\text{-}HOC_6H_4CH_2$; R_4 = Et
d, R_1 = Br; R_2 = H; $R_3 = m\text{-}HOC_6H_4CH_2CH_2$; R_4 = Et
26, $R_1 = R_2 = R_3 = CH_3$; R_4 = Et
27, $R_1 = R_2 = R_3 = CH_3$; $R_4 = m\text{-}CH_3OC_6H_4CH_2CH_2$

4a-Flavin Pseudobases 25a–d, 26, and 27. Pseudobases of **25a**, **25c**, and **26** were obtained as pale yellow solids by stirring 10–15 mg of the corresponding flavinium perchlorates (**20a**, **20c**, and **24**) for 1 h in 4–10 mL of 0.1 M phosphate buffer (pH 6.8–7.1). These were collected by filtration, washed with a little water, and desiccated in vacuo over Drierite. In the preparation of **25b** and **25d** from **20b** and **20d**, a pyridine (0.5–1 mL) solution of the flavinium salt (10 mg) was taken up with 4 mL of 0.1 M phosphate buffer and stirred for 20 min. Then the solution was made slightly acidic with acetic acid, and the pale yellow precipitate was collected by filtration and dried. **27** was obtained by stirring 8.6 mg of **23** in 0.5 mL of pyridine, 2 mL of acetone, and 4 mL of a 0.1 M phosphate buffer (pH 6.8) solution at room temperature for 5 days; the brown-yellow solid was collected by filtration, washed, and dried as previously.

λ_{max}'s (nm) and absorbance ratios (parentheses) for **25a,c,d**, **26**, and **27** are as follows: **25a** (ethanol) 272 (1), 307 (0.465), 350 (0.39); **25c** (*tert*-butyl alcohol) 276 (0.98), 280 (1.0), 306 (0.766), 349 (0.83); **25d** (methanol 249 (sh) (1), 255 (0.955), 350 (0.274); **26** (ethanol) 282 (0.855), 305 (0.834), 355 (0.928); **27** (methanol) 273 (1.0), 280 (0.99), 303 (sh) (0.73), 361 (0.739).

Rearrangement of pseudobase (3×10^{-5} M) in the presence of excess base (KOH at 150-fold excess) in methanol was found to be pseudo first order. For **25a**, **25d**, and **26** the rate constants were in the range of (2–4) $\times 10^{-4}$ s^{-1}, while for pseudobase **27**, which possesses a bulky N^5 substituent, $k_{obsd} \cong 5 \times 10^{-5}$ s^{-1}. The spectra at completion of reaction were determined and found to be superimposable upon the spectra of the spirohydantoins. The conversion of pseudobases to spirohydantoin is quantitative.

When **26** was allowed to solvolyze under anaerobic conditions in dry *t*-BuOH with an excess of *t*-BuOK or KOH, the pseudobase disappearance again followed a first-order rate law and with about the same rate constant as in ethanol ($k_{obsd} = 9.74 \times 10^{-4}$ s^{-1} with *t*-BuO^- as base and 2.1×10^{-3} s^{-1} with dry KOH). The products were also identical as shown spectrally and by TLC (Merck) in three different solvents (1:1 (v/v) benzene–ethyl acetate, R_f 0.86; 19:1 (v/v) chloroform–ethanol, R_f 0.18; benzene, R_f 0.11).

The procedure for the isolation of spirohydantoin **28b** formed from pseudobase **25b** is general. One hundred and fifty milligrams of pseudobase **25b** was rearranged in methanol by addition of a 150-fold excess of KOH (as 1.0 M in KOH), neutralization of the reaction mixture by addition of acetic acid, and removal of the solvent under reduced pressure. The residue was triturated with dry chloroform, and the chloroform filtrate was spotted on preparative thin-layer chromatogram plates (Analtech) and developed six times (10:7:3 (v/v) benzene–cyclohexane–

Table II. Crystal Data for the 10a-Spirohydantoin (28b)

formula: $C_{22}N_4O_4H_{23}Br$	D_{calcd} = 1.493 g cm^{-3}
formula weight: 487.35	D_{measd} = 1.49 g cm^{-3}
crystal system: monoclinic	crystal size: 0.075 × 0.075 × 0.12 mm
a = 11.277 (2) Å	λ(Cu Kα) = 1.5418 Å
b = 18.349 (3) Å	μ(Cu Kα) = 26.47 cm^{-1}
c = 5.302 (1) Å	F(000) = 500
β = 98.78 (1)°	Z = 2
V = 1084.3 (3) Å^3	space group: *Pa*

ethyl acetate). The band containing the ring-contracted product was scraped from the plates. The product was extracted into ethyl acetate which was then removed under vacuum. The resulting residue was recrystallized from ethanol to provide the spirohydantoin **28b** as coloreless fine needles: mp 313–314 °C dec (darkening at ~270 °C); NMR ($CDCl_3$) δ 1.27 (3 H, t, J = 6.5 Hz, N CH_2CH_3), 3.05 (3 H, s, NCH_3), 3.81 (3 H, s, OCH_3), 4.07 (2 H, q, J = 6.5, N CH_2CH_3); IR (KBr disk) $\bar{\nu}_{C=O}$ 1790, 1730, 1670, and 1640, $\bar{\nu}_{OCH_3}$ 1250 cm^{-1}; UV (ethanol) λ_{max} (ε) 228 (41 110), 263 (7310), 272 (sh) (6670), 279.5 (5790), 309 (6680) nm (see Figure 4); UV (after addition of 1 drop of 1 N NaOH to 3 mL of the ethanolic solution) λ_{max} (ε) 233 (40 950), 272 (18 060), 279.5 (7400), 314 (6800) nm; exact mass measurement calcd for $C_{22}H_{23}O_4N_4{}^{81}Br$, 488.08834; found, 488.09077.

A single crystal for mounting and X-ray analysis was obtained by dissolving the analytical sample of the spirohydantoin at reflux in a dry cosolvent of chloroform (minor component) and carbon tetrachloride. The solution was allowed to cool from 50 °C to room temperature in a Dewar bottle and set aside for 30 days. Solvent was then decanted and the crystals were washed with 7:1.5 (v/v) CCl_4–$CHCl_3$ and dried in vacuo.

X-ray Data Collection for 28b. The crystal structure of the 10a-spirohydantoin (**28b**) was determined in order to establish the chemical formula of this compound. Crystal data are listed in Table II. Three-dimensional data were collected on a Syntex automated diffractometer using a graphite monochromator (Cu Kα radiation). The θ–2θ scan technique was used and intensities were measured to $(\sin\theta)/\lambda$ = 0.61 Å^{-1}. There was no fall-off in intensity during the data collection as judged from regular periodic measurements of four standard reflections. Values for $\rho(I)$ were derived from counting statistics and measured instrumental uncertainties. All reflections gave a measurable intensity and were used in the subsequent refinement. A total of 1987 intensity data were converted to structure amplitudes by application of Lorentz and polarization factors. An absorption correction was applied, assuming that the crystal was an ellipsoid of revolution. The data were then placed on an absolute scale with a Wilson plot.

Structure Determination and Refinement for 28b. The structure was solved by analysis of the Patterson map which was dominated by vectors involving bromine atoms. This was refined by anisotropic least-squares methods. In the final stages of refinement, when hydrogen atoms had been located from a difference map, these were refined isotropically. All reflections measured were used in the refinement. The weighting scheme ($w = 1/\sigma_F{}^2$) was $\sigma_F = 0.375 \pm 0.0272(F - 10.3)$ for $F > 10.3$, $\sigma_F = 0.375$ for $3.0 < F < 10.3$, and $\sigma_F = 0.375 + 0.153(F - 3)$ for $F < 3.0$. The quantity minimized was $\sum w\{|F_o| - |F_c|\}^2$. Final residuals were R = 0.03 and R_w = 0.04.

Atomic scattering factors for bromine, oxygen, nitrogen, and carbon atoms were those given by Cromer and Waber;[33] those for hydrogen atoms were from Stewart, Davidson, and Simpson.[34] The computer programs used were developed in this laboratory by H.L.C. or were modified (UCLALS4).[35] Coordinates derived from the refinement and temperature factors are available as supplementary material.

Spectra of spirohydantoins were obtained from the pure compounds for **28a–c** and **29**, from the product in the reaction solution on rearrangement of the pseudobase of **28d**, **29**, and **30**, and from the product of solvolysis of the 4a-hydroperoxyflavin in *tert*-butyl alcohol.

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Supplementary Material Available: Coordinates for molecule **28b**, spectral data for spirohydantoins **28a–d**, **29**, and **30**, and mass spectral data of the products from the photolysis of 4a-flavin *n*-propyl peroxides and of the products from the decomposition of 4a-hydroxyflavins in *t*-BuOK (5 pages). Ordering information is given on any current masthead page.

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