One yellow dye has been isolated from such a solution. The conditions under which it changes into the red dye have been determined.

A mechanism has been proposed to account for the series of reactions which result in formation of the red dye, and the evidence is discussed.

ROCHESTER 4, NEW YORK RECEIVED JULY 26, 1944

[CONTRIBUTION FROM THE CHEMISTRY LABORATORY OF INDIANA UNIVERSITY]

o-Phenylenemalonamide

BY R. L. SHRINER AND P. G. BOERMANS[1]

The reaction between *o*-phenylenediamine and malonic acid has been found to produce a compound with the empirical formula $C_9H_8N_2O_2$. This compound has been obtained by a number of investigators,[2,3,4] but the only data bearing on its structure were the formation of a sparingly soluble sodium salt[2] and hydrolysis to regenerate *o*-phenylenediamine. Both Meyer[2] and Phillips[3] assigned the seven membered ring structure shown by formula I. This compound sublimed at about 360° and was quite insoluble in most solvents. Because of this low solubility and also since the reaction involved two bifunctional molecules[5] a polyamide structure was also suggested as a possibility.[4] The present paper reports experimental evidence that the compound does have the ring structure (I).

Because of the extreme insolubility of compound I in all solvents, its molecular weight could not be directly determined. To surmount this difficulty the dimethyl derivative (II) was prepared by treatment of compound I with methyl iodide and sodium ethoxide in alcoholic solution. Analysis revealed that methyl groups had replaced two hydrogen atoms. Although this dimethyl derivative had a lower melting point (255°) than I and was more soluble, good values for its molecular weight could not be obtained. By using *p*-nitrotoluene as a solvent, approximate values were found which indicated that this compound was not polymeric.

To determine whether the methyl groups were affixed to the nitrogen atoms or to the methylene group, the compound was hydrolyzed with dilute sulfuric acid. The expected products would be either *o*-phenylenediamine or N,N′-dimethyl-*o*-phenylenediamine. Neither of these was obtained but upon addition of an excess of potassium hydroxide, 1,2,3-trimethyl-2-hydroxy-2,3-dihydrobenzimidazole (III) was obtained. It is suggested that the acid hydrolysis opened the ring with subsequent loss of carbon dioxide to form N-acetyl-N,N′-dimethyl-*o*-phenylenediamine, which then cyclized to give III. Compound III has been described previously by Pinnow,[6] Fischer,[7] and Niementowski.[8]

For purposes of comparison compound III was prepared by a series of known reactions. 2-Methylbenzimidazole, prepared from *o*-phenylenediamine and acetic anhydride, was methylated with two moles of methyl iodide in a sealed tube at 100°. This method is similar to that of Fischer,[7] who carried out the methylation in two steps. The product, 1,2,3-trimethylbenzimidazole iodide (IV), was converted to the corresponding chloride (V) by shaking with silver chloride in hot methanol. Treatment of this chloride (V) with hot potassium hydroxide caused the precipitation of compound III.

Although compounds IV and V are quaternary ammonium salts, compound III is not a quaternary type of compound. This is shown by its ready solubility in the non-polar solvents, ether and benzene, but slight solubility in water. It is also stable to heat whereas quaternary ammonium bases decompose when heated.

Samples of compound III obtained by the two methods described above proved identical in every respect and a melting point of a mixture of the two samples showed no depression.

Experimental

***o*-Phenylenemalonamide.**—A mixture of 10.8 g. of *o*-phenylenediamine (0.1 mole) and 5.2 g. (0.05 mole) of malonic acid in 50 ml. of approximately 4 *N* hydrochloric acid was refluxed in an oil-bath at 125–130° for two and one-half hours with vigorous stirring. The mixture was cooled, filtered and insoluble product washed with cold water. The white granular product weighed 5.37 g. (61.8%). As previously reported,[2,3,4] it was insoluble in water, dilute acid, dilute alkalies, and organic solvents. The compound sublimes with partial decomposition near 360°.

Anal. Calcd. for $C_9H_8N_2O_2$: C, 61.34; H, 4.58; N, 15.91. Found: C, 61.39; H, 4.49; N, 15.88.

When equimolecular amounts of *o*-phenylenediamine and malonic acid are used in this same reaction, there forms in addition to the *o*-phenylenemalonamide, 2-amino-malonanilic acid as described by Phillips.[3]

Lower yields of *o*-phenylenemalonamide were obtained when diethylmalonate was used instead of the free acid, and when the ratio of *o*-phenylenediamine to malonic acid

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[1] From a thesis submitted to the Faculty of the Graduate School in partial fulfillment of the requirements for the degree, Doctor of Philosophy, in the Department of Chemistry, Indiana University.

[2] Meyer, *Ann.*, **327**, 1 (1903); **347**, 17 (1906); **415**, 29 (1918).

[3] Phillips, *J. Chem. Soc.*, 172, 2393 (1928); THIS JOURNAL, **64**, 187 (1942).

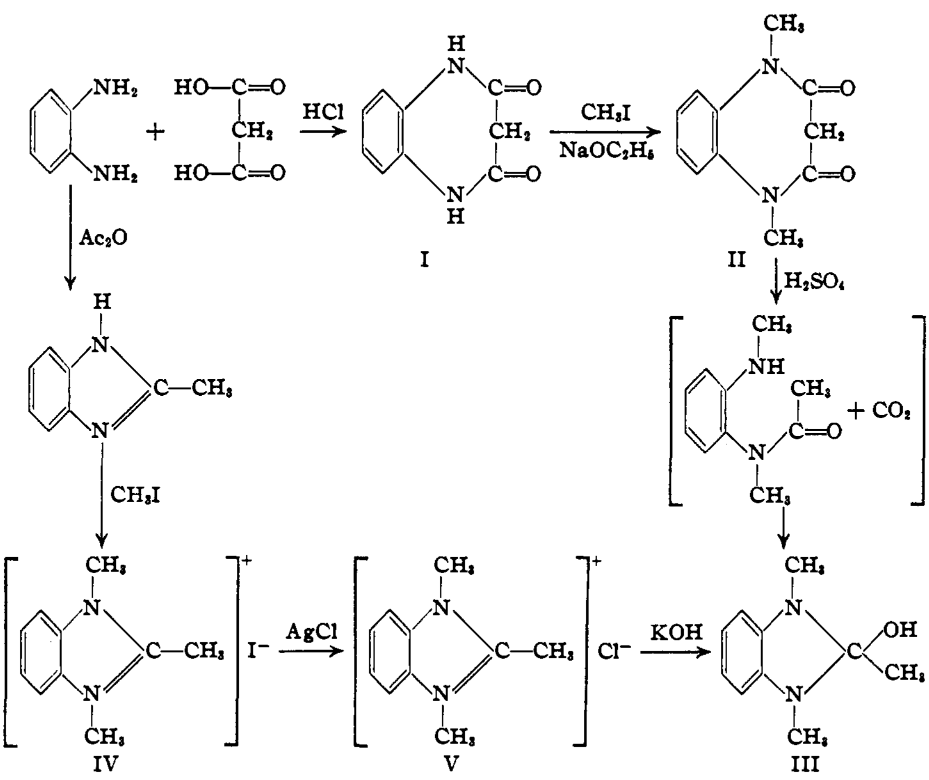
[4] Shriner and Upson, *ibid.*, **63**, 2277 (1941); **64**, 187 (1942).

[5] Carothers, *Trans. Faraday Soc.*, **32**, 41 (1936); *Chem. Rev.*, **8**, 353 (1931).

[6] Pinnow and Sämann, *Ber.*, **32**, 2191 (1899).

[7] Fischer, *ibid.*, **25**, 2826 (1892); *J. prakt. Chem.*, **73**, 427 (1906)

[8] Niementowski, *ibid.*, **20**, 1888 (1899).



was less than 2, and when the acidity of the hydrochloric acid was less than 4 *N*.

N,N'-Dimethyl-o-phenylenemalonamide.—A mixture of 11.72 g. of *o*-phenylenemalonamide and 10 ml. of methyl iodide in a 500-ml. flask containing 200 ml. of absolute ethanol to which 3.05 g. of sodium had previously been added, was refluxed for sixty hours. About every twelve hours a few ml. of methyl iodide was added. The *o*-phenylenemalonamide which did not react (7.65 g.) was filtered from the warm solution and the filtrate thoroughly cooled. The precipitate which formed was filtered, washed with water, and recrystallized from hot 95% ethanol. The fine, white needle-like crystals weighed 1.73 g. (37%) and melted at 255° (cor.).

Anal. Calcd. for $C_{11}H_{12}N_2O_2$: C, 64.67; H, 5.93; N, 13.73. Found: C, 64.70; H, 6.05; N, 13.72.

The molecular weight of this compound was determined cryoscopically using *p*-nitrotoluene as a solvent (K = 7.79). Three determinations gave values of 195, 191, 192 for the molecular weights; calcd. for $C_{11}H_{12}N_2O_2$, mol. wt., 204. The results indicate that the compound is not polymeric.

1,2,3-Trimethylbenzimidazole Iodide.—To 5.0 g. of 2-methylbenzimidazole, prepared according to the procedure of Phillips,[8] contained in a Pyrex tube of 30 mm. diameter was added 10.7 g. of methyl iodide. During this addition the tube was held in a dry ice-bath. The tube was sealed off to a length of 25 cm. and held at 97–100° in a steam jacket for forty-eight hours. After cooling, the tube was opened and the hard, reddish product removed. Its weight of 10.93 g. showed that the calculated amount of hydrogen iodide (4.85 g.) has been lost. The product was recrystallized twice from hot ethanol in 53% yield (5.62 g.) and gave a melting point of 256–259° (cor.). Fischer[7] reported a melting point of 254° for this compound.

Anal. Calcd. for $C_{10}H_{13}N_2I$: C, 41.66; H, 4.55; N, 9.60. Found: C, 41.21; H, 4.44; N, 9.65.

1,2,3-Trimethylbenzimidazole Chloride.—To 0.49 g. of 1,2,3-trimethylbenzimidazole iodide dissolved in 5.6 ml. of absolute methanol in a 25-ml. Erlenmeyer flask, a small excess of dry silver chloride was added. The flask was stoppered and placed in a water-bath at 60–65° for several hours with frequent shaking. Occasional cooling to room temperature gave a precipitate of starting material but, as the reaction progressed, the amount of precipitated diminished, which indicated formation of the more soluble chloride. The suspension remained at room temperature for two days before the silver iodide was removed by filtration. Evaporation of the methyl alcohol from a watch glass left 0.29 g. (87%) of crystalline residue which melted at 226–231° (cor.). This is in fair agreement with the melting point of 225–230° given by Pinnow[6] for this compound.

1,2,3-Trimethyl-2-hydroxy-2,3-dihydrobenzimidazole.—To 10 ml. of 20% sulfuric acid in a 50-ml. Erlenmeyer flask, 0.9 g. of N,N'-dimethyl-*o*-phenylenemalonamide was added and the mixture heated so that it refluxed. After thirty minutes all of the compound had dissolved. The heating was continued for a total of three hours. The cooled solution was made neutral to litmus with potassium hydroxide and extracted with three 10-ml. portions of ether. The ether extract was dried with potassium hydroxide pellets and the ether evaporated. No residue remained, indicating that no N,N'-dimethyl-*o*-phenylenediamine had been formed by the reaction.

To the neutral aqueous solution was added a large excess of strong potassium hydroxide solution. Fine, white crystals of 1,2,3-trimethyl-2-hydroxy-2,3-dihydrobenzimidazole separated from the solution. After removal by filtration they were washed with cold water and recrystallized from hot water to give 0.48 g. (61%) of the compound. It was insoluble in dilute alkali, slightly soluble in cold water, soluble in hot water, ether, benzene. ethanol, and dilute acids, and gave a melting point of 165–165.5° (cor.).

Anal. Calcd. for $C_{10}H_{14}N_2O$: C, 67.36; H, 7.92; N, 15.67. Found: C, 67.34; H, 7.83; N, 15.27.

This compound was also prepared from 1,2,3-trimethylbenzimidazole chloride after the method described by

Pinnow.[6] To 0.20 g. of 1,2,3-trimethylbenzimidazole chloride, dissolved in 1.5 ml. of distilled water, 0.4 g. of potassium hydroxide was added. A flocculent precipitate formed upon addition of the potassium hydroxide. The mixture was heated to boiling, cooled and filtered to give 0.15 g. (83%) of product, which after recrystallization from hot water melted sharply at 165° (cor.), which checked the value reported by previous investigators.

The melting points of the products obtained by both methods and of a mixture of the two products were determined simultaneously. All these samples melted at exactly the same temperature and in the same manner.

Summary

o-Phenylenediamine and malonic acid react to give *o*-phenylenemalonamide, a compound containing a seven-membered ring. Alkylation of this compound produced a N,N′-dimethyl derivative which underwent rearrangement on treatment with sulfuric acid to produce 1,2,3-trimethyl-2-hydroxy-2,3-dihydrobenzimidazole.

BLOOMINGTON, INDIANA RECEIVED JULY 13, 1944

[CONTRIBUTION FROM BACTERIOLOGY SECTION, IOWA AGRICULTURAL EXPERIMENT STATION, AND CHEMISTRY DEPARTMENT, IOWA STATE COLLEGE, AND DEPARTMENT OF BACTERIOLOGY, TUSKEGEE INSTITUTE]

The Degradation of Heavy-Carbon Butyric Acid from the Butyl Alcohol Fermentation[1]

BY H. G. WOOD, R. W. BROWN, C. H. WERKMAN AND C. G. STUCKWISCH

The present investigation was part of a study in which C^{13} compounds were used as tracers for determining the mechanism of the butyl alcohol fermentation. When $CH_3C^{13}OOH$ was added to a fermentation of corn mash by the butyl alcohol bacteria, butyl alcohol was formed which contained heavy carbon. This alcohol was separated from the other neutral products of the fermentation by oxidation to the corresponding acid and was isolated by the distillation procedure of Schicktanz, *et al.*[2]

For an understanding of the mechanism of formation of the butyl alcohol, it was necessary to know not only the position of the heavy carbon in the carbon chain but also the concentration of heavy carbon in each position. Such information is essential in deciding whether two molecules of acetic acid unite, for example, as follows

$$2CH_3C^{13}OOH \longrightarrow CH_3C^{13}OCH_2C^{13}OOH + H_2O$$

$$CH_3C^{13}OCH_2C^{13}OOH + 8H \longrightarrow CH_3C^{13}H_2CH_2C^{13}H_2OH + 2H_2O$$

or, whether the acetic acid unites with an intermediate compound formed from the cornstarch. In this latter case probably only one position in the molecule would contain a concentration of heavy carbon in excess of the normal, since that portion of the molecule arising from the starch would have a normal concentration of C^{13}. Moreover, both types of reactions might occur simultaneously. The relative concentration of C^{13} in the respective positions of the molecule would then be a measure of the relative rates of the two reactions and would also designate the parts of the molecule which arise preferentially from the acetic acid and from an intermediate formed from the starch carbon.

Because of the limited amount of heavy carbon compound available it was necessary to employ a degradation procedure that could be conducted on approximately two millimoles of butyric acid. With this quantity of material it is difficult to use a degradation involving a number of reactions with pure and dry compounds. For this reason a reaction which could be conducted in aqueous solution of the acid was sought and the oxidation of butyrate following the procedure of Allen and Witzemann[3] was accordingly investigated. These authors have found that, under proper conditions of oxidation by hydrogen peroxide, there are approximately the following yields of compounds from ammonium butyrate

Carbon dioxide..........	48%
Acetic acid.............	24%
Acetone.................	27%
Acetaldehyde (?)........	8%

The aldehyde was not positively identified as acetaldehyde.

The oxidation offered possibility of giving the desired degradation because the acetone, if proved to arise from α, β and γ positions, could be used as a measure of the average C^{13} in these three positions, and by difference from the whole butyrate molecule the C^{13} in the carboxyl group could be calculated. Furthermore, by degradation of the acetone by the iodoform reaction, the C^{13} could be located in other positions.

$$CH_3C^{13}H_2CH_2C^{13}OOH \longrightarrow CH_3C^{13}OCH_3 + C^{13}O_2$$

$$CH_3C^{13}OCH_3 \xrightarrow{NaOI} CHI_3 + CH_3C^{13}OOH$$

It is evident from the above equations that the beta position of the butyric acid is equivalent to the carboxyl group of the acetic acid which is formed in the iodoform reaction. No further degradation is needed to determine the C^{13} in the carboxyl group of the acetic acid because the C^{13} of the iodoform has the same value as the methyl group of the acetic acid. C^{13} of the carboxyl carbon can, therefore, be calculated when the C^{13} of the whole molecule of acetic acid is known. The C^{13} value of the iodoform likewise will be a measure of the average value of both the α and γ positions of butyric acid since the

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[1] Journal Paper No. 1224 of the Iowa Agricultural Experiment Station, Ames, Iowa. Project No. 746.

[2] Schicktanz, Steeles and Blaisdell, *Ind. Eng. Chem.*, *Anal. Ed.*, 12, 320 (1940).

[3] Allen and Witzemann, THIS JOURNAL, 63, 1922 (1941).