specific reaction velocity is a linear function of the reciprocal of the initial virus concentration.

The specific reaction velocity varies in a complex way with changes in temperature. Between 0 and 23° the rate decreases as temperature is increased, but above 23° the rate increases as temperature is increased. This behavior was explained by assuming that the denaturation of tobacco mosaic virus in concentrated urea can proceed by several parallel reactions, some with negative differential rate-temperature coefficients and some with positive coefficients. Both types of reaction were visualized as taking place in two stages: first, a reversible reaction between urea and virus to form a readily denaturable complex and, second, the denaturation of the complex. The important difference between the two types of postulated reactions is that in the one with negative coefficients the ΔH values of the equilibrium reactions are greater than the energies of activation of the denaturation steps, while in the reactions with the positive coefficients the opposite is true.

It was found that the specific reaction velocity varied approximately with the 8.1th power of the urea concentration at 0° and with the 5.7th power at 45°. This observation is evidence in favor of the validity of the assumptions made to explain the complex rate-temperature dependence.

It was observed that the specific reaction velocity varied with about the 1.5th power of the reciprocal of the hydrogen ion activity. This can be explained as meaning that on the average about 1.5 protons must be dissociated by a virus particle before it denatures in urea.

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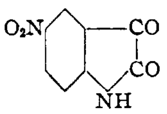
[CONTRIBUTION FROM THE DEPARTMENT OF CHEMISTRY OF WESTERN KENTUCKY STATE TEACHERS COLLEGE]

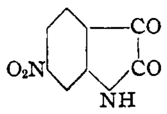
The Nitration of Isatin

BY WARD C. SUMPTER AND WILLIS F. JONES

The results of the several studies which have been made of the nitration of isatin are somewhat conflicting and confusing. The first nitration of isatin was carried out by Baeyer[1] through the action of potassium nitrate on a solution of isatin in concentrated sulfuric acid. Baeyer reported the melting point of his product as 226–230° and assumed that it was 5-nitroisatin (I). Liebermann and Krauss[2] prepared nitroisatin in essentially the same manner but reported the melting point as 245° (dec.).



I



II

Procedures for the nitration of isatin are given in German Patent 221,529 and the melting point reported as 253–255° when the nitration is effected by nitric acid in sulfuric acid solution and 248–250° when the nitration is accomplished by the action of potassium nitrate on a solution of isatin in sulfuric acid.

In 1924 Rupe and Stocklin[3] published a paper in which they claimed that 6-nitroisatin (II) was obtained when isatin was nitrated by treating its solution in concentrated sulfuric acid with the calculated quantity of fuming nitric acid (sp. gr. 1.5). They reported the melting point of their product as 244° and stated that it was not identical with the product obtained by following the procedure of Baeyer.

In 1925 Calvery, Noller and Adams[4] employing a procedure essentially the same as that used by Rupe and Stocklin, obtained a product melting at 254–255° which they assumed to be 5-nitroisatin. They cited Baeyer's paper and German Patent 221,529 but seemingly were not aware of the fact that Rupe had claimed the preparation of 6-nitroisatin by a procedure practically identical with their own.

Subsequently Rupe and Kersten[5] presented what appeared on its face to be definite proof of the structure of Baeyer's 5-nitroisatin and of Rupe's 6-nitroisatin.[6] They did not offer any explanation for the difference in their own results and those of Calvery, Noller and Adams but

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(1) Baeyer, *Ber.*, **12**, 1312 (1879).

(2) Liebermann and Krauss, *ibid.*, **40**, 2492 (1907).

(3) Rupe and Stocklin, *Helv. Chim. Acta*, **7**, 557–566 (1924).

(4) Calvery, Noller and Adams, THIS JOURNAL, **47**, 3059 (1925).

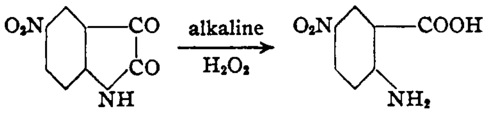
(5) Rupe and Kersten, *Helv. Chim. Acta*, **9**, 578 (1926).

(6) In his second paper Rupe used a different system of numbering and designated the compound as 4-nitroisatin. Structural formulas in the paper show that he meant 6-nitroisatin.

simply mentioned in a footnote that the latter workers had obtained a product melting at 254–255° and designated as 5-nitroisatin by using the same nitration procedure. The fact that this was not in accord with his own results apparently caused Rupe no concern.

The present work was undertaken with the view of clearing up this confliction in results. To this end the procedures of Baeyer, of Calvery, Noller and Adams and of Rupe for the nitration of isatin were carefully repeated. The same product was obtained in each case and the melting point was that reported by Calvery, Noller and Adams. The identity of the products was established by melting point methods. For further proof of identity samples of the nitroisatin prepared by each of the three procedures were converted into the phenylhydrazone, the three preparations so obtained being identical.

In view of the fact that isatin normally substitutes first in position 5 it was felt that the product was undoubtedly 5-nitroisatin. In order to confirm this conclusion and to provide further proof of the identity of the several nitroisatin preparations samples of the nitroisatin prepared by each of the three procedures were converted into 5-nitroanthranilic acid (III) by treatment with alkaline hydrogen peroxide. The three samples of 5-nitroanthranilic acid obtained in this manner proved to be identical with one another and with an authentic sample of 5-nitroanthranilic acid.[7] These four samples of 5-nitroanthranilic acid all yielded the same acetyl derivative when treated with acetic anhydride.



I III

It follows from the above evidence that the product obtained by nitrating isatin according to the procedures of Baeyer, of Calvery, Noller and Adams and of Rupe is 5-nitroisatin.

Experimental

5-Nitroisatin (I).—The substance was prepared: A, by the procedure of Baeyer; B, by the procedure of Rupe and Kersten; C, by the procedure of Calvery, Noller and Adams. The yields averaged 85% of the theoretical; m. p. 254–255°. Identity of the three preparations was established by melting point methods.

Samples of nitroisatin prepared by methods A, B and C were converted into the phenylhydrazone; m. p. 295°.[8] The identity of the three samples of the phenylhydrazone was established by melting point methods.

5-Nitroanthranilic Acid. (III).—Seven grams of nitroisatin (m. p. 254–255°) was treated with 150 ml. of 10% sodium hydroxide and 150 ml. of 3% hydrogen peroxide added. After standing for thirty minutes the reaction mixture was acidified and the precipitate collected. The product was crystallized from ethyl alcohol or acetic acid, from which it separated as light yellow needles; m. p. 278° (dec.). The yields averaged 86% of the theoretical. Samples made from the three nitroisatin preparations (A, B and C) were identical as shown by melting point methods. The samples were also identical with an authentic sample of 5-nitroanthranilic acid.[7]

Samples of nitroanthranilic acid prepared from the three nitroisatin preparations and a fourth authentic sample of 5-nitroanthranilic acid were converted into the acetyl derivative by heating with acetic anhydride. The four samples obtained in this manner melted at 221°[9] separately and when mixed.

This work has been supported by a research grant (A. A. A. S.) received through the Kentucky Academy of Science.

Summary

The nitration of isatin according to the procedure of Rupe gives 5-nitroisatin identical with that obtained by the procedures of Baeyer and of Calvery, Noller and Adams and not 6-nitroisatin as reported by Rupe and co-workers.

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[7] Bogert and Scatchard, THIS JOURNAL, **41**, 2052 (1919).

[8] Schunck and Marchlewski (*Ber.*, **28**, 546 (1895)) reported 284°; Rupe and Apotheker (*Helv. Chim. Acta*, **9**, 1049 (1926)) reported 286°.

[9] Bogert and Cook, THIS JOURNAL, **28**, 1451 (1906) reported 221.5°.