E. Chromic Anhydride in Acetic Acid.—The natural 7-hydroxy-β-isosparteine (0.48 mmole) in 5 ml of glacial acetic acid was treated with 200 mg (2 mmoles) of chromic anhydride at 55–65° for 16 hr. The mixture was cooled in an ice bath, made strongly basic, and extracted with chloroform. The same hydroxy lactam was isolated which was formed under the conditions of B and C above.

Registry No.—I, 10146-70-0; II, 10146-71-1; III, 10146-72-2; (−)-7-hydroxy-β-isosparteine monoperchlorate, 10182-05-5; 7-hydroxy-β-isosparteine monohydroiodide, 10146-73-3; 7-acetoxy-β-isosparteine, 3279-73-0; 7-acetoxy-β-isosparteine diperchlorate, 10146-75-5; (−)-β-isosparteine monoperchlorate, 10146-76-6.

Acknowledgments.—We wish to express appreciation to Dr. W. T. Huffman and Mr. Moran for the collections of plant material; to Drs. Charles Piper Smith and John M. Fogg, Jr., for the classification of the botanical specimens; to Drs. J. M. H. Pinkerton and L. K. Steinrauf for thier interest in this problem and for making available to us the results of their X-ray crystallographic analysis in advance of publication; and to Edward F. Szymanski for nmr studies of the alkaloid salts. We thank also the Rohm and Haas Co., the Sharp and Dohme Laboratories, Smith, Kline and French Laboratories, and the National Institutes of Health for financial assistance.

Flavins. XIII. Rearrangement Reactions of 1,3,10-Trialkylflavinium Salts[1]

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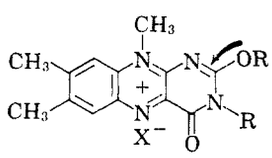
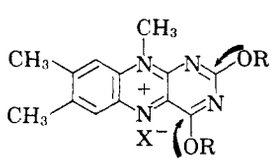
Spirohydantoins have been found to result from reactions of several nucleophiles with 1,3,10-trimethylflavinium perchlorate (**3**). Observations regarding an incorrectly formulated "alloxan-anil" have been reinvestigated and interpreted in the light of Clark-Lewis' findings and the results presented herein.

Mager and Berends' recent inference[4] on operative intermediates in the leucoflavin autoxidation scheme has prompted us to report chemical studies related to the proposed scheme. In previous publications,[5,6] we noted that the 2(O),3(N)-dialkyl- and 2(O),4(O)-dialkylflavinium salts (*e.g.*, **1** and **2**, respectively) reacted



1



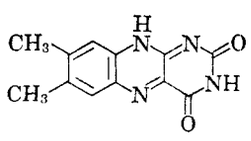
2

with hydroxyl ion and/or ammonia at positions denoted by arrows (by simple nucleophilic addition–elimination processes). The present article deals with the reactions of the 1(N),3(N)-dialkylflavinium salt **3** with several nucleophiles, namely hydroxyl ion, ammonia, and borohydride ion. The 1,3,10-trimethylflavinium salt **3** reacted with these agents (the latter under a specific set of conditions) to undergo a skeletal rearrangement reaction leading to the spirohydantoins **4**, **5**, and **6**, respectively (Scheme I). The experimental conditions for the preparation of **6** require special mention, for an isomeric tetrahydroflavin **7** was produced when a suspension of **3** in absolute methanol was treated with an excessive quantity of borohydride. The spirohydantoin **6** was obtained by intermittently treating a stirred aqueous suspension of **3** with *very small amounts* of sodium borohydride. After each addition, a quantity of **3** gradually dissolved, imparting a transient orange color to the solution. The white spirohydantoin **6** crystallized during the course of the reaction, but the difference in the crystalline forms (and colors thereof) allowed one to easily determine by microscopic examination the point of complete solution of **3**.

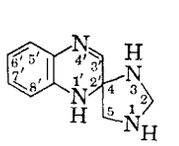
The infrared spectrum of each of the spirohydantoins **4**, **5**, and **6** contained a sharp band in the 3279–3311-cm^{-1} region (ν_{NH}) and two bands in the 1761–1770- and 1709–1715-cm^{-1} regions. The latter two absorption bands are attributable to the 4-oxo and 2-oxo groups, respectively, of the hydantoin ring.[7] The nmr spectrum (60 Mc, benzene-d_6, 65° for solubility requirement) of the spirohydantoin **6** contained an AB quartet (J = 11.5 cps) with resonances centered at 2.48 and 3.05 ppm. This AB spin system is assignable to a nonequivalent methylene group.[8]

The identity of the spirohydantoin system was further substantiated by comparing, and finding identical, the product obtained by rearranging the alloxazinium salt **11** with the known **12**, which was obtained from the ureide **13** by the procedure of Clark-Lewis[9a] (eq 1).

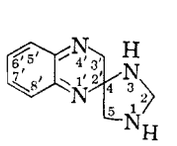
(1) In common usage, the term "flavin" represents derivatives of 7,8-dimethylisoalloxazine (*e.g.*, 7,8-dimethylbenzo[*g*]pteridine-2,4(3H,10H)-dione). For simplicity of terminology in the text, we have employed "spirohydantoin" for derivatives of the spiro[imidazolidine-4,2′(1′H)-quinoxaline] and spiro[imidazolidine-4,2′(3′H)-quinoxaline] systems.



7,8-dimethylbenzo[*g*]-pteridine-2,4(3H,10H)-dione



spiro[imidazolidine-4,2′(1′H)-quinoxaline]



spiro[imidazolidine-4,2′(3′H)-quinoxaline]

(2) National Science Foundation Postdoctoral Fellow, 1963–1964.

(3) Center for Research in Pharmacology and Toxicology, University of North Carolina, Chapel Hill, N. C. 27514.

(4) H. I. X. Mager and W. Berends, *Biochim. Biophys. Acta*, **118**, 440 (1966).

(5) P. Hemmerich, C. Veeger, and H. C. S. Wood, *Angew. Chem.*, **77**, 699 (1965); *Angew. Chem. Intern. Ed. Engl.*, **4**, 671 (1965).

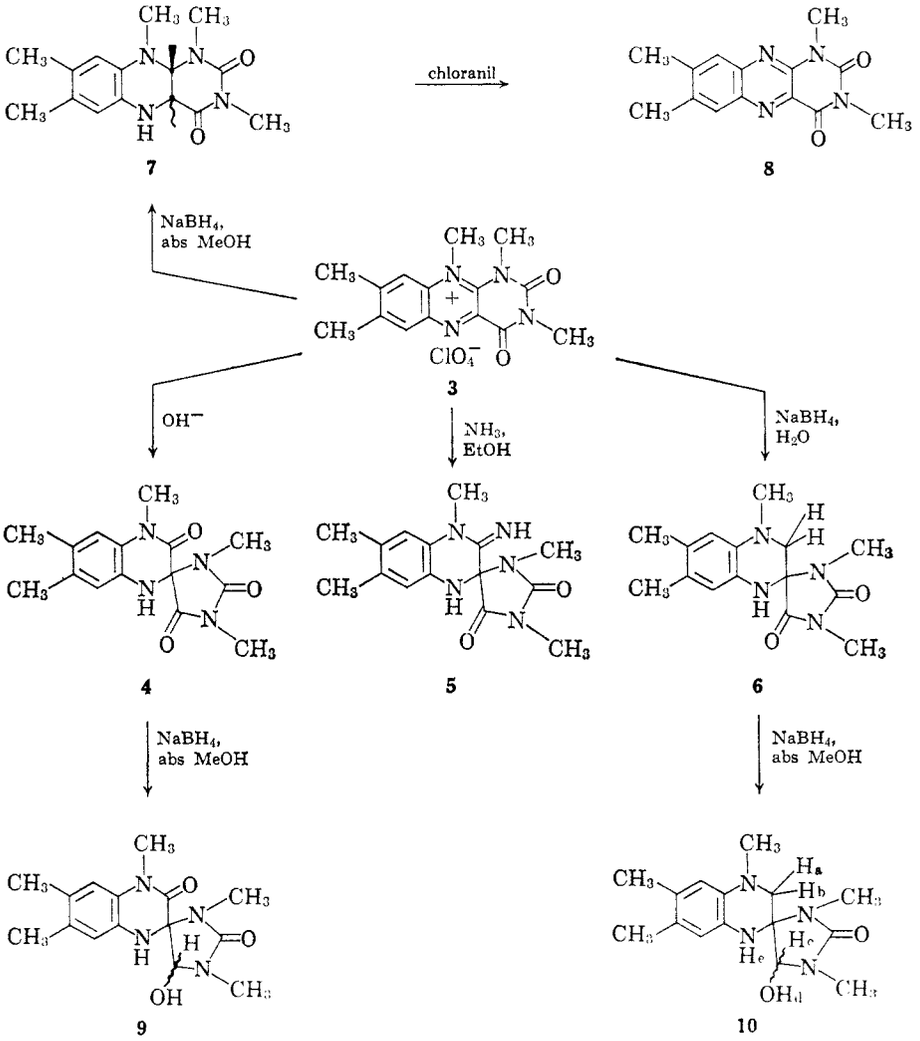
(6) K. H. Dudley and P. Hemmerich, *Helv. Chim. Acta*, **50**, 355 (1967).

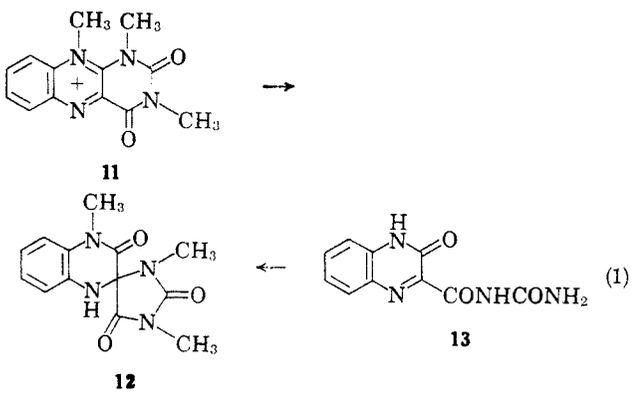
(7) A. R. Katritzky and A. P. Ambler in "Physical Methods in Heterocyclic Chemistry," Vol. II, A. R. Katritzky, Ed., Academic Press Inc., New York, N. Y., 1963, p 228.

(8) R. C. Cookson, T. A. Crabb, J. J. Frankel, and J. Hudec, *Tetrahedron, Suppl.*, **7**, 355 (1966).

(9) (a) J. W. Clark-Lewis, *J. Chem. Soc.*, 422 (1957); (b) J. W. Clark-Lewis and M. J. Thompson, *ibid.*, 430 (1957); (c) F. E. King and J. W. Clark-Lewis, *ibid.*, 3379 (1951).

SCHEME I





The infrared spectrum of the tetrahydroflavin **7** (isomeric with **6**) contained a sharp band at 3333 cm^{-1} (ν_{NH}) and strong bands at 1701 and 1661 cm^{-1}, which are interpreted as 5,6-dihydropyrimidine ring carbonyl absorptions.[10] The nmr spectrum (60 Mc, deuteriochloroform) showed an AB quartet (J = 4.0 cps) with resonances centered at 4.54 and 4.15 ppm. Although the positions of the carbonyl absorptions contained in the infrared spectra of **6** and **7** established a radical difference in structural features, additional chemical evidence was desired to preclude the possibility of **6** and **7** being *cis*- and *trans*-tetrahydroflavins.

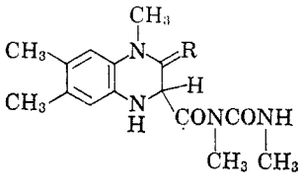
The tetrahydroflavin **7** (stereochemistry unknown), in contrast to the spirohydantoin **6**, was promptly dehydrogenated by chloranil to 1,3-dimethyllumichrome **8**, as evidenced by thin layer chromatography (tlc). It was subsequently found that the spirohydantoins **4** and **6**[11] underwent reaction at room temperature when exposed to a solution of sodium borohydride in absolute methanol. The analytical data and integration of the nmr spectrum of **10** indicated that the products contained two more hydrogen atoms. The infrared spectra (KBr disks), which contained two bands in the 3225–3350-cm^{-1} region and only intense, broad bands at 1660–1680 cm^{-1} in the carbonyl region, clearly indicated that reduction occurred in the hydantoin ring. Ring open products of type **14** have been eliminated on the evidences of the infrared spectra (*e.g.*, the spectra of **9** and **10**), which did not contain absorptions characteristic of amide II bands[12] and the nmr spectrum (*e.g.*, the nmr of **10**), which did not contain signals characteristic of an ABX spin system.[13] Alternative products of type **15** have also been precluded on the bases of these infrared spectra (absence of amide II bands) and the nmr spectrum (absence of a signal char-

(10) M. Horák and J. Gut, *Collection Czech. Chem. Commun.*, **26**, 1680 (1961).

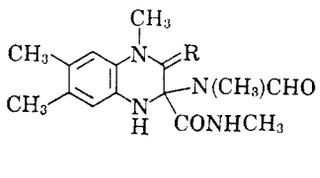
(11) The spirohydantoin **5** also reacted with sodium borohydride, but characterization of the product was unintentionally overlooked by K. H. D.

(12) L. J. Bellamy, "The Infrared Spectra of Complex Molecules," John Wiley and Sons, Inc., New York, N. Y., 1958, pp 216–221.

(13) The nmr spectrum of 2-phenyl-1,2,3,4-tetrahydroquinoxaline contained an ABX system; see J. Figueras, *J. Org. Chem.*, **31**, 803 (1966).



14, R = O, H_2

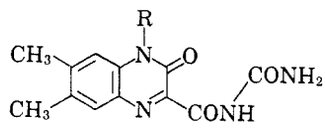


15, R = O, H_2

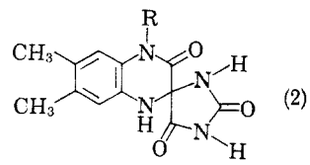
acteristic of a formyl proton). The nmr spectrum of **10** (100 Mc, deuteriochloroform) contained an AB quartet centered at 3.04 and 3.75 ppm (two protons assigned to H_a and H_b, J = 11.0 cps), the downfield doublet of which showed line broadening (presumably due to a long-range interaction with H_d). Other pertinent resonances were observed at 3.93 ppm (broad singlet, two protons assigned to H_d and H_e) and 4.78 ppm (doublet, one proton assigned to H_c and coupled with H_d, J = 3.5 cps). Deuterium exchange caused the disappearance of the broad singlet at 3.93 ppm, collapse of the doublet at 4.78 ppm to a sharp singlet, and sharpening of the doublet (centered at 3.75 ppm) of the AB quartet. The appearance of a singlet at 4.79 ppm and sharpening of the AB quartet (signal at 3.75 ppm) also resulted from spin–spin decoupling of the broad, two-proton signal at 3.93 ppm. The structures **9** and **10** have been assigned on the basis of this spectrum. The observed carbonyl absorptions (infrared spectra, 1660–1680 cm^{-1}) are in accord with the spiroimidazoline-2-one assignment,[7] but the absorptions at 3290 and 3350 cm^{-1}, which have been assigned to ν_{OH} of **9** and **10**, respectively, are unusually low.

The Ureides.—This class of compounds has been studied extensively by Clark-Lewis,[9,14] whose work led to the acceptance of the ureide structure in preference to that of the alloxan–anil.[15] As early as 1945, Tishler, *et al.*,[16] obtained a white compound (not analyzed), mp 148°, by dissolving the ribityl ureide **17** in dilute alkali, keeping this solution at room temperature for a few minutes, and reacidifying. Upon boiling the uncharacterized product, mp 148°, in water, the Tishler group isolated another substance having mp 218° (*Anal.* Found: C, 50.56; H, 5.71; N, 13.80.). Although we could anticipate the fate of the ureide **17** in dilute alkali,[9] this latter conversion to the compound melting at 218° was especially provocative, since the spirohydantoins **4**, **5**, and **6** were observed stable to such conditions. In fact, compound **4** could be recrystallized from water or 2 *N* perchloric acid, while compound **5**, insoluble in water, formed a crystalline perchlorate salt (ν_{CO} 1786 and 1724 cm^{-1}).

We reinvestigated the observations of the Tishler group and have found that alkaline solutions of **16** and **17**, upon acidification, yield the white spirohydantoins **18** and **19**, respectively (eq 2). In each case the analytical data indicated the presence of one water of hydration. The infrared spectra of **18** and **19** contained bands (1775–1765- and 1740–1730-cm^{-1} regions) assignable to carbonyl groups of hydantoin systems. Regarding the observed lability of **19**,[16] we have found that the spirohydantoins **18** and **19** undergo ring opening in hot 50% aqueous acetic acid to give the parent ureides



16, R = CH_3
17, R = ribityl



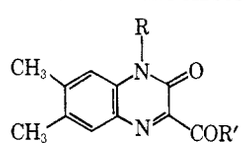
(2)

18, R = CH_3
19, R = ribityl

16 and **17**. Inasmuch as this reverse ring-opening reaction in neutral or acidic media conflicted with the chemical properties of **4**, **5**, and **6**, we examined more closely the infrared and visible light absorption spectra of the ureides **16** and **17**.

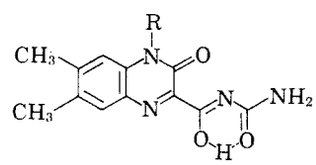
The infrared spectrum (KBr disk) of ureide **16** contained a sharp intense band at 3413 cm^{-1} (assignable to an intramolecular hydrogen-bonded hydroxyl group)[17] a medium band at 3268 cm^{-1} (ν_{NH}), and carbonyl absorptions at 1718 and 1689 cm^{-1}; the intensities of the 3413-, 1718-, and 1689-cm^{-1} absorptions were approximately equal. The light absorption spectra of the ureides **16** and **17** in methanol showed a 16-mμ bathochromic shift of the long wavelength band when compared with the spectra of the derivatives **20**, **21**, and **22** (Table I). In the light of these physical data, we

TABLE I
ULTRAVIOLET AND VISIBLE LIGHT ABSORPTION MAXIMA

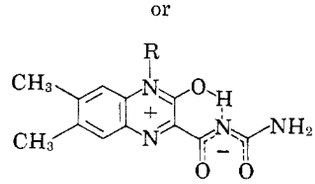


Compd	R	R′	λ_{max}^{MeOH}, mμ	$\lambda_{max}^{CHCl_3}$, mμ
20	Ribityl	NH_2	386, 316	390, 320
21	Ribityl	$NHNH_2$	386, 318	
22	CH_3	$NHNH_2$	386, 318	389, 320
16	CH_3	$NHCONH_2$	402, 326	
17	Ribityl	$NHCONH_2$	402, 326	408, 332

feel that the ureides **16** and **17** are better expressed as the iminol tautomers **16′** and **17′** and suggest that the



or



16′, R = CH_3
17′, R = ribityl

(14) R. M. Cresswell, A. C. Hill, and H. C. S. Wood, *J. Chem. Soc.*, 698 (1959).
(15) G. W. H. Cheeseman, *Advan. Heterocyclic Chem.*, **2**, 203 (1963).
(16) M. Tishler, J. W. Wellman, and K. Ladenburg, *J. Am. Chem. Soc.*, **67**, 2165 (1945).
(17) M. Tichy, *Advan. Org. Chem.*, **5**, 115 (1965).

pH-dependant stabilities of the hydrogen bonds are the important factors in the observed spirohydantoin–ureide interconversions. Accordingly then, the failure of the spirohydantoins **4**, **5**, and **6** to undergo similar ring-opening reactions in neutral or acidic media may be attritubed to N-alkylation in the hydantoin ring, which prohibits formation of such iminol tautomers for stabilizing the ureide forms.

Experimental Section[18]

The abbreviations A, B, C, etc., represent the solvent systems employed for elution of thin layer chromatograms: solvent system A, benzene–isopropyl ether–chloroform (4:4:1); B, benzene–isopropyl ether–chloroform (2:2:1); C, benzene–isopropyl ether–ethanol (4:4:0.25); D, benzene–isopropyl ether–ethanol (4:4:1); E, benzene–isopropyl ether–ethanol (2:2:1); F, *n*-butyl alcohol–acetic acid–water (6:2:2).

1′,4′-Dihydro-1,3,4′,6′,7′-pentamethylspiro[imidazolidine-4,2′(3′H)-quinoxaline]-2,3′,5-trione (4).—To a stirred suspension of 200 mg of flavinium perchlorate **3**[6] in 5.0 ml of water was added 40 mg of potassium cyanide. After 1 hr, another 40 mg of potassium cyanide was added, the mixture diluted to 10 ml, and stirring continued for an additional 2 hr. The white crystalline product (130 mg, mp 202–210°) was collected, washed with water, and vacuum dried. The compound crystallized from ethanol–water as a mixture of needles and prisms, mp 202–204°, and was once obtained by recrystallization from water as a pure needle form, mp 184–185°. The infrared spectra of the two samples were identical and an admixture gave mp 184–185°. When a sample was recrystallized from 2 *N* perchloric acid, the infrared spectrum showed no change. The compound moved as one zone (developed with iodine vapors) in solvent systems B, C, and D. Infrared bands appeared at 3300, 1762, 1720, 1632, 1610 (w), and 1520 cm^{-1}; ultraviolet, λ_{max}^{MeOH} 226 mμ (ε 23,600), 308 (4800); nmr (60 Mc, deuteriochloroform), two singlets at δ 6.76 (1) and 6.56 (1) assigned to two aromatic ring H, a broad singlet at 4.90 (1) assigned to N–H, three singlets at 3.45 (3), 3.02 (3), and 2.83 (3) assigned to three N–CH_3, and two singlets at 2.21 and 2.17 (6) assigned to two aromatic ring CH_3.

Anal. Calcd for $C_{15}H_{18}N_4O_3$ (302.3): C, 59.6; H, 6.0; N, 18.5. Found: C, 59.4; H, 6.1; N, 18.6.

3′,4′-Dihydro-3′-imino-1,3,4′,6′,7′-pentamethylspiro[imidazolidine-4,2′(1′H)-quinoxaline]-2,5-dione (5).—A suspension of 400 mg of **3**[6] in 50 ml of absolute ethanol was treated briefly with dry ammonia until a clear yellow-orange solution formed. Ammonium chloride (5 mg) was suspended in the solution and the mixture kept at 0°. After 1 day white crystals had begun to separate; after 2 days the precipitate had acquired a pink color and was collected. The compound was recrystallized from ethanol: yield, 160 mg; mp 242–244°. A chromatogram (solvent system B) showed one zone (developed with iodine vapors). Infrared bands appeared at 3300, 3200, 1761, 1725, 1625, and 1590 cm^{-1}; ultraviolet, λ_{max}^{MeOH} 230 mμ (ε 22,000), 258 (13,900), 306 (4600).

Anal. Calcd for $C_{15}H_{19}N_5O_2$ (301.3): C, 59.8; H, 6.4; N, 23.2. Found: C, 60.0; H, 6.2; N, 23.1.

A perchlorate salt, mp 261° (decomposed with gas evolution), was prepared by recrystallizing compound **5** from 2 *N* perchloric acid. Infrared bands appeared at 1786, 1724, and 1669 cm^{-1}.

Anal. Calcd for $C_{15}H_{20}ClN_5O_6$ (383.3): C, 44.8; H, 5.0; N, 17.4. Found: C, 44.7; H, 5.0; N, 17.2.

3′,4′-Dihydro-1,3,4′,6′,7′-pentamethylspiro[imidazolidine-4,2′(1′H)-quinoxaline]-2,5-dione (6).—To a well-stirred suspension of 900 mg of **3** in 50 ml of water were added, intermittently, very small portions of sodium borohydride. Each addition of borohydride caused a transient orange color and coagulation of the starting material at the surface of the solution. Small volumes of ethanol (total volume, 30 ml) were added concurrently with the borohydride to disperse coagulated material and to reduce the foaming. The pH of the solution never became higher than 6. During the additions of borohydride, white crystals (clusters of small needles) separated and the point of complete solution of **3** was determined by microscopic examination of the nature of the suspension. To the finished reaction was added 30 ml of water and the suspension allowed to stand at 0° for 1 hr. The collected product was washed with water. The compound crystallized from approximately 12 ml of methanol (first at 25°, then at −20°) as beautiful elongated needles (350 mg), mp 202–204°, which moved as one zone (developed with iodine vapors) in solvent system C. Infrared bands appeared at 1763, 1710, 1620 (w), and 1510 cm^{-1}; ultraviolet, λ_{max}^{MeOH} 222 mμ (ε 34,200), 312 (8100); λ_{sh}^{MeOH} 250 mμ (ε 9350); nmr (60 Mc, benzene-d_6, 65°); two singlets at δ 6.35 (1) and 6.20 (1) assigned to two aromatic ring H, broad peak at 3.40 (1) assigned to NH, a doublet (J = 11.5 cps) centered at 3.05 assigned to one nonequivalent methylene proton, two singlets at 2.90 (3) and 2.77 (3) assigned to two –NCH_3, a doublet (J = 11.5 cps) centered at 2.48 one peak of which was obscured by a –NCH_3 singlet at 2.38 (4), and two singlets at 2.14 (3) and 2.10 (3) assigned to two aromatic –CH_3. When the spectrum (60 Mc) was measured in deuteriochloroform, only four sharp signals were seen. These were found at δ 6.48 and 6.42 (two aromatic ring H) and 3.07 and 3.03 (two –NCH_3). A very broad NH resonance was found at 4.21. Very suppressed broad peaks were seen at 3.24, 2.98, and 2.12 and assigned to –NCH_2–, –NCH_3, and two aromatic ring –CH_3, respectively.

Anal. Calcd for $C_{15}H_{20}N_4O_2$ (288.3): C, 62.48; H, 6.99; N, 19.43; NCH_3, 15.63. Found: C, 62.45; H, 6.94; N, 19.55; NCH_3, 16.05.

The Tetrahydroflavin 7.—A total of 300 mg of sodium borohydride was added in three portions to a suspension of 400 mg of **3**[6] in 20 ml of absolute methanol. The dissolution of **3** was followed almost immediately by the separation of a white crystalline product. The reaction mixture was diluted with 15 ml of methanol and heated under reflux for 15 min. After cooling to room temperature, the mixture was diluted with 40 ml of water and allowed to stand at 0° for 1.5 hr; the product (200 mg), mp 223–228°, was collected and dried. A sample recrystallized from methanol gave a sharp mp 223–224°. When a colorless solution of the compound in chloroform was applied to a chromatogram and dried at room temperature, the spot turned orange, but had no fluorescence under the ultraviolet lamp. After elution in solvent system C, the (single) zone was bright yellow and still nonfluorescent. The yellow color became more intense upon spraying with 6 *N* hydrochloric acid–hydrogen peroxide reagent and then provided a blue fluorescence when examined under the ultraviolet lamp. Infrared bands appeared at 3333, 1701, 1661, 1608 (m), and 1575 (vw) cm^{-1}. In contrast to the infrared spectra of all other compounds in this study, this spectrum contained a well-resolved ν_{CH} region, in order of decreasing intensity, at 2900, 2850, 2940 and 2870, 2780, and 3010 cm^{-1}. The ultraviolet spectra showed λ_{max}^{MeOH} 218 mμ (ε 27,500), 310 (4450); λ_{sh}^{MeOH} 248 (5500); nmr (60 Mc, deuteriochloroform), a singlet at δ 6.48 (2) assigned to two aromatic ring H, a doublet (J = 4.0 cps) centered at 4.52 (1) assigned to –NCHCO–, a doublet (J = 4.0 cps) centered at 4.14 (1) assigned to –NCHN–, a very broad singlet at 3.77 (1) assigned to NH, three singlets at 3.26 (3), 3.16 (3), and 2.81 (3) assigned to three –NCH_3 groups, and a singlet at 2.12 (6) assigned to two aromatic ring –CH_3 groups.

Anal. Calcd for $C_{15}H_{20}N_4O_2$ (288.3): C, 62.48; H, 6.99; N, 19.43. Found: C, 62.56; H, 6.92; N, 19.58.

Dehydrogenations of the Spirohydantoin 6 and the Tetrahydroflavin 7. A. Dehydrogenation with Chloranil.—Samples (8–10 mg) of **6** and **7** were each mixed with 10 mg of freshly recrystallized chloranil in 2.5 ml of toluene containing 1 drop of acetic acid and the resulting mixtures heated under reflux (oil bath). For establishing the identities of the reaction products, authentic samples were simultaneously eluted (tlc) and the mobilities, fluorescing properties, and reactivity toward developing agents compared. After 1.5 hr, a chromatogram (solvent system C) of the reaction solution prepared from the tetrahydroflavin **7** showed a predominant zone of dimethyllumichrome **8** (blue fluorescence under ultraviolet source) and two unidentified very weak fluorescing zones (pale yellow and pale blue) near the origin. Leuco compounds were not present, as evidenced by treatment of the chromatogram with 1:1 6 *N* hydrochloric acid–3% hydrogen peroxide solution. After 2.0 hr, a chromatogram (solvent system C) of the reaction solution prepared from the spirohydan-

(18) Melting points (uncorrected) were determined by the capillary method using a copper block. Infrared spectra were recorded using a Beckman IR-8 spectrophotometer; samples were prepared in the form of pressed KBr disks. Ultraviolet and visible light absorptions curves were measured on a Beckman DB recording spectrophotometer. Nmr spectra were determined on Varian Models A-60 and A-100 using tetramethylsilane (TMS) as an internal standard; chemical shift values are expressed as δ (ppm). Thin layer chromatography plates were coated with MN-silica gel S, which contains a starch binder.

toin **6** showed a weak but definite zone of **8** and a faster moving, unidentified weak purple fluorescing zone. Treatment of the chromatogram with 1:1 6 *N* hydrochloric acid–3% hydrogen peroxide developed a characteristic bright yellow, nonfluorescing zone of **6**.

B. Dehydrogenation with Sulfur.—Samples of **6** and **7** were intimately mixed with sulfur, fused at ~175° for 30 min, and then the temperature was raised to ~200°. The mixtures were cooled, dissolved in chloroform, and examined chromatographically. A chromatogram of the solution prepared from the fusion mixture of **7** contained a zone of **8** (established using solvet system A), 3-methyllumiflavin (established using solvent system F), and an unidentified green-yellow fluorescing zone (seen using solvent system F). A chromatogram (solvent system A) of the solution prepared from the fusion mixture of **6** showed a very weak zone of **8**, an unidentified strong purple fluorescing zone near the origin, and an unidentified orange fluorescing zone at the origin.

1′,4′-Dihydro-5-hydroxy-1,3,4′,6′,7′-pentamethylspiro[imidazolidine-4,2′(3′H)-quinoxaline]-2,3′-dione (9) (Tentative Structure).—The spirohydantoin **4** (250 mg) was dissolved in 5 ml of warm methanol, treated with a total of 100 mg of sodium borohydride, and the mixture kept at room temperature. After 10 min, tlc (solvent system C) indicated complete reaction of **4**. The product was induced by scratching to crystallize, 5.0 ml of water was added, and the product (220 mg, chromatographically pure in solvent system E) was collected. After recrystallization from methanol, the compound gave mp 220–222° (to a red oil); infrared bands at 3290, 3260, 1680, 1620 (vw), and 1508 cm^{-1}.

Anal. Calcd for $C_{15}H_{20}N_4O_3$ (304.3): C, 59.19; H, 6.62; N, 18.41. Found: C, 59.02; H, 6.76; N, 18.43.

3′,4′-Dihydro-5-hydroxy-1,3,4′,6′,7′-pentamethylspiro[imidazolidine-4,2′(1′H)-quinoxalin]-2-one (10) (Tentative Structure).—The spirohydantoin **6** (150 mg) was dissolved in 6.0 ml of warm methanol, supercooled to room temperature, and 25 mg of sodium borohydride was added. The reaction was monitored by tlc (solvent system C) and after 45 min the solution was filtered, using 2.0 ml of methanol to complete the transfer. The filtrate was diluted with 8.0 ml of water and crystallization of the product was induced by scratching. The product separated as well-defined needles (93 mg), mp 198–200°, and moved as one zone (developed with iodine vapors) in solvent system E. The compound decomposed in chloroform–isopropyl ether (attempted recrystallization) and was also sensitive to "peroxide-containing" chromatographic solvents. For analysis the compound was recrystallized from methanol–water: infrared bands at 3350, 3333, broad maximum from 1680 to 1660, 1618 (vw), and 1498 cm^{-1}. In addition to the signals mentioned in the text for the nmr spectrum (100 Mc, deuteriochloroform), singlets for the three $-NCH_3$ groups were observed at δ 2.84, 2.83, and 2.74 (3 each). The two aromatic ring $-CH_2$ groups were seen at 2.16 and 2.11 (3 each) and the ring protons at 6.47 and 6.40 (1 each). In the deuterium-exchange experiment, the water peak appeared at 4.65 and corresponded to two protons.

Anal. Calcd for $C_{15}H_{22}N_4O_2$ (290.4): C, 62.04; H, 7.64; N, 19.30. Found: C, 61.74; H, 7.72; N, 19.32.

1,3,10-Trimethylalloxazinium Perchlorate (11).—A solution of 1.0 g of 5-acetyl-1,3,10-trimethylleucoalloxazine[19] in 25 ml of ethanol was filtered into a solution of nitrous acid prepared by adding 1.0 g of sodium nitrite to 25 ml of 2 *N* perchloric acid. A deep yellow solution formed immediately and the product was induced to crystallize by scratching. The mixture was allowed to stand overnight at 0°; the product (950 mg), mp 256–258°, was collected, washed sucessively with water, ethanol, and ether, and vacuum dried. The compound moved as one zone in solvent system F: infrared bands appeared at 1730, 1672, 1605, 1585, and 1567 cm^{-1}.

Anal. Calcd for $C_{13}H_{13}ClN_4O_6$ (356.7): C, 43.77; H, 3.67; N, 15.71. Found: C, 43.64; H, 3.55; N, 15.74.

1′,4′-Dihydro-1,3,4′-trimethylspiro[imidazolidine-4,2′(3′H)-quinoxaline]-2,3′,5-trione (12).—To a stirred suspension of 200 mg of the alloxazinium salt **11** in 5.0 ml of water was added 30 mg of potassium cyanide and the product (100 mg) collected after 1.25 hr. After recrystallization from water, the compound gave mp 155–157° or 170–171° (Kofler hot stage). If the compound was recrystallized from methanol, it gave mp 188–190° and did not depress the melting point of **12**, mp 188–190° (lit.[9a] mp 194°), prepared by the method of Clark-Lewis.[9a] The compounds were identical by comparison of infrared spectra and by tlc (solvent system B, developed with iodine vapors).

Anal. Calcd for $C_{13}H_{14}N_4O_3$ (274.3): C, 56.93; H, 5.15; N, 20.43; O, 17.50. Found: C, 56.84; H, 5.27; N, 20.76; O, 17.38.

4,6,7-Trimethyl-3-oxoquinoxaline-2-carboxyureide (16).—A suspension of 2.0 g of N-3,4-trimethyl-6-(*p*-carboxyphenylazo)-aniline[20] in 100 ml of ethanol was hydrogenated at atmospheric pressure over palladium on charcoal. After consumption of 2 moles of hydrogen, the light brown solution was filtered into a suspension of 2.0 g of alloxan hydrate in 50 ml of water. Bright yellow needles of the ureide began separating immediately. After 1.5 hr at room temperature, the solution was filtered and the product washed thoroughly and successively with water, ethanol, and ether. The compound (1.2 g) was contaminated with a small quantity of lumiflavin (tlc, solvent system F). After recrystallization from glacial acetic acid, the product was uniform and gave mp 245–255° (lit.[21] mp 251–252°); infrared bands at 3413, 3268, 1718, 1689, 1638, 1618, and a doublet at 1591 and 1580 cm^{-1}; ultraviolet, λ_{max}^{MeOH} 218 mμ (ϵ 24,000), 240 (20,800), 326 (9500), 402 (6500).

Anal. Calcd for $C_{13}H_{14}N_4O_3$ (274.3): C, 56.9; H, 5.2; N, 20.4. Found: C, 56.8; H, 5.0; N, 19.4, 19.2.

4-Ribityl-6,7-dimethyl-3-oxoquinoxaline-2-carboxyureide (17).—From hydrogenation of 2.0 g of freshly recrystallized N-ribityl-3,4 dimethyl-6-(*p*-carboxyphenylazo)aniline in 100 ml of ethanol, and following the procedure for **16** and isolating the product after the mixture had been kept at 0° for 18 hr, there was obtained 1.6 g, mp 225–240° after recrystallization from 50% aqueous acetic acid (lit.[16] mp 237–240°), of chromatographically pure **17** (solvent system F). The infrared spectrum contained an intense carbonyl absorption at 1689 cm^{-1}, which was two times the intensities of other bands in the 1500–1600-cm^{-1} region; ultraviolet, λ_{max}^{MeOH} 216 mμ (ϵ 23,800), 240 (20,400), 326 (9600), 402 (6550).

Anal. Calcd for $C_{17}H_{22}N_4O_7$ (394.4): C, 51.8; H, 5.6; N, 14.2. Found: C, 52.1; H, 5.7; N, 14.2.

1′,4′-Dihydro-4′,6′,7′-trimethylspiro[imidazolidine-4,2′(3′H)-quinoxaline]-2,3′,5-trione (18).—To a stirred suspension of 130 mg of **16** in 20 ml of 1:1 ethanol–water was added 1 ml of 2 *N* sodium hydroxide. Within a few minutes a clear yellow solution formed, which was quickly filtered and acidified with drops of glacial acetic acid until a white gelatinous substance began to separate. Separation of the product (noncrystalline under the microscope) was complete within 5 min. The product was filtered and washed free of its yellow color with water and then carefully with small amounts of methanol and ether. The product (applied as its dilute ammonia solution) moved as one zone (developed with iodine vapors) in solvent system F. After recrystallization from methanol–water, the substance amounted to 70 mg, mp 236–244°. During drying (24 hr at 80° (0.08 mm)) for analysis, the sample acquired a yellow color which proved (tlc) to be due to the reversal of the compound to its parent ureide **16**. Development of the chromatogram (iodine vapors) showed, however, that the sample consisted mostly of the spirohydantoin **18**: infrared bands appeared at 3333, 3205, 1769, 1730, 1636, and 1612 cm^{-1}; ultraviolet, λ_{max}^{MeOH} 226 mμ (ϵ 26,800), 306 (6400).

Anal. Calcd for $C_{13}H_{14}N_4O_3 \cdot H_2O$ (292.3): C, 53.4; H, 5.5; N, 19.2. Found: C, 53.8; H, 5.2; N, 19.4.

A sample of the spirohydantoin **18** was suspended in 1 ml of glacial acetic acid and brought to boiling. The dissolution of **18** was accompanied by the formation of a deep yellow color. After 1 hr at room temperature, tlc (solvent system F) showed that **18** had been completely converted to the ureide **16**, which was isolated by dilution of the acetic acid solution with water and compared by mixture melting point behavior. This reverse conversion to **16** could also be observed by diluting a stock solution of **18** in methanol with 2 *N* hydrochloric acid and measuring the light absorption spectrum.

1′,4′-Dihydro-6′,7′-dimethyl-4′-(ribo-2,3,4,5-tetrahydroxypentyl)spiro[imidazolidine-4,2′(3′H)-quinoxaline]-2,3′,5-trione (19).—A suspension of 120 mg of the ureide **17** in 10 ml of water was treated with 1 ml of 2 *N* sodium hydroxide, the mixture held until a yellow solution formed, and then the product was isolated as described in the procedure for **18**. The white noncrystalline

(19) P. Hemmerich, B. Prijs, and H. Erlenmeyer, *Helv. Chim. Acta*, **43**, 372 (1960).

(20) P. Hemmerich, S. Fallab, and H. Erlenmeyer, *ibid.*, **39**, 1242 (1956).

(21) R. Kuhn and K. Reinemund, *Ber.*, **67**, 1932 (1934).

product (50 mg), double mp 160–170° and 210–215° (lit.[16] mp 148°), was chromatographically uniform in solvent system F (developed with iodine vapors) when applied as its dilute ammonia solution. The compound developed a yellow color when dried for analysis; infrared bands appeared at 3333 (protruding from the intense hydroxyl absorption), 1773, 1739, 1644, and 1618 cm^{-1}.

Anal. Calcd for $C_{17}H_{22}N_4O_7 \cdot H_2O$ (412.8): C, 49.5; H, 5.9; N, 13.6. Found: C, 49.8; H, 5.6; N, 13.8.

A sample of **19** (50 mg) in 10 ml of water was heated to boiling and, upon dissolution of **19**, an intense yellow solution formed, which was filtered free of a very small amount of insoluble material. The filtrate was boiled for 5 min and cooled; the yellow crystalline product (45 mg), mp 225–243° (lit.[17] mp 218°), was collected and dried. The compound was chromatographically pure and identical with **17** in solvent system F and did not depress the melting point of **17**. The same results were obtained employing a 50% aqueous acetic acid solution.

4-Ribityl-6,7-dimethyl-3-oxoquinoxaline-2-carboxamide (20).—Ethyl 4-ribityl-6,7-dimethyl-3-oxaquinoxaline-2-carboxylate[16] (500 mg) was dissolved in 30 ml of concentrated ammonia; the solution was filtered and let stand at room temperature for 1 hr. The copious crop of pale yellow needles (300 mg, chromatographically pure in solvent system F), mp 209–211°, was collected and washed successively with water, ethanol, and ether; an infrared band appeared at 1667 cm^{-1}.

Anal. Calcd for $C_{16}H_{21}N_3O_6$ (351.4): C, 54.7; H, 6.0; N, 12.0. Found: C, 54.8; H, 6.2; N, 11.8.

4-Ribityl-6,7-dimethyl-3-oxoquinoxaline-2-carboxyhydrazide (21). A.—A solution of 250 mg of the ureide **17** in 1.5 ml of anhydrous hydrazine was heated at 95° for 5 min and diluted with water; the pale yellow needles were collected and recrystallized from water: yield, 70 mg; mp 230–236°.

B.—A solution of ethyl 4-ribityl-6,7-dimethyl-3-oxoquinoxaline-2-carboxylate[16] (300 mg) in 2.0 ml of anhydrous hyrazine was warmed at 60–70° for 1 min and diluted with water; the product (260 mg, chromatographically pure, solvent system F) was collected and washed well with water and then with methanol and ether. The analytical sample, mp 231–237°, was prepared by recrystallization from water; an infrared band appeared at 1661 cm^{-1}.

Anal. Calcd for $C_{16}H_{22}N_4O_6 \cdot H_2O$ (384.4): C, 50.00; H, 6.3; N, 14.6. Found: C, 50.00; H, 6.4; N, 14.7.

4,6,7-Trimethyl-3-oxoquinoxaline-2-carboxyhydrazide (22).—A suspension of 100 mg of the ureide **16** in 1 ml of anhydrous hydrazine was heated (oil bath) during 15 min to 115° and then held at 115° for 20 min. (A copious crop of yellow needles separated as the temperature reached 100°.) The mixture was allowed to cool, 10 ml of water added, and the product (70 mg) collected and washed with water. The compound was chromatographically pure (solvent system F) and the analytical sample, mp 273–276°, was prepared by recrystallization from water; an infrared band appeared at 1661 cm^{-1}.

Anal. Calcd for $C_{12}H_{14}N_4O_2$ (246.3): C, 58.51; H, 5.73; N, 22.75. Found: C, 58.60; H, 6.00; N, 23.32.

Comments on the "Unnatural Reactivity" of the Isoalloxazine System.—As we have noted previously,[6] the flavin system (7,8-dimethylisoalloxazine) and the isoalloxazine system often do not exhibit the same chemical properties. One very striking example was observed during the course of this study. When the alloxazinium salt **11** was subjected to treatment with sodium borohydride under each set of conditions required for the preparations of **6** and **7**, the isolated products were found to be identical. Although this product has not been throughly investigated (*i.e.*, nmr and dehydrogenation experiments), it is tentatively regarded as a tetrahydroisoalloxazine of the nature of **7**.

The Sodium Borohydride Reduction of 11. A.—A suspension of 0.3 g of **11** in 5 ml of methanol was treated with two 0.1 g-portions of sodium borohydride. After the vigorous reaction had subsided and a white crystalline product had separated, the mixture was diluted with an equal volume of water and the product collected and recrystallized from ethanol (yield, 0.12 g); the compound softens at 210° and fuses at 213–216°. The compound exhibited chromatographic properties like those of **7**. Infrared bands appeared at 1718 and 1709 (doublet), 1611 and 1587 (doublet), and a strong band at 1661 cm^{-1}. The ν_{CH} region was not well defined, but one sharp ν_{NH} was seen at 3333 cm^{-1}.

Anal. Calcd for $C_{13}H_{16}N_4O_2$ (260.3): C, 59.98; H, 6.20; N, 21.53. Found: C, 60.07; H, 6.34; N, 21.77.

B.—From attempts to prepare a spirohydantoin of type **6**, there were isolated only small amounts of the compound described immediately above under procedure A. Thus, from 0.28 g of **11** suspended in 15 ml of water and by adding small quantities of borohydride with precaution as described under the procedure for **6**, there was obtained 20 mg of product, which was identical with that obtained from procedure A by comparison of infrared spectra, melting point, and R_f values in solvent system C (zones further developed with iodine vapors).

Registry No.—**4**, 13681-51-1; **5**, 13641-01-5; **5** perchlorate, 13698-39-0; **6**, 13641-02-6; **7**, 13641-08-2; **9**, 13641-09-3; **10**, 13698-41-4; **11**, 13641-10-6; **12**, 13641-11-7; **16**, 13641-12-8; **17**, 13641-13-9; **18**, 13641-14-0; **19**, 13717-34-5; **20**, 13698-42-5; **21**, 13698-43-6; **22**, 13641-15-1.

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