this kind may help explain the nature of reactions in frozen systems, which may, in turn, provide clues for explaining complex reactions in restricted biological systems. Moreover, since several bimolecular organic transformations in ice appear to be new examples of solid-state reactions, their characterization will require understanding of the reactant and product diffusion processes and the role of crystal imperfections, e.g., lattice faults, vacant sites, and channels.

Several possible explanations have been offered for the enhanced reaction rates in ice: increased proton mobility,[1] imposition of a favorable positional orientation between reactants,[1] and participation of the ice crystal surface as replacement for a catalyst molecule.[5] Another important factor may be dielectric behavior, the dielectric constant of ice being markedly lower than that of water. This could promote aggregation of the catalyst through hydrogen bonding, resulting in a concerted attack such as found with covalently linked imidazole residues.[13]

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Isotuboflavine and Norisotuboflavine. Two New Alkaloids Isolated from *Pleiocarpa mutica* Benth.[1]

H. Achenbach and K. Biemann

Contribution from the Department of Chemistry, Massachusetts Institute of Technology, Cambridge, Massachusetts. Received April 29, 1965

Three new β-carboline derivatives were isolated from the stem bark of Pleiocarpa mutica Benth. One of them was identified as 1-carbomethoxy-β-carboline. For the other two, named isotuboflavine and norisotuboflavine, structures VIII and VII, respectively, were deduced, mainly on the basis of mass spectrometric evidence.

Since the reports[2-4] of the occurrence of hypotensically active alkaloids in the roots of *Pleiocarpa tubicina* Stapf and in the leaves, roots, and seeds of *Pleiocarpa mutica* Benth. these plants have been investigated in various laboratories[5-15] particularly by Schmid, *et al.*[5-12] During the past few years, more than 20 indole alkaloids have been isolated from these sources and the structures of most of them were determined.[6-13] The majority of the alkaloids possess the aspidofractine skeleton and the most prominent representative of this group is pleiocarpine (I).[6] In addition to these hexacyclic alkaloids there occur others, some of which are

also found in other *Apocynaceae*, such as 1,2-dehydroaspidospermidine,[10] quebrachamine,[10] eburnamine,[5] and related compounds as well as alkaloids[12] with the akuammicine–condylocarpine carbon skeleton. Most recently[8] pleiocarpamine had been shown to represent a variant of the general pattern of indole alkaloids.

In addition to these indole and dihydroindole derivatives, two β-carboline alkaloids had been isolated: *P. mutica* gave flavocarpine (II),[13] and from the root bark of *P. tubicina* a very small amount of tuboflavine (III)[11] was obtained.

In the course of a detailed investigation of the alkaloids present in an extract of the stem bark of *P. mutica* we have isolated a few milligrams of three additional β-carboline alkaloids. The determination of their structures will be discussed in this paper.

On repeated careful chromatography of the crude extracts on alumina and silicic acid, four optically inactive ultraviolet fluorescing substances were isolated. One of these was identified as tuboflavine (III)[11] on the basis of spectral data and its melting point. Another component which also exhibited the typical ultraviolet spectrum of a β-carboline had a molecular weight of 226 and the infrared, n.m.r. and mass spectrum was most consistent with the presence of a carbomethoxy group. The compound was subsequently identified as 1-carbomethoxy-β-carboline (IV).[16] To our knowledge this compound has not yet been isolated from a natural source.

The two other substances had properties very similar to those of tuboflavine but did not seem to be identical with any alkaloid previously isolated from this plant material or any other source. Both were obtained in the form of yellow needles which melted at 282–284 and 262–264°, respectively. From a determination of the accurate mass of these compounds their elemental composition was deduced as $C_{15}H_{10}N_2O$ and $C_{16}H_{12}N_2O$,

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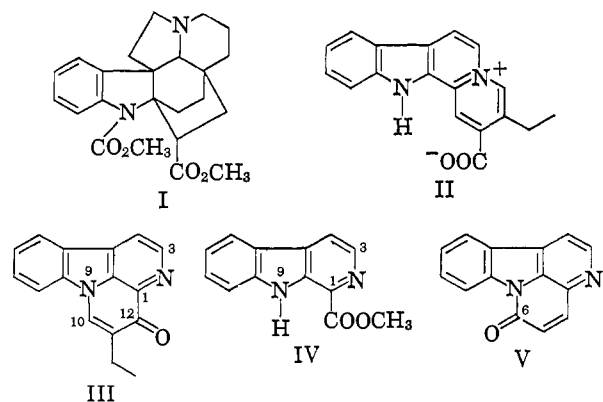(10) B. W. Bycroft, D. Schumann, M. B. Patel, and H. Schmid, *ibid.*, 47, 1147 (1964).

(11) C. Kump, J. Seibl, and H. Schmid, *ibid.*, 46, 499 (1963).

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(15) F. A. Hochstein and B. Korol, Abstracts of Papers, 140th National Meeting of the American Chemical Society, Chicago, Ill., Sept. 1961, p. 11-O.

(16) H. R. Snyder, H. G. Walker, and F. X. Werber, *J. Am. Chem. Soc.*, 71, 527 (1949).

I II III IV V

abundant ions corresponding to $C_{11}H_8N_2$ and $C_{11}H_6N_2$ indicates a carboline system in these new alkaloids. This assumption was confirmed by alkaline cleavage of the higher homolog (isotuboflavine) to β-carboline-1-carboxylic acid which also proves the position of the carbonyl group at C-9 and requires the presence of the structural element VI in both compounds. The remain-

VI

respectively.[17] From this difference in elemental composition as well as the fact that they exhibited identical ultraviolet spectra, very similar infrared spectra, and analogous fragmentation in the mass spectrometer (see Figures 1 and 2), one could conclude that these two alkaloids are homologs and they were thus named isotuboflavine and norisotuboflavine. The ultraviolet spectra are similar to that of tuboflavine (III) but show some distinct differences. Like III they show a bathochromic shift upon addition of acid.[11] It should be

ing three and four carbon atoms, respectively, are placed as shown in VII for norisotuboflavine and in VIII for isotuboflavine on the basis of the experiments discussed below.

The difference in the mass spectra of III (Figure 3) and VIII (Figure 2) is in agreement with a difference in the position of the ethyl group. While both compounds exhibit a loss of CO, indicating the presence of a cyclic carbonyl, they differ mainly in the abundance of the $M - CH_3$ ion as well as the mass of the most abundant $C_nH_mN_2$ fragment, which is $C_{13}H_8N_2$ in III and $C_{11}H_8N_2$ in VIII.

The loss of a methyl group from III leads to A with a positive charge which can be stabilized by the indole nitrogen while the analogous ion (B) from VIII is destabilized by the carbonyl carbon. The same argument
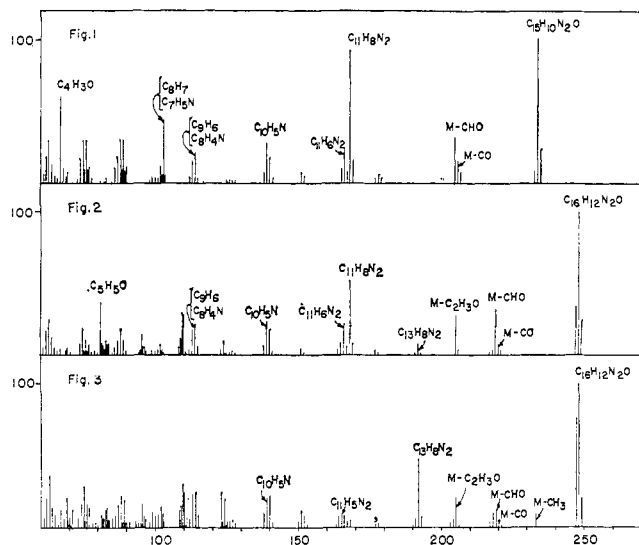
Fig. 1
Fig. 2
Fig. 3

Figure 1. Mass spectrum of norisotuboflavine (VII).
Figure 2. Mass spectrum of isotuboflavine (VIII).
Figure 3. Mass spectrum of tuboflavine (III).

noted that the ultraviolet spectrum is rather different from the isomeric canthinone chromophore (V).[20] The infrared spectra of both alkaloids exhibited the typical pyridone band at 1620 cm.$^{-1}$ which is present in the spectrum of III and the absence of bands in the –NH region is equally typical. In addition to these indications deduced from absorption spectra, the presence of

VII, R = H
VIII, R = CH$_3$

$(III)^+ \xrightarrow{-CH_3}$ A

$(VIII)^+ \xrightarrow{-CH_3}$ B

explains the difference in the M–H abundance. After the loss of CO, the loss of CH_3 leads to a $M - C_2H_3O$ ion which in either case is in resonance with the aromatic system and its abundance is thus of similar magnitude.

The most revealing aspect is the retention of two carbon atoms of ring D in III to lead to the $C_{13}H_8N_2$ ion. This ion is, on the other hand, very weak in VII and VIII, in favor of the abundant $C_{11}H_8N_2$ ion, which requires loss of ring D with transfer of two hydrogens.

The proximity of the alkyl group to the aromatic ring A in VII and VIII seems to be the reason for this difference, as it permits cleavage of the N-9-C-10 bond with transfer of hydrogen.
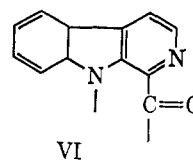
(17) The mass spectra of these compounds were determined both with a conventional, single-focusing instrument as well as with a high resolution mass spectrometer using techniques[18] which permit the determination of the elemental composition of all ions.[19] Those of importance for the present discussions are given in the text and figures.

(18) (a) P. Bommer, W. McMurray, and K. Biemann, and (b) D. Desiderio and K. Biemann, 12th Annual Conference on Mass Spectrometry and Allied Topics, Montreal, June 1964.

(19) K. Biemann, P. Bommer, and D. Desiderio, Tetrahedron Letters, No. 26, 1725 (1964).

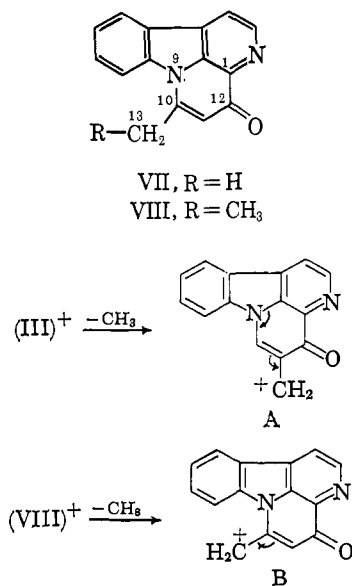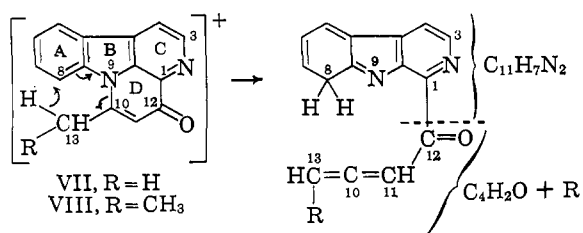(20) H. F. Haynes, E. R. Nelson, and J. R. Price, Australian J. Sci. Res., A5, 387 (1952).

$$\text{VII, R = H} \quad \text{VIII, R = CH}_3 \qquad \longrightarrow \qquad C_{11}H_7N_2$$

$$C_4H_2O + R$$

Rupture of the C-1–C-12 bond with rearrangement of a hydrogen atom (at C-11?) to the aromatic system leads then to the $C_{11}H_8N_2$ ion while simple cleavage of this bond with retention of the positive charge at the carbonyl group gives rise to C_4H_8O in VII and C_5H_5O in VIII.

The absence of an alkyl group at C-10 in III prevents the initial process and this molecule thus loses the ethyl group as C_2H_4 after loss of CO, to give a highly aromatic $C_{13}H_8N_2$ ion, such as IX.

IX
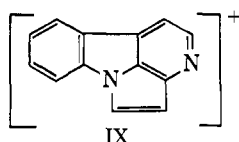
Further confirmation of the identity of the chromophore as well as an explanation for the small differences between the ultraviolet spectra of III on the one hand and of VII and VIII on the other was obtained upon their catalytic hydrogenation. Both alkaloids take up 2 moles of hydrogen and the ultraviolet spectra of the tetrahydro derivatives are now identical with the one of tetrahydrotuboflavine. Thus the differences in the ultraviolet spectra are due to the steric influence of the alkyl group at C-10, which prevents coplanarity of the chromophore in VII and VIII, but not in III.

The mass spectra of the tetrahydro derivatives X and XI exhibit very strong peaks caused by the loss of ethyl and methyl, respectively, and in both cases this fragmentation is followed by the loss of 18 mass units (H_2O). The corresponding fragments (m/e 223 and 205) are of much lower abundance in the mass spectrum of tetrahydrotuboflavine, which is consistent with the view that in X and XI, and therefore also in VII and VIII, the alkyl groups are adjacent to nitrogen. The

X, R = H
XI, R = CH₃
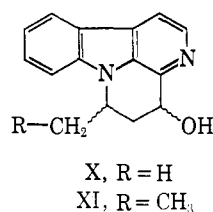
presence of the alcoholic hydroxyl group is confirmed by the production of a monoacetyl derivative on treatment with acetic anhydride and pyridine.

The n.m.r. spectrum of isotuboflavine (VIII) is in complete agreement with the proposed structure. It shows a single olefinic proton at 7.08 p.p.m. with weak allylic coupling ($J = 0.9$ c.p.s.) while the olefinic proton (at C-10) in tuboflavine (III) appears at 8.80 p.p.m. because of the proximity of the benzene ring. Conversely the signals for the ethyl group are found at lower field in VIII (quartet at 3.55 p.p.m., triplet at 1.69 p.p.m.) than in III (quartet at 2.92 p.p.m., triplet at 1.40 p.p.m.).

The presence of such isomers (tuboflavine, isotuboflavine) as well as homologs (isotuboflavine and norisotuboflavine among the alkaloids of a single species) further illustrates the great flexibility of the biogenetic scheme that must be the basis for the formation of indole alkaloids.

Experimental

Melting points are uncorrected and were taken on a Kofler micro hot stage. Unless stated otherwise, spectra were determined as follows: ultraviolet spectra, in methanol using a Cary Model 14 recording spectrophotometer; infrared spectra, in chloroform solution using a Perkin-Elmer Model 337 spectrophotometer; n.m.r. spectra, in trifluoroacetic acid with tetramethylsilane as an internal standard, using a Varian A 60 spectrometer; low resolution mass spectra, with a CEC Model 21-103C mass spectrometer using a direct inlet system; high resolution mass spectra, with a double-focusing mass spectrometer (CEC 21-110) using a photographic plate for recording.[18]

All solutions were dried with anhydrous sodium sulfate and evaporated on a rotary evaporator at 50° (aspirator vacuum). Thin layer chromatography was performed on silica gel G. The R_f values are listed in Table I.

Table I. R_f Values of the Alkaloids and Their Derivatives

Compd.	Solvent A[a]	Solvent B[b]	Ultraviolet fluorescence
Tuboflavine	0.43	0.14	Light blue
Isotuboflavine	0.35	0.085	Light blue
Norisotuboflavine	0.31	0.07	Light blue
Tetrahydrotuboflavine	0.47	0.17	Blue
Tetrahydroisotuboflavine	0.43	0.14	Blue
Tetrahydronorisotuboflavine	0.39	0.12	Blue
Acetyltetrahydrotuboflavine	0.73	0.46	Blue
Acetyltetrahydroisotuboflavine	0.72	0.44	Blue
Acetyltetrahydronorisotuboflavine	0.71	0.41	Blue
1-Carbomethoxy-β-carboline	0.70	0.48	Light blue

[a] Methanol–chloroform (1:9). [b] Chloroform–ethyl acetate–methanol (15:4:1).

Isolation of Alkaloids. Two 130-g. portions of the basic fraction of a methanol extract of about 20 kg. of powdered bark of *P. mutica* Benth. which had been depleted of pleiocarpine by crystallization was chromatographed on alumina (1800 g., Woelm, neutral, activity III) and eluted with methanol–benzene, 1:25 (chromatogram A). The corresponding fractions (250 ml. each) of these two chromatograms were combined and fractions A4 through A10 were rechromatographed again on alumina (1800 g., Woelm, neutral activity II) using the same eluent (chromatogram B). Fractions A1–A3 and B4–B20 were separated further on silicic acid (1500 g., 100 mesh), which on elution with chloroform resulted in more than 100 fractions (chromatogram C), each 500 ml. in volume.

1-Carbomethoxy-β-carboline (IV). Fractions C85 to C90 contained a substance, fluorescing very intensively in ultraviolet light and exhibiting a yellow color on treatment with dilute sulfuric acid. This compound was separated from impurities by thin layer chromatography (methanol–chloroform 3% v./v.) and

after sublimation (130° at 0.05 mm.), 25 mg. of crystalline material was obtained which proved to be identical in all physical constants with 1-carbomethoxy-β-carboline[11,16]: white needles, m.p. 166° (lit.[16] 165–168°); infrared ν_{max} 3440 (s), 1700 (s), and 1630 (s) cm.$^{-1}$; ultraviolet λ_{max} 245 mμ (log ϵ 4.17) 257 (4.16), 274 (4.21), 300 (3.96), and 368 (3.69); λ_{min} 228 mμ (log ϵ 3.96) 251 (4.14), 263 (4.13), 286 (3.80), and 327 (3.11); mass spectrum m/e 226 (M$^+$), 194, 168, 166, 140, and 114; and n.m.r. (in chloroform solution) δ 8.55 and 8.05 (2 H, AB pattern, $J = 5$ c.p.s.), 8.2–7.3 (4 H, aromatic protons), and 4.10 (3 H, singlet, OCH$_3$).

Tuboflavine (III). Fractions B21 to B26 (inclusively) were rechromatographed on silicic acid (450 g., 100 mesh) (chromatogram D). Upon elution with methanol–chloroform (increasing from 1:30 to 1:4) fractions D1 through D4 (100 ml. each) were combined and evaporated, and the residue was dissolved in chloroform and extracted with hydrochloric acid (200 ml., 0.1 N.). The aqueous layer was adjusted to pH 10 with ammonia and re-extracted with chloroform, and the chloroform solution was dried and evaporated. Upon addition of acetone, the residue (220 mg.) crystallized immediately. Recrystallization from methanol gave yellow needles identical with tuboflavine (III)[11] in all physical constants: m.p. 207° (lit.[11] 207–208°); C$_{16}$H$_{12}$N$_2$O (M$^+$ found: 248.097, calcd. 248.0949); ν_{max} 1640 (sh), 1620 (s), and 1585 (s) cm.$^{-1}$; λ_{max} 216 mμ (log ϵ 4.57), 262 (4.36), 288 (4.37), 320 (3.72), and 399 (3.98); λ_{min} 238 mμ (log ϵ 3.97), 274 (4.20), 308 (3.61), and 347 (3.15); mass spectrum m/e 248.097 (M$^+$), 247.087, 233.073, 219.092, 205.076, 192.069, and 140.050; and n.m.r. δ 9.25 and 8.90 (2 H, AB pattern, $J = 6$ c.p.s.), 8.85–7.5 (5 H, 4 aromatic protons), 2.92 (2 H, quartet, $J = 7.5$ c.p.s.), and 1.40 (3 H triplet, $J = 7.5$ c.p.s.).

Isotuboflavine (VIII) and Norisotuboflavine (VII). Fractions D11 to D20 (inclusively) were combined with B27 and B28, evaporated to dryness (total dry weight 800 mg.), chromatographed on silicic acid (170 g., 100 mesh) (chromatogram E), and eluted with methanol–chloroform (1:30). Fractions of 25 ml. each were collected and E13 through E19 contained two bases, both of which showed fluorescence in ultraviolet light. These were extracted from the chloroform solution with 0.2 N hydrochloric acid and re-extracted upon addition of ammonia. The alkaloids could be separated by repeated chromatography on silicic acid, using methanol–chloroform (1:30) as the eluent. Both alkaloids were precipitated from concentrated chloroform solutions upon addition of acetone. Recrystallization from methanol gave isotuboflavine (20 mg.), yellow needles, m.p. 263–265°, and norisotuboflavine (3 mg.), yellow needles, m.p. 282–284°. *Isotuboflavine (VIII)* was characterized as follows: C$_{16}$H$_{12}$N$_2$O (M$^+$ found 248.095, calcd. 248.0949); infrared ν_{max} 1650 (m), 1620 (s), and 1570 (m) cm.$^{-1}$ (in KBr); ultraviolet λ_{max} 260 mμ (log ϵ 4.39), 282 (4.38), 320 (3.74), and 385 (4.02); λ_{min} 238 mμ (log ϵ 3.98), 2.69 (4.24), 304 (3.64), and 343 (3.41); λ_{infl} 225 mμ (log ϵ 4.23); mass spectrum m/e 248.095 (M$^+$), 247.087, 219.093, 205.076, 192.069, 168.069, 166.053, 139.040, 114.035, and 81.034; and n.m.r. δ 9.20 and 8.88 (2 H, AB pattern, $J = 6$ c.p.s.), 8.9–7.6 (4 H, 4 aromatic protons), 7.08 (1 H, triplet, $J = 0.9$ c.p.s.), 3.55 (2 H, quartet, $J =$

7.5 c.p.s.), and 1.69 (3 H, triplet, $J = 7.5$ c.p.s.). *Norisotuboflavine (VII)* was characterized as follows: C$_{15}$H$_{10}$N$_2$O (M$^+$ found 234.077, calcd. 234.0793); infrared ν_{max} 1645 (m), 1620 (s), and 1570 (m) cm.$^{-1}$ (in KBr); ultraviolet λ_{max} 259 mμ (log ϵ 4.42), 282 (4.38), 321 (3.76), and 387 (4.02); λ_{min} 238 mμ (log ϵ 4.02), 268 (4.24), 305 (3.66), and 344 (3.39); λ_{infl} 225 mμ (log ϵ 4.26); and mass spectrum m/e 234.077 (M$^+$), 205.075, 168.070, 166.053, 139.042, 114.034, and 67.019.

Tetrahydro Derivatives. Approximately 1 mg. of the alkaloid was dissolved in 2.5 ml. of aqueous ethanol (80% v./v.) and hydrogenated on palladium charcoal (10 mg., 10% Pd). After 1 hr. the catalyst was filtered off, the solution was evaporated to dryness, and the product was purified by thin layer chromatography. The eluents were chloroform–ethyl acetate–methanol (15:4:1) for tuboflavine and methanol–chloroform (1:9) for isotuboflavine and norisotuboflavine, respectively. None of the products was obtained in crystalline form. *Tetrahydrotuboflavine*[21] had the following properties: ultraviolet λ_{max} 218, 237, 292, 350, and 365 mμ; λ_{min} 227, 276, 308, and 359 mμ; λ_{infl} 253, 261, and 285 mμ; and mass spectrum m/e 252 (M$^+$), 223, 205, 196, 182, 168, 140, and 114. *Tetrahydroisotuboflavine (XI)*: ultraviolet λ_{max} 218, 238, 292, 352, and 366 mμ; λ_{min} 228, 276, 304, and 357 mμ; λ_{infl} 253, 261, and 285 mμ; and mass spectrum m/e 252 (M$^+$), 223, 205, 168, and 140. *Tetrahydronorisotuboflavine (X)*: mass spectrum m/e 238 (M$^+$), 223, 205, 168, and 140.

Acetylation of the Tetrahydro Derivatives. Approximately 0.5 mg. of the tetrahydro derivatives was dissolved in pyridine (0.5 ml.) and acetic anhydride (0.5 ml.). The mixture was kept at 80° for 2 hr. and evaporated to dryness, and the residue was purified by thin layer chromatography using methanol–chloroform (1:9). *Acetyltetrahydrotuboflavine* had the following properties: ultraviolet λ_{max} 238, 292, 352, and 366 mμ; λ_{min} 224, 275, 308, and 360 mμ; λ_{infl} 252, 261, and 285 mμ; and mass spectrum m/e 294 (M$^+$), 251, and 205. *Acetyltetrahydroisotuboflavine* was characterized by its mass spectrum: m/e 294 (M$^+$), 251, and 205. *Acetyltetrahydronorisotuboflavine*: mass spectrum m/e 280 (M$^+$), 237, 205.

Cleavage of Isotuboflavine by Alkali.[11] Isotuboflavine (1.35 mg.) was dissolved in a few drops of dioxane (peroxide-free), potassium hydroxide (1 ml., 1 N) was added, and the mixture was kept at 85°. After 12 hr. the reaction mixture was acidified with dilute hydrochloric acid and evaporated almost to dryness. After adjusting to pH 10 with ammonia and extraction with chloroform, the aqueous solution was reacidified with hydrochloric acid and evaporated to dryness. The last traces of excess hydrochloric acid were removed over potassium hydroxide in a vacuum desiccator. The dry hydrochloride was dissolved in water, an excess of diazomethane (in ether) was added, and the mixture was shaken. After standing at room temperature for 2 hr., the mixture was evaporated to dryness and the residue was extracted with chloroform. After

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(21) The same compound was obtained with different by-products by treatment of tuboflavine with lithium aluminum hydride in boiling tetrahydrofuran for 12 hr.[11]

evaporation and sublimation (130° at 0.05 mm.), white crystals (0.3 mg.) of m.p. 162–163° were obtained. The melting point remained unchanged upon admixture of authentic 1-carbomethoxy-β-carboline. R_f values and ultraviolet and mass spectra were identical with those of 1-carbomethoxy-β-carboline.

Stability Test of Tuboflavine. The alkaloid was dissolved in a mixture of chloroform and methanol; aqueous ammonium hydroxide solution, silicic acid, and alumina (Woelm, neutral) were added. This mixture was allowed to stand at room temperature for 4 weeks with occasional shaking. After this time on separation and esterification, no 1-carbomethoxy-β-carboline could be found.

Acknowledgment. We are indebted to Dr. Frank A. Hochstein, Chas. Pfizer and Co., for the alkaloid extracts. This work was supported by a grant from the National Institutes of Health, Public Health Service (GM 05472), and from the National Science Foundation (G-21037).

Photochemical Degradation of Flavins. II. The Mechanism of Alkaline Hydrolysis of 6,7-Dimethyl-9-formylmethylisoalloxazine[1,2]

Pill-Soon Song, Eddie C. Smith, and David E. Metzler

Contribution from the Department of Biochemistry and Biophysics, Iowa State University, Ames, Iowa. Received May 17, 1965

The alkaline degradation of 6,7-dimethyl-9-formylmethyl-isoalloxazine (FMF), an intermediate in the photolysis of riboflavin, was studied kinetically. The reaction followed pseudo-first-order kinetics to give lumiflavin with a less significant competing reaction yielding lumichrome. A mechanism of the carbon–carbon bond cleavage in the alkaline cleavage of FMF has been proposed on the basis of the kinetics and product identification. The significance of the reaction is pointed out in connection with the photolysis of riboflavin.

Introduction

Anaerobic photolysis of a solution of riboflavin with visible light leads to reduction of the isoalloxazine ring with the production of a hypothetical "leuco-deuteroflavin."[3,4] Subsequent aeration causes a return of the yellow color of oxidized flavins and the presumed formation of "deuteroflavin." Recently, Smith and Metzler have isolated from a photobleached and reoxidized riboflavin solution the compound 6,7-dimethyl-9-formylmethylisoalloxazine[1] (9-formylmethylflavin, FMF) which possesses all of the chemical characteristics of the postulated "deuteroflavin," including a faster rate of photobleaching and a rapid conversion to lumiflavin in basic solutions.[5] The present communication is concerned with the mechanism of the alkaline hydrolysis of FMF, an important reaction in the photochemical degradation of riboflavin.

(1) Part I: E. C. Smith and D. E. Metzler, *J. Am. Chem. Soc.,* **85**, 3285 (1963).

(2) Supported by Grant No. G-12339 from the National Science Foundation. Abbreviations used throughout this paper: RF, riboflavin; FMF, 9-formylmethylflavin; LF, lumiflavin; LC, lumichrome.

(3) R. Kuhn, H. Rudy, and T. Wagner-Jauregg, *Ber.,* **66**, 1150 (1933).

(4) G. Oster, J. S. Bellin, and B. Holmstrom, *Experientia,* **18**, 249 (1962).

(5) The term "deuteroflavin" is no longer adequate because the 2′-keto derivative of riboflavin which also fits the description of "deuteroflavin" has been isolated by Terao [M. Terao, *Tohoku Igaku Zassi,* **59**, 441 (1959)].

Experimental Section

Materials. FMF (6,7-dimethyl-9-formylmethylisoalloxazine) was made in this laboratory.[1] All the inorganic compounds used were analytical reagent grade obtained from Mallinckrodt Chemical Co. Silica Gel G for thin-layer chromatography was obtained from E. Merck, A.G., and thin-layer plates were prepared as previously reported.[1]

Kinetic Measurements. The rate of disappearance of FMF in borate buffer and unbuffered solutions in the dark was followed by measuring the decrease in absorbance at 445 mμ under various conditions of pH and ionic strength (μ) using a Cary Model 15 recording spectrophotometer. It must be noted that, since the spectrum of LF shows a marked dependence on temperature, probably due to a complexation equilibrium of LF (unpublished observation), it is important to maintain the same temperature for making spectral measurements of reaction mixture and LF solution. All measurements were made at 25 ± 1°. The pH of reaction mixtures was measured by the Beckman pH meter and the desired pH was obtained with 0.1 or 0.2 N NaOH solution. The amount of NaOH solution to bring about the desired pH was predetermined. The kinetic measurements were then followed immediately after adjusting the pH of the solutinos. The pH of the reaction mixture during the reaction was nearly unchanged both in buffered and unbuffered solutions. The pH of the reaction mixture in unbuffered solution at the end of the reaction was slightly lower.

Identification of Products. In addition to thin-layer chromatography which was employed as previously described,[1] 1 l. of alkali-treated reaction mixture (about 1 × 10⁻⁴ mole/l. of FMF) was extracted three–five times with about 200 ml. of chloroform, and the chloroform extracts were evaporated. The residue was then dissolved in about 200 ml. of water and extracted with an equal volume of chloroform. The chloroform