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Abstract

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A New Method for the Synthesis of Alloxazines

On the Condensation of Aromatic *o*-Diamines with Violuric Acid and Its Thio Analogues

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Aromatic *o*-diamines in a neutral or acetic-acid medium, as we have shown (^{1}), react with violuric acid with formation of ureides of 2-oxyquinoxalinecarbonic-3 acid, the reaction proceeding without liberation of water.

In the present investigation we have established that boric acid in an alcoholic solution of acetic acid, upon interaction of the same starting substances, causes the reaction of alloxazine formation, proceeding with liberation of one molecule of water. Thus, from 4,5-dimethyl-*o*-phenylenediamine (I) and violuric acid, lumichrome (III) was obtained, along with the ureide of 6,7-dimethyl-2-oxyquinoxalinecarbonic-3 acid (V).

$$\text{I. } R = CH_3 \qquad \text{II. } R = H$$

$$\text{I, II} + \text{violuric acid} \xrightarrow[-NH_2OH]{} \begin{cases} \text{III. } R = CH_3 \\ \text{IV. } R = H \end{cases}$$

$$\xrightarrow[-H_2O]{}$$

$$\begin{matrix} 1.\ CH_3COOH \\ 2.\ CH_3COOH + H_3BO_3 \end{matrix} \qquad \text{V. } R = CH_3$$

Identification of the substances and determination of their ratio in the mixture as a function of the amount of boric acid (Table 1) were carried out by paper chromatography and by studying their ultraviolet absorption spectra and comparing them with pure substances and literature data ($^{2-7}$).

Under the conditions indicated above, lumichrome is formed as a result of the reaction between 6,7-dimethyl-*o*-phenylenediamine and violuric acid, and not as the product of its possible deoximation—alloxan—which is not detected even in negligible traces in control experiments carried out with 8 moles of boric acid under the usual condensation conditions, but in the absence of 6,7-dimethyl-*o*-phenylenediamine (the determination was carried out according to (^{1})).

In aqueous acetic acid, upon condensation of compound I with violuric acid in the presence of 8 moles of boric acid, lumichrome becomes the sole reaction product (Table 1). With a decrease in the amount of boric acid, the reaction gives a mixture of lumichrome (III) and the ureide of chinoxalinecarboxylic acid (V).

The reaction is very strongly catalyzed by protons. On heating in dilute hydrochloric acid, the yield of lumichrome reaches 70% (Table 2).

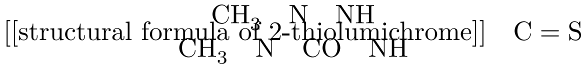
Table 1

Effect of the amount of boric acid on the yield and ratio of III and V in the condensation of violuric acid (1 mole) and 4,5-dimethyl-*o*-phenylenediamine (1.1 moles) in acetic acid solution

Amount of boric acid, mole	Yield, % III	Yield, % V	Molar ratio III : V
In alcoholic solution	**In alcoholic solution**	**In alcoholic solution**	**In alcoholic solution**
0	0	42.3	–
0.5	5.8	25.2	1 : 4.3
1.5	6.6	18.3	1 : 2.8
2.5	9.5	17.5	1 : 1.8
8.0	8.3	15.3	1 : 1.8
In aqueous solution	**In aqueous solution**	**In aqueous solution**	**In aqueous solution**
0	0	31.0	–
0.5	7.0	19.0	1 : 2.7
1.5	8.25	18.3	1 : 2.2
2.5	9.5	17.2	1 : 1.8
5.0	20.6	6.9	1 : 0.33
8.0	28.0	0	–

Analogously to the reaction with a substituted aromatic *o*-diamine, violuric acid also reacts with the amino groups of *o*-phenylenediamine (II); under the same reaction conditions alloxazine (IV) was obtained in 73.5% yield (Table 2). The optimum yield of alloxazines is observed with a 10% excess of aromatic *o*-diamines.

From 4,5-dimethyl-*o*-phenylenediamine, by condensation with 2-thiovioluric acid in an alcoholic solution of hydrogen chloride, 2-thiolumichrome (VI) was obtained in 58% yield



$$\begin{array}{l} \qquad\qquad CH_3 \quad N \quad NH \\ \text{[[structural formula of 2-thiolumichrome]]} \quad C = S \\ \qquad\qquad CH_3 \quad N \quad CO \quad NH \end{array}$$

(VI)

It should be noted that substituted *o*-phenylenediamine under the same conditions reacts with alloxan and violuric acid differently. In strongly diluted hydrochloric acid or in aqueous acetic acid in the presence of boric acid, alloxan forms the ureide of oxychinoxalinecarboxylic acid considerably more readily than alloxazine (7), whereas violuric acid reacts with greater difficulty, with formation either exclusively of alloxazine or of its mixture with the ureide of oxychinoxalinecarboxylic acid. It was of interest to determine which of the nitrogen atoms of the amino group of *o*-phenylenediamine (variant "a") or of the isonitroso group of violuric acid

Table 2

Effect of hydrochloric acid concentration on the yield of compounds III and IV

Yield, %	0.3	0.6	1.0	2.0	3.0	4.0
HCl concentration, equiv/L	**0.3**	**0.6**	**1.0**	**2.0**	**3.0**	**4.0**
Lumichrome	16.6	24.8	29.8	49.8	70.0	45.1
Alloxazine	20.7	33.0	60.0	65.4	73.5	52.7

(variant "b") participates in the formation of the pyrazine ring. This question was investigated with the aid of violuric acid with a labeled nitrogen atom (N^{15}) of the isonitroso group, which was obtained under model conditions (8) by nitrosation of barbituric acid with $NaN^{15}O_2$, synthesized by reduction of $NaN^{15}O_3$ (containing 10.7 atom % N^{15}) with lead sulfide.

method (^{9}). The alloxazine obtained from labeled violuric acid was burned by the Kjeldahl method; the ammonium salt was oxidized with sodium hypobromite to nitrogen, whose isotopic composition was then determined mass-spectrometrically.

Found, at. %: N^{15} 0.417; 0.419
$C_{10}H_6N_2O_4$. Calculated, at. %: N^{15} 0.38 (variant "a"), 2.96* (variant "b").

Comparison of the experimental and calculated values makes it possible to conclude that the condensation proceeds as a result of nucleophilic addition of the N atom of the amino group of *o*-phenylenediamine to the electrophilic carbon atom at position 5 of violuric acid, with elimination of the isonitroso group in the form of hydroxylamine and subsequent or simultaneous closure of the pyrazine ring of the alloxazine molecule.

It should be noted that, in the interaction of violuric acid with *m*-phenylenediamine, in contrast to the reaction with *o*-phenylenediamine, its isonitroso group reacts not with the amino group of the *m*-diamine, but enters into an electrophilic substitution reaction at the carbon atom ([10,11]) at which the maximum electron density is concentrated (coincident ortho-, para-orientation of both amino groups); in this case, one nitrogen atom of *m*-phenylenediamine and the nitrogen atom of the isonitroso group of violuric acid participate in the formation of the pyrazine ring. In the molecule of *o*-phenylenediamine, a sufficient shift of electron density is not created toward any of the carbon atoms (noncoincident orientation of the amino groups) required for electrophilic substitution, and it enters into the nucleophilic addition reaction through the N atom of the amino group.

Experimental Part

Interaction of 4,5-dimethyl-*o*-phenylenediamine (I) with violuric acid. A mixture of 1.57 g of violuric acid, 1.62 g of compound I, and 40 ml of acetic acid in the presence of boric acid (amounts—see Table 1) is boiled for 5 hr. The yellow precipitate that separates is filtered off and washed with boiling water and with alcohol (2 × 20 ml). The precipitate is chromatographed together with standards on paper (grade "M") in an ascending stream in system 1 (*n*-butanol—pyridine—water, 6 : 4 : 3). Spots of two substances are obtained: lumichrome (III) with R_f 0.75 (blue-white fluorescence) and the urend of 6,7-dimethyl-2-oxyquinoxalinecarbonic acid-3 (V) with R_f 0.67 (yellow-green fluorescence). The spots are cut out, eluted with alcohol, and the contents of compounds III and V in the eluates are determined fluorometrically relative to analytically pure samples of the corresponding compounds (Table 1).

6,7-Dimethylalloxazine, lumichrome (III). 1) A mixture of 1.57 g of violuric acid, 1.62 g of 4,5-dimethyl-*o*-phenylenediamine (I), 4.95 g of boric acid (in molar ratios 1 : 1.1 : 8), and 40 ml of an aqueous solution of acetic acid (1 : 1) is boiled for 5 hr; the precipitated solid is filtered off and washed with boiling water and alcohol (2 × 20 ml). This gives 0.68 g (28%) of lumichrome (III)—lemon-yellow needles, not melting up to 360° (from glacial acetic acid, 1 : 75). R_f 0.67 (blue-white fluorescence) in system 2 (*n*-butanol—acetic acid—water, 4 : 1 : 5); according to ([6]), R_f 0.69; according to ([3]), R_f 0.72. Absorption spectrum in alcohol: $\lambda_{\max}$ 250 mμ (ε $3.03 \cdot 10^4$), 338 mμ (ε $1.01 \cdot 10^4$), and 385 mμ (ε $0.96 \cdot 10^4$)—corresponds to the data of ([3]).

Found, %: C 59.26, 59.11; H 4.44, 4.39; N 23.01, 23.38
$C_{12}H_{10}O_2N_4$. Calculated, %: C 59.49; H 4.16; N 23.13

$$* \frac{10.7 + 3 \cdot 0.38}{4} = 2.96.$$

When the amount of boric acid is decreased, the reaction proceeds nonuniformly and leads to a mixture (see Table 3) of lumichrome (III) and the ureide of 6,7-dimethyl-2-oxyquinoxalinecarbonic-3-acid (V)—lemon-yellow needles with m.p. 255–257° with decomp. (recrystallized from glacial acetic acid), corresponding to the data of (1). R_f 0.65 in system 2 (yellow-green fluorescence); according to (12) R_f 0.63. Absorption spectrum in alcohol: $\lambda_{\max}$ 239 mμ (ε $2.40 \cdot 10^4$), 325 mμ (ε $1.11 \cdot 10^4$), and 403 mμ (ε $0.71 \cdot 10^4$)—corresponds to the data of (1).

2) From 1.57 g of violuric acid and 1.62 g of 4,5-dimethyl-*o*-phenylenediamine (I) in 40 ml of 3 *N* HCl solution, under the same reaction and isolation conditions, 1.7 g (70%) of lumichrome (III) is obtained—lemon-yellow needles. R_f 0.67 in system 2; according to (6) R_f 0.69. Absorption spectrum in alcohol: $\lambda_{\max}$ 250 mμ (ε $2.90 \cdot 10^4$), 338 mμ (ε $0.89 \cdot 10^4$), and 385 mμ (ε $0.66 \cdot 10^4$)—corresponds to the data of (3).

Found % : C 59.64, 59.66; H 4.31, 4.33; N 22.87, 23.03
$C_{12}H_{10}O_2N_4$. Calculated % : C 59.49; H 4.16; N 23.13

Alloxazine (IV). From 1.57 g of violuric acid, 1.28 g of *o*-phenylenediamine (II), and 40 ml of 3 *N* HCl solution, by the method described above, 1.55 g (73.5%) of alloxazine is obtained—lemon-yellow needles, not melting up to 360° (from glacial acetic acid, 1 : 250). R_f 0.78 in system 1; according to (7) R_f 0.74, R_f 0.55 in system 2. Absorption spectrum in 1 *N* sodium carbonate solution: $\lambda_{\max}$ 250 mμ (ε $3.10 \cdot 10^4$), 326 mμ (ε $0.66 \cdot 10^4$), and 385 mμ (ε $0.63 \cdot 10^4$)—corresponds to the data of (7).

Found % : C 55.85, 56.13; H 2.97, 2.88; N 25.86, 25.84
$C_{10}H_6O_2N_4$. Calculated % : C 55.08; H 2.81; N 26.16

6,7-Dimethyl-2-thioalloxazine, 2-thiolumichrome (VI). From 1.73 g of 2-thiovioluric acid, 1.62 g of 4,5-dimethyl-*o*-phenylenediamine (I), and 40 ml of 3 *N* alcoholic hydrogen chloride solution, as described above, 1.50 g (58%) of 2-thiolumichrome is obtained—yellow needles (from ethylene glycol); m.p. above 350°. R_f 0.77 in system 2; according to (5) R_f 0.80. Absorption spectrum in alcohol: $\lambda_{\max}$ 224 mμ (ε $2.45 \cdot 10^4$), 261 mμ (ε $1.79 \cdot 10^4$), 295 mμ (ε $3.43 \cdot 10^4$), 361 mμ (ε $0.75 \cdot 10^4$), and 410 mμ (ε $1.19 \cdot 10^4$)—corresponds to the data of (5).

Found % : S 12.13; N 22.26
$C_{12}H_{10}N_4OS$. Calculated % : S 12.41; N 21.72

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Note: Figure translations are in progress. See original paper for figures.