

hexene oxides, and the stereochemistry of these oxides and certain of their derivatives, are described.

4. Various transformation products of the cyclohexene derivative have been described,

among them the corresponding dibenzoylxylylene and α,α' -diphenyl- β,β' -(3,6-dimethylbenzo)-furan. It is believed that the methods described are general for the preparation of compounds of these types.

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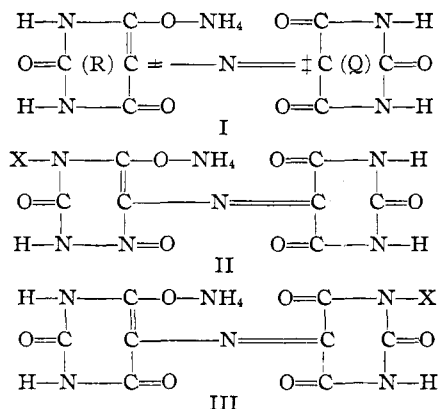
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[CONTRIBUTION FROM JONES CHEMICAL LABORATORY, UNIVERSITY OF CHICAGO]

Structure of the Murexides and the Alloxantines¹

By N. M. WINSLOW²

The structure of the dye murexide (I) was established independently by Stieglitz,³ Moehlau,⁴ and Piloty.⁵ Acid hydrolysis of the dye gives uramil and alloxan in semiquantitative yields. Hydrolysis should, therefore, provide a means of distinguishing isomeric N-substituted murexides of general structures II and III.⁶ Hydrolysis of II should give N-substituted uramil and alloxan, while similar treatment of III should give uramil and N-substituted alloxan. However, if II and III exist in tautomeric equilibrium, or if resonance occurs between the two forms, then any preparative procedure calculated to yield either II or III would give the same mixture of the two, and hydrolysis of the product would yield four products: N-substituted uramil, N-substituted alloxan, uramil, and alloxan. The present investigation is concerned with the two isomeric N-phenylmurexides, 1-phenylmurexide (II, X = C₆H₅) and 1'-phenylmurexide (III, X = C₆H₅).



(1) A part of a dissertation submitted by the author in partial fulfillment of the requirements for the degree of Doctor of Philosophy at the University of Chicago in 1936. The investigation was carried out under the direction of the late Professor Julius Stieglitz.

(2) Present address: Lakewood, Ohio.

(3) Stieglitz, *Am. Chem. J.*, **31**, 661 (1904).

(4) Moehlau, *Ber.*, **37**, 2686 (1904).

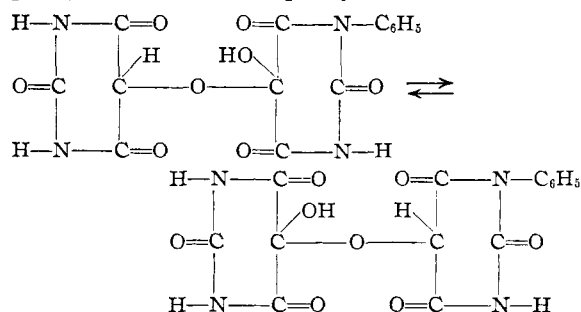
(5) Piloty, *Ann.*, **333**, 22 (1904).

(6) The murexides decompose without melting. A study of absorption spectra should furnish confirmatory evidence.

1-Phenylmurexide was prepared readily from 1-phenyluramil and alloxan in ammonium carbonate solution. When the dye was hydrolyzed within forty-eight hours of its preparation, 1-phenyluramil and alloxan, the latter determined as alloxantine, were recovered in yields representing 70 and 56%, respectively, of the theoretical.

The synthesis of 1'-phenylmurexide in a manner analogous to the method used for the preparation of 1-phenylmurexide was attempted without success, due to the relative insolubility of uramil in ammonium carbonate solution and the instability of the murexide in alkali sufficiently strong to dissolve uramil. There remained the alternative method of preparing the corresponding 1'-phenylalloxantine and treating this compound with ammonia.

Pure 1'-phenylalloxantine was prepared by the condensation of N-phenylalloxan with dialuric acid in hot aqueous solution. But chemical purity, as established by elementary analysis, was not the only requirement of 1'-phenylalloxantine intended for use in the preparation of 1'-phenylmurexide; it must also maintain its tautomeric identity. Quite obviously if a tautomeric equilibrium existed, treatment of the alloxantine with ammonia would give only a mixture of 1-phenylmurexide and 1'-phenylmurexide.



When 1'-phenylalloxantine was hydrolyzed with aqueous potassium acetate, dialuric acid, pre-

precipitated immediately as the potassium salt, was obtained in 98% yields. This quantitative yield of potassium dialurate showed that 1'-phenylalloxantine maintained its identity under the conditions of preparation, a conclusion in entire agreement with the results of Stieglitz and Nightingale,⁷ obtained with 1-methylalloxantine and 1'-methylalloxantine.

In this connection it was of interest to study the isomeric 1-phenylalloxantine, prepared by heating 1-phenyluramil with alloxan solution containing hydrochloric acid. Hydrolysis of the pure material with sodium acetate produced 74.5% of the theoretical yield of N-phenyldialuric acid, giving additional confirmation of the views of Stieglitz and Nightingale regarding the asymmetrical structure of alloxantines and the maintenance of identity by isomeric N-substituted alloxantines. One further observation lends strong support to the conclusion of these authors. When 1-phenylalloxantine, which presumably is hydrolyzed to N-phenyldialuric acid and alloxan, was treated with potassium acetate, potassium dialurate was obtained.⁸ However, it was precipitated slowly, as contrasted with immediate precipitation when 1'-phenylalloxantine was treated with potassium acetate, and the total amount obtained was approximately 67% of the theoretical. Their explanation of the formation of potassium dialurate from the hydrolysis of the methylalloxantine prepared from alloxan and methyluramil, as the result of a slow inter-(or intra-?) molecular oxidation and reduction, thus was fully confirmed by our own work with the alloxantine prepared from the alloxan and phenyluramil.

By treatment of 1'-phenylalloxantine, which had been shown to maintain its identity under the conditions of preparation, with ammonia, 1'-phenylmurexide was obtained. Hydrolysis of this dye gave 96.5% of the theoretical yield of uramil. N-Phenylalloxan could not be isolated as a hydrolysis product because of its instability under the acid conditions of hydrolysis.

Since the two N-phenylmurexides synthesized by different procedures gave different hydrolysis products, it must be concluded that the two isomers maintain their identity at least for a

limited length of time. Both resonance and rapid tautomerism between the two are ruled out, but this conclusion in no way precludes the probability that each of the dyes will in the course of time be transformed into identical equilibrium mixtures which would give identical hydrolysis products.

Similar results have been obtained with two other pairs of isomeric N-substituted murexides: Stieglitz and Slimmer,³ later Stieglitz and Eckstein,⁹ and finally Stieglitz and Tartar,¹⁰ prepared the two isomers 1,3-dimethylmurexide and 1',3'-dimethylmurexide; hydrolysis of the former yielded dimethyl uramil and alloxan, while uramil and dimethylalloxan were obtained from the latter as hydrolysis products. More recently,¹¹ the isomers 1,3-diphenylmurexide and 1',3'-diphenylmurexide were prepared and similarly shown to yield different hydrolysis products.

These investigations were undertaken in connection with the Stieglitz theory of color production in organic molecules.¹² It is the fundamental concept of this theory that color of organic molecules is due to partial absorption of white light by vibration of electrons held in proper restraint between their own atomic nucleus in a reducing group (the auxochrome) and a nearby strongly positive group (the chromophore). For example, in the structure (I) of the dye murexide, it is, in particular, the pair of electrons indicated by—in C^+ , held in loose restraint in the reducing uramil group R, which are attracted both by the positive nucleus of C^+ itself and by the alloxan group Q whose oxidizing power may be considered centered in C^+ . According to the views of Stieglitz, the color-producing vibrations of these electrons are intra-atomic, that is, between the different energy levels of an atom (C^+), rather than interatomic, that is, between two different atoms (C^+ and C^+), leading to tautomeric equilibrium.

While results obtained with the N-substituted murexides discussed above do not establish the source of color in the molecules, they are in complete agreement with the Stieglitz theory. On the other hand, early theories¹³ holding that the color of dye molecules is due to electronic movements involved in tautomeric equilibrium are

(9) Unpublished results, University of Chicago, 1905.

(10) H. V. Tartar, "The Constitution of Murexide and the Theory of Dyes," Doctoral Dissertation, University of Chicago, 1920.

(11) R. M. Cole, Doctoral Dissertation, University of Chicago, 1936.

(12) Stieglitz, *Proc. Natl. Acad. Sci.*, **9**, 303 (1923); *J. Franklin Inst.*, **198**, 35 (1924).

(13) Armstrong, *Proc. Chem. Soc.*, **2**, 27 (1888); Baeyer, *Ann.*, **354**, 153 (1907); Willstaetter, *Ber.*, **41**, 1458, 3245 (1908).

(7) D. N. Nightingale, "Studies in the Murexide and Alloxantine Series," Doctoral Dissertation, University of Chicago, 1928; cf. Nightingale, *THIS JOURNAL*, **59**, 806 (1937).

(8) The potassium salt of N-phenyldialuric acid is soluble in water.

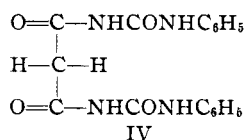
definitely disproved. Resonance involving the valence bonds of the central nitrogen atom in the murexide molecule is also eliminated as the source of color. Nothing is indicated concerning the bonds of other atoms in the molecules.

The results are of more recent interest in connection with the theory of resonance. If the structure (I) of the parent substance is correct, an N-substituted murexide would be expected to exist as a resonance hybrid of II and III. The fact that three different isomeric pairs of N-substituted murexides have been shown to maintain their identity presents an interesting problem.

Experimental

Preparation of Intermediate Compounds.—N-Phenylbarbituric acid was prepared by refluxing a mixture of malonic acid (50 g.), phenylurea¹⁴ (64 g.), acetic anhydride (100 cc.) and dry chloroform (700 cc.) for twenty-four hours. The chloroform was removed by distillation, and from the dry residue a 40% yield of N-phenylbarbituric acid was obtained by extraction with cold aqueous sodium carbonate.

The material undissolved by cold aqueous sodium carbonate contained a small amount of symmetrical acetylphenylurea, but consisted chiefly of malonyl dimonophenylurea (IV):



This compound was obtained in the pure condition by recrystallization from acetone.

Anal. Calcd. for $\text{C}_{17}\text{H}_{16}\text{O}_4\text{N}_4$: C, 60.00; H, 4.71; N, 16.20. Found: C, 60.02; H, 5.11; N, 16.21.

The malonyl dimonophenylurea was dissolved in 500 cc. of 0.6 M sodium carbonate by digestion for one hour on a steam-bath. Chilling of the solution precipitated about 25% of the phenylurea originally used in the preparation, and the addition of hydrochloric acid gave an additional 20% yield of N-phenylbarbituric acid.

For the purposes of this investigation, N-phenylbarbituric acid prepared as described proved to be sufficiently pure, melting from 254.5 to 258.5°. A pure sample, obtained by recrystallization from dioxane, melted at 262°. The recorded¹⁵ melting point is 262°.

1-Phenyluramil was prepared from a solution of sodium phenylviolurate obtained by digestion at 50° of N-phenylbarbituric acid (5 g.) with 100 cc. of water containing sodium nitrite (1.5 g.). The solution was reduced with hydrogen sulfide, acidified with 50 cc. of concentrated hydrochloric acid, warmed to 45°, and filtered from precipitated sulfur. 1-Phenyluramil crystallized slowly after the addition of four volumes of alcohol; yield 1.7 g.

Anal. Calcd. for $\text{C}_{10}\text{H}_9\text{O}_3\text{N}_3$: N, 19.18. Found: N, 19.25.

(14) Davis and Blanchard, *THIS JOURNAL*, **51**, 1791 (1929).

(15) Macbeth, Nunan and Traill, *J. Chem. Soc.*, **129**, 1, 1252 (1926).

N-Phenylalloxan was prepared by oxidation at 0° of 1-phenyluramil (0.50 g.) with 2 cc. of nitric acid (sp. gr. 1.42). The reaction mixture was dried *in vacuo*, and N-phenylalloxan was recovered by extraction with ether and precipitation from the ether solution with four volumes of ligroin. Yields varied from 60 to 90% of the theoretical.

Anal. Calcd. for $\text{C}_{10}\text{H}_9\text{O}_3\text{N}_2$: C, 50.95; H, 3.36; N, 11.66. Found: C, 50.65; H, 3.31; N, 11.94.

Preparation of Alloxantines.—1-Phenylalloxantine was prepared by heating at 50° for six hours a suspension of 1-phenyluramil (1 g.) in a solution of alloxan monohydrate (0.9 g.)¹⁶ in 12 cc. of water containing 1 cc. of concentrated hydrochloric acid. The product was collected on a filter, washed with cold water and dried *in vacuo*; yield 1.4 g.

Anal. Calcd. for $\text{C}_{14}\text{H}_{10}\text{O}_3\text{N}_4$: N, 15.47. Found: N, 15.50.

A solution of dialuric acid was obtained by reduction of alloxan monohydrate (1.00 g.) dissolved in 12.5 cc. of water with hydrogen sulfide at 98°. When the hot solution, filtered from the precipitated sulfur, was mixed with a hot solution of N-phenylalloxan (0.50 g.) in 6 cc. of water, 1'-phenylalloxantine crystallized over a period of about ten minutes. The product was washed with cold water, alcohol, and ether; yield 0.8 g.

Anal. Calcd. for $\text{C}_{14}\text{H}_{10}\text{O}_3\text{N}_4$: N, 15.47. Found: N, 15.42.

Preparation of Murexides.—1-Phenylmurexide was prepared by mixing a solution of alloxan monohydrate (2 g.) in 10 cc. of water with a solution of 1-phenyluramil (2 g.) in 10 cc. of 20% ammonium carbonate solution. The mixture was heated for thirty minutes at 50° in an atmosphere of hydrogen. The product was purified by repeated extraction with 15-cc. portions of 95% alcohol; yield 1.1 g.

Anal. Calcd. for $\text{C}_{14}\text{H}_{12}\text{O}_6\text{N}_6$: N, 23.33. Found: N, 23.57.

1'-Phenylalloxantine (1.5 g.) suspended in absolute benzene at 50°, was converted to 1'-phenylmurexide by passing a stream of dry ammonia gas through the suspension for three hours. The murexide was purified by washing with benzene and heating in air for ten hours at 50°; yield 1.5 g.

Anal. Calcd. for $\text{C}_{14}\text{H}_{12}\text{O}_6\text{N}_6$: N, 23.33. Found: N, 23.71.

Hydrolysis of Alloxantines and Murexides.—A suspension of 1-phenylalloxantine (0.500 g.) in 19 cc. of water containing sodium acetate (0.35 g.) was heated on a steam-bath until the alloxantine dissolved. From the clear hydrolysate, acidified with 1 cc. of 50% acetic acid, basic lead N-phenyldialurate¹⁷ was precipitated by the addition of 0.600 g. of lead acetate [$\text{Pb}(\text{C}_2\text{H}_3\text{O}_2)_2 \cdot 3\text{H}_2\text{O}$]; yield 0.455 g., theoretical 0.611 g.

Anal. Calcd. for $\text{C}_{10}\text{H}_9\text{O}_3\text{N}_2\text{Pb}$: N, 6.32; Pb, 46.73. Found: N, 6.67; Pb, 46.50.

1'-Phenylalloxantine was hydrolyzed in the same manner as 1-phenylalloxantine except that potassium acetate was

(16) Biltz and Heyn, *Ann.*, **413**, 60 (1917); *J. Chem. Soc.*, **112**, I, 289 (1917).

(17) This compound was also prepared from N-phenyldialuric acid obtained by reduction of N-phenylalloxan with hydrogen sulfide at 98°.

Anal. Calcd. for $\text{C}_{10}\text{H}_9\text{O}_3\text{N}_2\text{Pb}$: N, 6.32; Pb, 46.73. Found: N, 6.91; Pb, 46.74.

used instead of sodium acetate. When the hydrolysate was chilled in ice, potassium dialurate was obtained; yield 0.247 g. from 0.500 g. of 1'-phenylalloxantine, theoretical 0.252 g.

Anal. Calcd. for $C_8H_5O_4N_2K$: N, 15.38. Found: N, 15.48.

1-Phenylmurexide (1.000 g.), suspended in 100 cc. of oxygen-free water by the passage of a slow stream of hydrogen, was hydrolyzed with 16 cc. of approximately molar hydrochloric acid added a few drops at a time over a period of forty-five minutes. The solid material remaining when the color of the solution had been completely discharged was brought upon a filter and identified as 1-phenyluramil by its qualitative reactions and nitrogen content; yield 0.427 g., theoretical 0.605 g. From the filtrate from the 1-phenyluramil, concentrated at room temperature to 5 cc., alloxantine was obtained by reduction with stannous chloride; yield 0.205 g., theoretical 0.364 g.

Hydrolysis of 1'-phenylmurexide (1.000 g.) by the procedure used for the hydrolysis of 1-phenylmurexide yielded 0.383 g. of uramil; theoretical 0.397 g. Due to the instability of N-phenylalloxan¹⁸ in acid solution, only traces

(18) 1,1'-Diphenylalloxantine was obtained in 73% yields by reduction of a freshly prepared acid solution of N-phenylalloxan with stannous chloride. When the acid solution was allowed to stand for forty-five minutes before reduction, the yield was only 1.5%.

Anal. Calcd. for $C_{20}H_{14}O_8N_4$: N, 12.79. Found: N, 12.87.

of 1,1'-diphenylalloxantine could be obtained from the filtrate from the uramil by reduction with stannous chloride.

Summary

1. The isomers 1-phenylmurexide and 1'-phenylmurexide maintain their identity at least for a limited length of time under certain experimental conditions. 1-Phenylalloxantine and 1'-phenylalloxantine also maintain their isomeric identities.

2. These phenylmurexides constitute the third pair of isomeric N-substituted murexides for which maintenance of identity has been established. The importance of this fact in connection with the theories of color production and resonance in organic molecules has been discussed.

3. The following compounds not described in the literature have been prepared: 1-phenylmurexide, 1'-phenylmurexide, 1-phenylalloxantine, 1'-phenylalloxantine, 1,1'-diphenylalloxantine, 1-phenyluramil, N-phenylalloxan, and malonyl dimonophenylurea.

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[CONTRIBUTION FROM THE DEPARTMENT OF PHYSICAL CHEMISTRY, HARVARD MEDICAL SCHOOL]

The Influence of Ionic Strength and pH on Electrophoretic Mobility

BY BERNARD D. DAVIS AND EDWIN J. COHN

Electrophoretic mobilities are generally determined in the presence of buffer solutions. It seemed of importance to investigate further the influence of the valence of the buffer and of the ionic strength of the solution. Tiselius has noted^{1,2} the effect of ionic strength and of the valence of inorganic cations, and work in this Laboratory had indicated that the ionic strength of the buffer has a very profound influence on electrophoretic mobility. The influence of these variables also has been studied by Abramson³ and by Smith,⁴ who observed the movements of particles of quartz or collodion coated with egg albumin in an electric field. The latter studied especially the dependence of the isoelectric point upon the nature and concentration of neutral salts.

In the present investigation we have used the moving boundary method and have chosen horse carboxyhemoglobin as the protein to be investigated since it is a well characterized protein whose other properties have been investigated extensively.⁵⁻⁹ Mobility has been determined from pH 5.65 to 7.2. Hemoglobin was therefore present as cation in most of these systems, whereas most of the other blood proteins would be present as anions. The ionic strengths vary from 0.02, the concentration often used by Tiselius, to ionic strengths characteristic of physiological systems. Two sets of buffers were employed, namely, phosphates in which monovalent and bivalent anions, and citrates in which bivalent and trivalent anions, are present. The measurements reported demonstrate the great influence

(1) A. Tiselius, Dissertation, Upsala, 1930.

(2) A. Tiselius, *Trans. Faraday Soc.*, **33**, 524 (1937).

(3) H. A. Abramson, "Electrokinetic Phenomena and their Application to Biology and Medicine," Chemical Catalog Co., Inc., New York, 1934.

(4) E. R. B. Smith, *J. Biol. Chem.*, **108**, 187 (1935); *ibid.*, **113**, 473 (1936).

(5) R. M. Ferry and A. A. Green, *ibid.*, **81**, 175 (1929).

(6) E. J. Cohn and A. M. Prentiss, Loeb Memorial Vol., *J. Gen. Physiol.*, **8**, 619 (1927).

(7) J. L. Oncley, *THIS JOURNAL*, **60**, 1115 (1938).

(8) R. M. Ferry, E. J. Cohn and E. S. Newman, *ibid.*, **60**, 1480 (1938).

(9) M. Richards, *J. Biol. Chem.*, **122**, 727 (1938).