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Chemical Studies on Riboflavin and Related Compounds. I. Oxidation of Quinoxaline-2,3-diols as a Possible Model for the Biological Decomposition of Riboflavin*

Isao Saito and Teruo Matsuura

ABSTRACT: In connection with the biological decomposition of riboflavin, the oxidation of three quinoxaline-2,3-diol derivatives, *i.e.*, 1-ribityl-2,3-diketo-6,7-dimethyl-1,2,3,4-tetrahydroquinoxaline (I), 6,7-dimethylquinoxaline-2,3-diol (II), and quinoxaline-2,3-diol (IV), was examined by chemical means. The former two compounds are known to be catabolic intermediates of riboflavin. On oxidation with alkaline ferricyanide, IV gave *cis,trans*-muconic acid and probably a dimer VII, II gave 3,4-dimethyl-4-carboxymethyl-Δ^1-butenolide (VIII) and 6-carboxy-7-methylquinoxaline-2,3-diol (IX), and I gave II, VII, and IX. Whereas IV was resistant to photosensitized oxidation, both I and II were degraded destructively under similar conditions. However, II was found to be an intermediate in the photooxidation of I. Mechanisms of these oxidation reactions are discussed in connection with the enzymatic degradation of riboflavin.

The first significant observation on the biological decomposition of riboflavin was reported by Foster *et al.* (Foster, 1944; Yanagita and Foster, 1956), who demonstrated that *Pseudomonas riboflavinus* catalyzes the hydrolysis of riboflavin to lumichrome and ribitol, followed by the oxidation of the latter to carbon dioxide. Miles and Stadtman (1955) reported that a microorganism degrades riboflavin to 6,7-dimethyl-9-(2′-hydroxyethyl)isoalloxazine under anaerobic conditions. Recently the isolation of 6,7-dimethyl-9-(2′-carboxyethyl)isoalloxazine, related to the above isoalloxazine, from urine of sheeps was reported (Owen and Montgomery, 1962). Such types of the degradation of the side chain of riboflavin seem to be related to the photochemical degradation (for a review, see Hemmerich *et al.*, 1965) of riboflavin.

Another type of the biological decomposition of riboflavin has been investigated by Stadtman and collaborators (Smyrniotis and Stadtman, 1957; Smyrniotis *et al.*, 1958; Miles *et al.*, 1959; Tsai *et al.*, 1963; Harkness *et al.*, 1964). They demonstrated that a different strain of *P. riboflavinus* degrades the isoalloxazine portion of the molecule. Thus riboflavin is oxidized to 3,4-dimethyl-6-carboxy-α-pyrone (III) *via* 2,3-diketoquinoxaline derivatives, I and II, as shown in Scheme I.

Although the mechanism of this microbial degradation of riboflavin has not yet been established, it has been suggested that the metabolic conversion of I to II may involve a mixed function oxygenase enzyme and that the conversion of II to III is a multistep process in which the presence of molecular oxygen plus any one of several cosubstrates capable of pyridine nucleotide linked oxidation are required (Harkness *et al.*, 1964). In order to contribute to the elucidation of the mechanism of this biological oxidation, we have investigated the oxidation of the metabolic intermedi-

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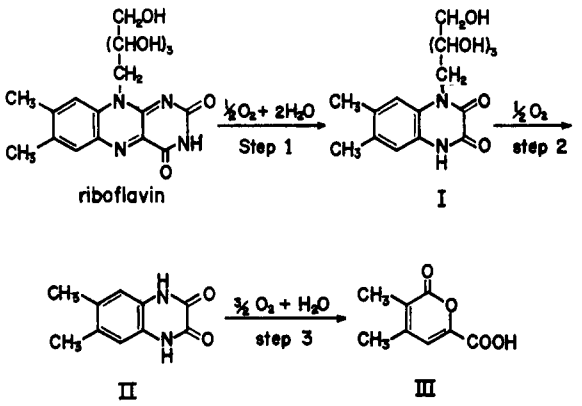
SCHEME I



ates, I and II, and also of a simple analog of II, quinoxaline-2,3-diol (IV), by chemical means. To our best knowledge, the chemical oxidation of quinoxaline-2,3-diol derivatives has not appeared in the literature, although quinoxaline itself undergoes oxidative cleavage of its benzene ring under drastic conditions to give pyrazine-2,3-dicarboxylic acid (Jones and McLaughlin, 1950).

Results

We first examined the autoxidation of I, II, and IV in alkaline media. These compounds were quite stable under the conditions employed. However, alkaline ferricyanide which is known to be a one-electron transfer oxidizing agent was found to be capable of oxidizing these quinoxalinediols. Quinoxaline-2,3-diol (IV) was slowly oxidized with alkaline ferricyanide in a nitrogen atmosphere to give two products. One was obtained in 27% yield and identified as *cis,trans*-muconic acid (V) through its spectral data and through its conversion to *trans,trans*-muconic acid by ultraviolet irradiation. The other product, which was soluble in aqueous alkali but insoluble in various organic solvents, was obtained in low yield and could not be isolated in pure form. Its ultraviolet spectrum (Figure 1) is similar to that of the starting material except for an additional band at 265 mμ which is usually present in biphenyl derivatives (Dieteren and Konigsberger, 1963). This suggests that the structure of the second product may be represented either by a 5,5′ (VI) or by a 7,7′ dimer (VII). Since the 5,5′ dimer (VI), which was obtained by an unambiguous synthesis, was not identical with the product, we tentatively assigned structure VII to the second product.

Oxidation of 6,7-dimethylquinoxaline-2,3-diol (II) with alkaline ferricyanide was easier than that of IV and resulted in the formation of a lactonic acid $C_8H_{10}O_4$ (16%), an acid $C_{10}H_8N_2O_4$ (20%), and polymers. Structure VIII was assigned to the lactonic acid on the basis of its spectral properties. The infrared (ν_{max}^{KBr} 1740, 1720, and 1640 cm^{-1}) and ultraviolet (λ_{max}^{EtOH}

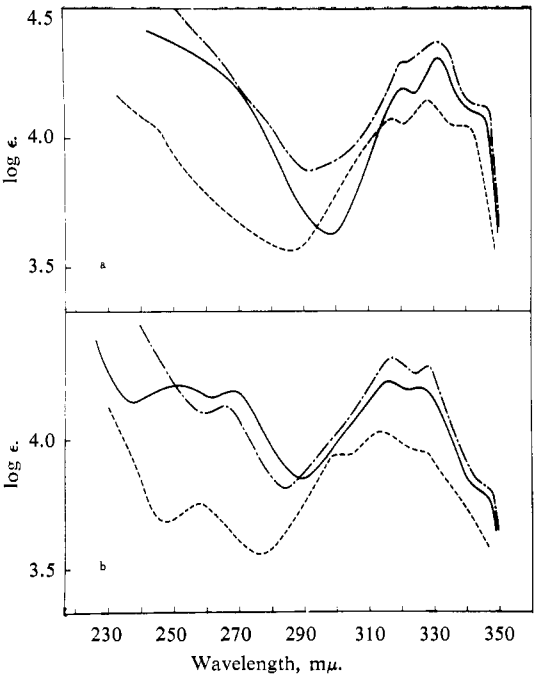
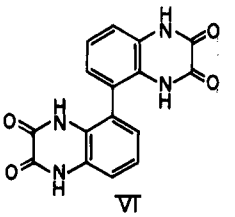
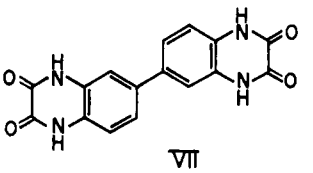


FIGURE 1: Ultraviolet absorption spectra of quinoxaline-2,3-diol (IV) (– – – –), (7,7′-biquinoxaline)-2,2′,3,3′-tetrol (VII) (———), and (5,5′-biquinoxaline)-2,2′,3,3′-tetrol (VI) (— - —). (a) In 0.1 N sodium hydroxide. (b) In 0.1 N hydrochloric acid.



VI



VII

212 mμ) absorption spectra showed an α,β-unsaturated γ-lactone ring and a carboxylic group. In the nuclear magnetic resonance spectrum,[1] signals of two methyl groups were observed; one is attributed to the 4-methyl group (δ 1.55, singlet) and the other to the 3-methyl group (δ 2.10, doublet, J = 1.5 cycles/sec). Signals of one vinyl proton (δ 5.82, quartet, J = 1.5 cycles/sec) and two carboxymethyl protons (δ 2.83, AB quartet, J_{AB} = 18.5 cycles/sec) were also observed.

[1] Chemical shifts are given as δ values expressed as parts per million. Tetramethylsilane was used as a reference.

TABLE I: Photosensitized Oxidation of Quinoxaline Derivatives I, II, and IV.

Substance	Solvent (N)	Reaction Time (hr)	Sensitizer[a]	Oxygen Consumed
IV	NaOH (2)	10	RB	None
II	Pyridine	8	RB	None
II	NaOH (2)	15	None	None
II	NaOH (2)	18	RB	4 mole equiv
II[b]	NaOH (2)	25	RB	4 mole equiv
I	NaOH (0.5)	10	RB	5 mole equiv

[a] Rose bengal (RB) was used as the sensitizer. [b] An aqueous coppper sulfate solution (*ca.* 10%) was used as a filter.

The spectral properties of the acid $C_{10}H_8N_2O_4$ led to its assignment as 6-carboxy-7-methylquinoxaline-2,3-diol (IX). The ultraviolet spectrum showed similar absorptions to that of II (Figure 2) and the infrared spectrum showed bands of a carboxylic group at 1690 cm^{-1}, a carbonyl group at 1675 cm^{-1}, and an amide group at 3500 cm^{-1}. The nuclear magnetic resonance spectrum showed the presence of two aromatic protons (δ 7.11 and 7.37) and a methyl group (δ 2.45, singlet) in the molecule. These spectral data are in agreement with structure IX.

Oxidation of 1-ribityl-2,3-diketo-6,7-dimethyl-1,2,3,4-tetrahydroquinoxaline (I) with ferricyanide was carried out in 0.1 N aqueous alkaline solution, since I is known to be more labile to alkali than II, yet stable in 0.1 N alkali. Thin layer chromatography showed that the reaction product consisted of at least eight compounds. Of these products three (II, VIII, and IX) were isolated. Some of the starting material was also detected. The results indicate that in the course of the ferricyanide oxidation I is converted to II by oxidative elimination of the ribityl side chain.

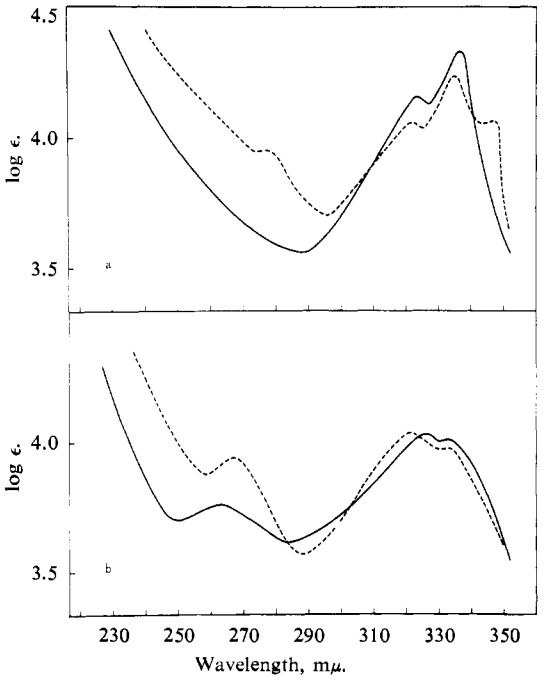


FIGURE 2: Ultraviolet absorption spectra of 6,7-dimethylquinoxaline-2,3-diol (II) (———) and 6-methyl-7-carboxyquinoxaline-2,3-diol (IX) (– – – –). (a) In 0.1 N sodium hydroxide. (b) In 0.1 N hydrochloric acid.

In the photooxidation of quinoxaline 2,3-diol (IV) in the presence or absence of rose bengal as a sensitizer no oxygen consumption was observed and the starting material (IV) was recovered quantitatively. Photosensitized oxidation of 6,7-dimethylquinoxaline-2,3-diol (II) in 2.0 N aqueous alkaline solution in the presence of rose bengal gave at least nine products, which were detected on a thin layer chromatogram, after 4 moles of oxygen had been consumed. In the absence of rose bengal the starting material (II) was recovered quantitatively (Table I).

Under similar conditions and in the presence of rose bengal 1-ribityl-2,3-diketo-6,7-dimethyl-1,2,3,4-tetrahydroquinoxaline (I) consumed 5 mole equiv of oxygen. When irradiation was interrupted after the consumption of 0.5 mole equiv of oxygen, paper chromatographic analysis (Figure 3) of the reaction mixture showed that it consisted of II, the unchanged starting material I, and an unidentified compound.

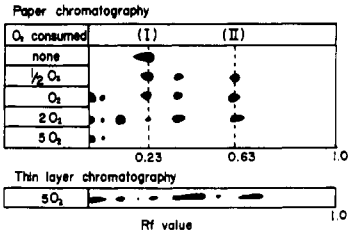
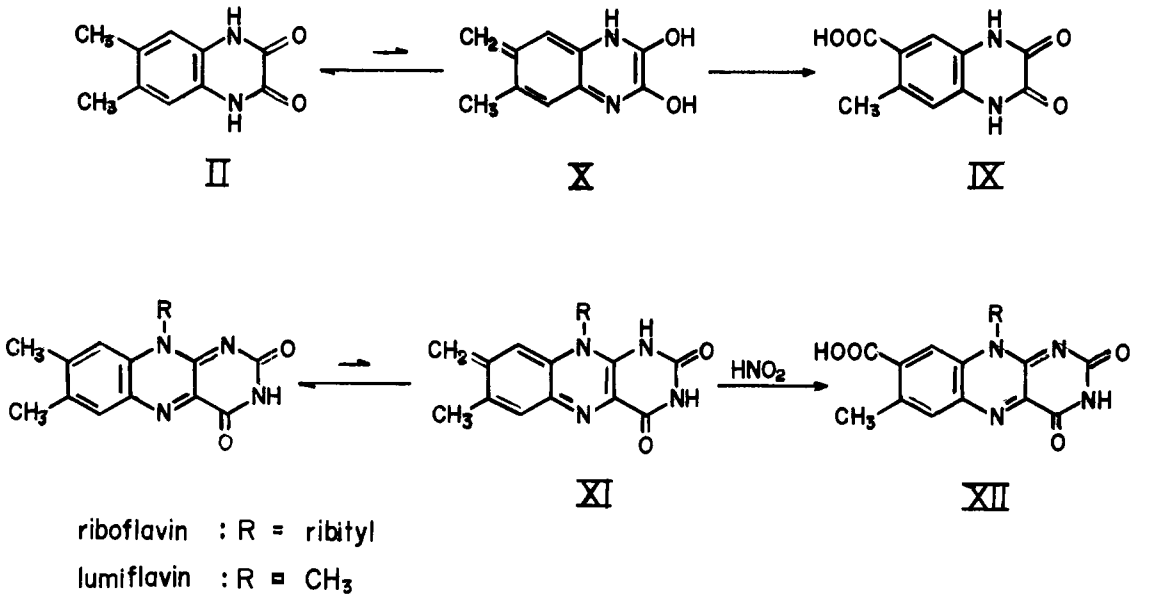


FIGURE 3: Chromatography of the photosensitized oxidation products of 1-ribityl-2,3-diketo-6,7-dimethyl-1,2,3,4-tetrahydroquinoxaline (I). Paper chromatography: 1-butanol saturated with 2 N ammonia. Thin layer chromatography: benzene–ethyl formate–formic acid (5:2:1).



After the consumption of 2 mole equiv of oxygen, II was isolated in 18% yield from the reaction mixture. When I was photooxidized until oxygen consumption ceased (5 mole equiv), II was no more found in the reaction mixture which was shown by thin layer chromatography to consist of at least seven products (Figure 3). These results demonstrate that one of the initial steps of the photosensitized oxidation of I is the oxidative deribitylation of I to II.

Discussion

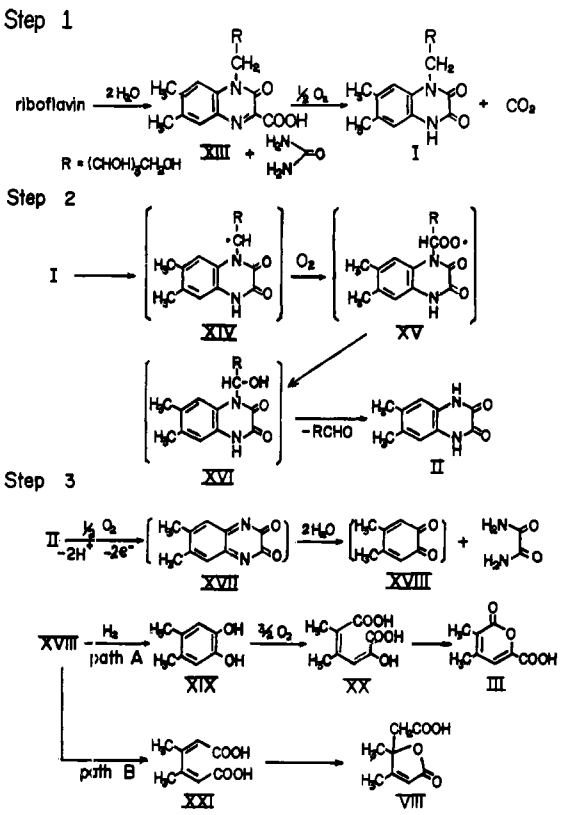
Alkaline ferricyanide oxidation and photosensitized oxygenation, which were chosen as moderate oxidizing methods for the quinoxaline-2,3-diols I, II, and IV, appear to be closely related to some biological oxidation. Alkaline ferricyanide is a one-electron transfer oxidizing agent. One-electron transfer oxidations are known to occur in the biosynthesis of various natural products such as alkaloids (for a review, see Scott, 1965). Photosensitized oxygenation of various biological systems, such as proteins and nucleic acids, is known as photodynamic action (Shugar and McLaren, 1964). Photosensitized oxygenation also seems to be closely related to oxygenases and to some oxidases. For example, it has been reported that tyrosine is photooxidized in the presence of a sensitizer to give 3,4-dihydroxyphenylalanine (Hara, 1960), representing a model for the enzymatic hydroxylation of tyrosine (Nelson and Dawson, 1944) and of 3,4-dimethylphenol (Mason *et al.*, 1955) by phenolases. Tryptophan is also photooxidized under similar conditions to give various degradation products (Yoshida and Kato, 1954a,b), including kynurenine and 3-hydroxykynurenine, which are known metabolites of tryptophan formed under the influence of tryptophan pyrrolase (Hayaishi *et al.*, 1957).

Whereas the reaction of quinoxaline-2,3-diol (IV) itself with alkaline ferricyanide is relatively slow, 6,7-dimethylquinoxaline-2,3-diol (II) is easily oxidized by ferricyanide. Such a difference between the reactivities of II and IV was also observed in the case of the photosensitized oxidation. This is probably due to hyperconjugation (as formula X) involving one of the methyl groups at the 6,7 positions of II. A similar hyperconjugation has been proposed for riboflavin (XI, R = ribityl) by Hemmerich *et al.* (1959). The formation of 6-carboxy-7-methylquinoxaline-2,3-diol (IX) in the ferricyanide oxidation of II is quite analogous to the oxidation of lumiflavin with nitrous acid, leading to 7,10-dimethylisoalloxazine-8-carboxylic acid (XII) (Hemmerich *et al.*, 1959).

As shown in Scheme I, the bacterial degradation of riboflavin leading to 3,4-dimethyl-6-carboxy-α-pyrone (III) involves the following three steps: (1) conversion of riboflavin to I, (2) deribitylation of I to II, and (3) degradation of II to III. A model reaction for step 1 has already been reported by Miles *et al.* (1959), who demonstrated that the alkaline hydrolysis of riboflavin followed by oxidation of the product XIII with peracetic acid yielded I, as shown in Scheme II. This result is consistent with the formulation by Stadtman and his group (Scheme I) which requires two molecules of water and one atom of oxygen in step 1.

The formation of II from I, either by ferricyanide oxidation or by photosensitized oxidation, represents a model for step 2. In connection with the mechanism of the deribitylation of I, it should be mentioned that there are a few reports on the oxidative dealkylation of tertiary amines. Perrine (1951) reported that tertiary *N*-methylamines such as 1,2,6-trimethylpiperidine are demethylated by the action of potassium ferricyanide, although the mechanism is not known. Furthermore, the photosensitized oxidation of certain amines or amides, which possess a partial structure of XXII, results in the removal of the *N*-alkyl group or in the formation of acylamines, as shown in Scheme III (Schenck, 1957; Lock and Sagar, 1966; Burnett and

SCHEME II

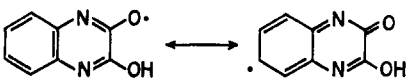


Riches, 1966). In the present model reactions for step 2, in particular in the photosensitized oxidation of I, a similar mechanism, which involves radical intermediates XIV and XV, is applicable. Recently extensive mechanistic investigations on riboflavin photochemistry have been carried out by Moore and Metzler (McBride and Moore, 1967; McBride and Metzler, 1967; and references cited therein). They suggested that the photochemical deribitylation of riboflavin might be initiated by an internal hydrogen abstraction from a CH on the ribityl chain by the photoexcited isoalloxazine group. Such a mechanism might not be applicable to the deribitylation of I in the photosensitized oxidation, because I is recovered unchanged on photooxidation without sensitizer. Since an energy transfer from the excited sensitizer to the substrate, which would cause excitation of the carbonyl group, appears unfavorable, it is concluded that an excited molecule of I is not involved in the present reaction. The radical XV may be converted into XVI which is then cleaved to form II. Since II and ribose have been found to be products in the enzymatic deribitylation of I (Tsai *et al.*, 1963), it is reasonable to assume that the enzymatic reaction also proceeds by an analogous mechanism.

For step 3, we suggest common intermediates (XVII and XVIII) in both the enzymatic and chemical degradation of II. Ferricyanide can abstract two electrons from the dianion of the quinoxalinediol (II) to form XVII, which in turn is hydrolyzed to 4,5-dimethyl-*o*-benzoquinone (XVIII) and oxamide. Further oxidative cleavage of XVIII with ferricyanide appears to proceed *via* path A, which is different from the enzymatic cleavage *via* path B. The 2,3-bond fission of catechols frequently occurs in biological systems involving metapyrocatechase enzymes (Hayaishi, 1962). Thus, it is reasonable to assume that, in the course of the bacterial decomposition of riboflavin, 4,5-dimethylcatechol (XIX) which might be enzymatically derived from the quinone XVIII, is cleaved to 3,4-dimethyl-6-carboxy-α-pyrone (III) *via* the acid XX.

In contrast to such an enzymatic cleavage of catechols, the chemical oxidation of catechols or *o*-quinones usually results in a 1,2-bond fission. For instance, in an alkaline ferricyanide oxidation 4,5-di-*t*-butylpyrogallol is cleaved at the bond between two hydroxy groups at the 3 and 4 positions to yield 3,6-di-*t*-butyl-6-carboxy-α-pyrone (Campbell, 1951). Therefore, it can be rationalized that the *o*-quinone XVIII is cleaved by alkaline ferricyanide to form β,β-dimethyl-*cis,cis*-muconic acid (XXI) which is then cyclized by an intramolecular Michael-type addition to the lactonic acid VIII.

The formation of *cis,trans*-muconic acid (V) in the alkaline ferricyanide oxidation of quinoxaline-2,3-diol (IV) may also be rationalized by a similar mechanism. The formation of the dimer VII in this oxidation can be explained by the coupling of a free radical (XXIII) which is produced by the removal of one electron from IV.



XXIII

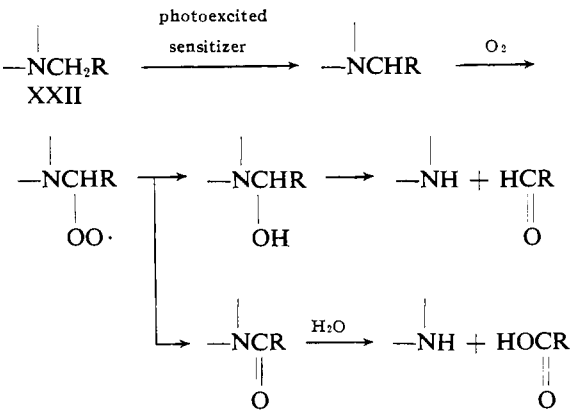
Experimental Section[2]

Materials. Quinoxaline-2,3-diol (IV) was prepared according to the method described by Newbald and Spring (1948); $\lambda_{max}^{0.1\ N\ NaOH}$ 315 (ϵ 12,000), 326 (14,500), 340 mμ (11,000). 6,7-Dimethylquinoxaline-2,3-diol (II) was prepared according to the method of Tsai *et al.* (1963); $\lambda_{max}^{0.1\ N\ NaOH}$ 321 (ϵ 13,000), 335 (17,500), and 345 mμ (12,000). 1-Ribityl-2,3-diketo-6,7-dimethyl-1,2,3,4-tetrahydroquinoxaline (I) was prepared according to a modification of the procedure of Miles *et al.* (1959). A mixture of 5 g of 2-(*N*-ribitylamino)-4,5-dimethylaniline and 100 g of diethyl oxalate was heated at 140–150° under nitrogen for 2 hr. Removal of diethyl oxalate followed by trituration of the residual oil with 100 ml of methanol yielded a grayish green solid. Two crystallizations from acetic acid afforded 1.5 g of I as colorless needles: mp 257–260°;[3] $\lambda_{max}^{0.1\ N\ NaOH}$

[2] Paper chromatography was done on Toyo Roshi No. 50 paper with 1-butanol saturated with 2 N ammonia.

[3] Miles *et al.* (1959) reports mp 262–266° with sintering at 259°.

SCHEME III



322 (ε 12,000), 334 (13,500), and 350 mμ (9500).

Anal. Calcd for $C_{15}H_{20}N_2O_6$: C, 55.55; H, 6.22; N, 8.64. Found: C, 55.15; H, 6.35; N, 8.85.

Alkaline Ferricyanide Oxidation of Quinoxaline-2,3-diol (IV). To a solution of 5.0 g (0.03 mole) of quinoxaline-2,3-diol in 500 ml of 2 N sodium hydroxide was added 30 g (0.09 mole) of potassium ferricyanide. The mixture was stirred under nitrogen for 24 hr. Acidification of the mixture with 12 N hydrochloric acid deposited unreacted starting material (3.0 g) which was collected by filtration. The filtrate was extracted with ether, and a yellow solid (0.48 g, 27% on the basis of the reacted IV), obtained after evaporating the extract, was recrystallized from ethyl acetate to give *cis,trans*-muconic acid: mp 188–190°;[4] λ_{max}^{EtOH} 255 mμ (ε 24,500) and ν_{max}^{Nujol} 900 and 720 cm^{-1}.

Anal. Calcd for $C_6H_6O_4$: C, 50.71; H, 4.26. Found: C, 50.48; H, 4.59.

Ultraviolet irradiation of this compound in the presence of iodine (Elvidge *et al.*, 1950) yielded *trans,trans*-muconic acid whose infrared spectrum was identical with that of an authentic sample.

The aqueous layer from the ether extraction was further extracted with phenol. After evaporation of the phenol, the residue showed two fluorescent spots (R_F values 0.58 and 0.40) on a paper chromatogram using 1-butanol saturated with 2 N aqueous ammonia. The substance corresponding to the spot of R_F 0.58 was identified as the starting material IV. The residue was adsorbed on cellulose power and eluted with 1-butanol saturated with 2 N aqueous ammonia. The eluate, which showed the spot of R_F 0.40 on a paper chromatogram, was neutralized with acetic acid and evaporated to dryness. The residual solid obtained was dissolved in 8 ml of water and the solution was acidified with 2 N hydrochloric acid to yield a solid (45 mg) which did not melt below 300°: $\lambda_{max}^{0.1\ N\ HCl}$ 265, 300, 314, and 328 mμ.

(5,5'-Biquinoxaline)-2,2',3,3'-tetrol (VI). 2,2',3,3'-Tetraaminobiphenyl (60 mg), which was synthesized according to the method of Dieteren and Konigsberger (1963), was suspended in 25 ml of diethyl oxalate. The mixture was heated at 120–130° under nitrogen for 3 hr. The resulting solid was dissolved in 3 ml of 0.5 N sodium hydroxide and acidified with 2 N hydrochloric acid to yield 38 mg of VI, which did not melt below 300°; ν_{max}^{Nujol} *ca.* 1700 cm^{-1} (br); nuclear magnetic resonance spectrum (dimethyl sulfoxide) δ 6.8–7.3 (six aromatic protons, multiplet), 10.8 (two NH protons, singlet), and 12.05 (two NH protons, singlet).

Anal. Calcd for $C_{16}H_{10}N_4O_4$: C, 59.66; H, 3.13; N, 17.39. Found: C, 59.71; H, 2.98; N, 17.98.

Alkaline Ferricyanide Oxidation of 6,7-Dimethylquinoxaline-2,3-diol (II). To a solution of 3.0 g (0.015 mole) of 6,7-dimethylquinoxaline-2,3-diol in 400 ml of 2 N sodium hydroxide was added 40 g (0.12 mole) of potassium ferricyanide and the mixture was stirred under nitrogen for 10 hr. The reaction mixture was adjusted to pH 2 with 2 N hydrochloric acid and extracted with 1-butanol. The extract was chromatographed on a silica gel column. Elution with chloroform–acetone (5:1) yielded a crystalline solid (0.41 g, 16%). Recrystallization from ethyl acetate gave the lactonic acid VIII as crystals: mp 97–98°; nuclear magnetic resonance spectrum ($CDCl_3$) δ 1.55 (three protons, singlet), 2.10 (three protons, doublet, J = 1.5 cycles/sec), 2.83 (two protons, AB quartet, J_{AB} = 18.5 cycles/sec), and 5.82 (1 proton, quartet, J = 1.5 cycles/sec); ν_{max}^{KBr} 1740, 1720, and 1640 cm^{-1}; λ_{max}^{EtOH} 212 mμ (ε 15,700).

Anal. Calcd for $C_8H_{10}O_4$: C, 56.46; H, 5.92. Found: C, 56.76; H, 5.72.

Further elution with acetone yielded a crystalline solid (0.70 g, 20%). Recrystallization from acetone–methanol gave 6-methyl-7-carboxyquinoxaline-2,3-diol (IX) as crystals, which did not melt below 300°. This compound was soluble in aqueous sodium bicarbonate; $\lambda_{max}^{0.1\ N\ HCl}$ 265 (ε 5900), 308 (6200), 330 (10,300), and 332 mμ (9500); ν_{max}^{Nujol} 1690 and 1675 cm^{-1}; nuclear magnetic resonance spectrum (D_2O–NaOD) δ 2.45 (three protons, singlet), 7.11 (one proton, singlet), and 7.37 (one proton, singlet).

Anal. Calcd for $C_{10}H_8N_2O_4$: C, 54.55; H, 3.66; N, 12.72. Found: C, 54.03; H, 3.23; N, 12.25.

Further elution with ethanol yielded polymers (0.80 g).

Alkaline Ferricyanide Oxidation of 1-Ribityl-2,3-diketo-6,7-dimethyl-1,2,3,4-tetrahydroquinoxaline (I). A solution of 0.50 g (1.5 mmoles) of I and 5 g (1.5 mmoles) of potassium ferricyanide in 100 ml of 0.1 N sodium hydroxide was stirred under nitrogen for 5 hr. The mixture was acidified with 2 N hydrochloric acid and extracted with 1-butanol. Paper chromatography of the extract showed four major and two minor spots in short-wave ultraviolet light. Thin layer chromatography on silica gel with benzene–ethyl formate–formic acid (5:2:1) showed at least eight spots. Of these products three were isolated by chromatography on cellulose powder with 1-butanol saturated with 2 N ammonia and identified by comparison with authentic

[4] Elvidge *et al.* (1950) reports mp 190–191°.

samples as 6,7-dimethylquinoxaline-2,3-diol (II) (infrared spectrum), the lactonic acid VIII (thin layer chromatography), and the acid IX (ultraviolet spectrum and paper chromatography). Some starting material I was also detected among the products. Other products were not obtained in a pure form.

Photosensitized Oxidation of 6,7-Dimethylquinoxaline-2,3-diol (II). In a typical run, a solution of 1.0 g (5.2 mmoles) of 6,7-dimethylquinoxaline-2,3-diol (II) and 20 mg of rose bengal in 100 ml of 2 N sodium hydroxide was irradiated at room temperature with a 100-w high-pressure lamp (Ushio Type UM 100) having a Pyrex cooling jacket. During the irradiation oxygen was bubbled through a sintered-glass joint which was attached at the bottom of the reaction vessel. Oxygen consumption was followed manometrically. After 450 ml (20 mmoles) of oxygen had been consumed, the reaction mixture was acidified with hydrochloric acid and extracted with 1-butanol. The extract showed at least nine spots on thin layer chromatogram (silica gel, benzene–ethyl formate–formic acid (5:2:1)). The products were isolated in impure form after chromatography on a silica gel column. Other results obtained under various conditions are summarized in Table I.

Photosensitized Oxidation of 1-Ribityl-2,3-diketo-6,7-dimethyl-1,2,3,4-tetrahydroquinoxaline (I). A solution of 1.2 g (3.8 mmoles) of I and 20 mg of rose bengal in 200 ml of 0.5 N sodium hydroxide was treated as described above. The irradiation was interrupted when 175 ml (8 mmoles) of oxygen had been consumed. One-half (100 ml) of the reaction mixture was acidified and extracted with 1-butanol. The butanol extract gave six fluorescent spots on a paper chromatogram (Figure 3). The major spots were identified as I and II. From the butanol extract, 80 mg of crystals was isolated by preparative paper chromatography (solvent, 2 N NH_4OH–1-butanol). The product was identified as II by comparison of its infrared spectrum with that of an authentic sample.

The other half of the mixture was further irradiated until a total of 10 mmoles of oxygen/3.8 mmoles of I had been consumed. The mixture was treated as described above. The butanol extract showed no more I and II on a paper chromatogram. On a thin layer chromatogram at least seven spots were detected (Figure 3). These products were not further investigated.

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