Chem. Pharm. Bull.
12 (1) 14 ~ 18

UDC 543.8[547.584] : 547.457.1

3. Tsutomu Momose, Akira Inaba, Kōnosuke Inoue, Kazumoto Miyahara, and Tae Mori : Organic Analysis. XLIX.*[1] Color Reaction of 3,4-, 4,5-, or 3,5-Dinitrophthalic Acid with Reducing Sugars.

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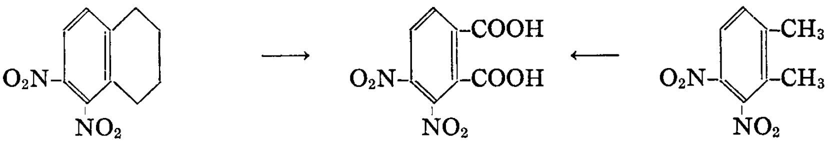
In a previous paper[1] of this series, the mechanism of the color reaction of 3,6-dinitrophthalic acid with reducing sugars was clarified by isolating the coloring matters in a crystalline form. The acid was firstly reduced to 3-hydroxylamino-6-nitrophthalic acid, and then changed to 3,3′-azobis-(6-nitrophthalic acid), giving wine red and orange red colors, respectively, in aqueous alkaline carbonate solution. There are still three isomers in the acid, and it is of much interest to study these color reactions with reducing sugars.

Preparation of 3,4-, 4,5-, and 3,5-Dinitrophthalic Acid

3,4-Dinitrophthalic acid was conveniently obtained by the oxidation of 5,6-dinitro-1,2,3,4-tetrahydronaphthalene with nitric acid, which was prepared[2] by the nitration of tetrahydronaphtalene. The acid, m.p. 213°, prisms, was also yielded from 3,4-dinitro-*o*-xylene[3] by the oxidation with dilute nitric acid in a fused tube.



4,5-Dinitrophthalic acid, m.p. 215~217°, prisms, was synthesized according to the literature,[4] except the last step of oxidation of 2-methyl-4,5-dinitrobenzoic acid was performed with a mixture of fuming nitric acid and fuming sulfuric acid in the usual method.

3,5-Dinitrophthalic acid, m.p. 226°, prisms, was prepared by the literature.[5] The acid was also obtained as a by-product from the mother liquid of 3,6-dinitrophthalic acid in the oxidation of 1,5-dinitronaphthalene with a mixture of fuming nitric acid and fuming sulfuric acid.[6]

Color Reaction of Dinitrophthalic Acids with Reducing Sugars

The reagent solution was prepared by dissolving each acid in 5% aqueous sodium carbonate in a concentration of 0.1%. One drop of the solution was mixed with a drop of reducing suger solution in a small test-tube, and heated on a water bath. The developed color and limit detection of glucose in one drop of water were tabulated in Table I, comparing with those of 3,6-dinitrophthalic acid.[7]

3,4-Dinitrophthalic acid showed a sensitive violet coloration in 1~2 minutes in the reaction, which turned gradually to yellow on prolonged heating. 4,5-Dinitrophthalic

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*[1] Part XLVIII : This Bulletin, **12**, 5 (1964)

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[1] T. Momose, A. Inaba : This Bulletin, **9**, 263 (1961).

[2] E. Kindermann : Ann., **426**, 43 (1922).

[3] A.W. Krossley, N. Renouf : J. Chem. Soc., **95**, 202 (1909).

[4] H. Goldstein, A. Tardent : Helv. Chim. Acta, **34**, 149 (1951).

[5] R. Eder, C. Widmer : *Ibid.*, **6**, 977 (1923).

[6] T. Momose, M. Torigoe : Yakugaku Zasshi, **71**, 977 (1951).

[7] T. Momose, A. Inaba : This Bulletin, **7**, 541 (1959).

acid behaved just the same as the 3,4-isomer. 3,5-Dinitrophthalic acid was less sensitive in the color reaction, and showed a yellow coloration with increasing intensity on prolonged heating. Table I shows that the detection of reducing sugars with 3,6-dinitrophthalic acid in the previous paper was reasonable when compared the limit of detection with those of others.

Mechanism of the Color Reaction

(1) 3,4-Dinitrophthalic acid : The developed violet color of glucose with 3,4-dinitrophthalic acid (I) was unstable and faded from the upper part of the test-tube where it contacted with the air. Its absorption curve was measured by adding sodium metaphosphate in the solution, and was shown in Fig. 1-A.

The coloring matter of the reaction was so unstable in its solution that the acidified reaction mixture was quickly extracted with ether, in which a small amount of sulfurous acid was added. The yielded substance, m.p. 169°, yellow prisms, gave the same absorption curve and maximum (Fig. 1-B) as the developed color in aqueous solution of sodium carbonate and sodium metaphosphate, and showed that it was the main coloring matter of the reaction.

This substance agreed with the formula $C_8H_6O_7N_2$, and reduced an ammoniacal solution of silver nitrate. Further reduction of the substance with glucose in aqueous

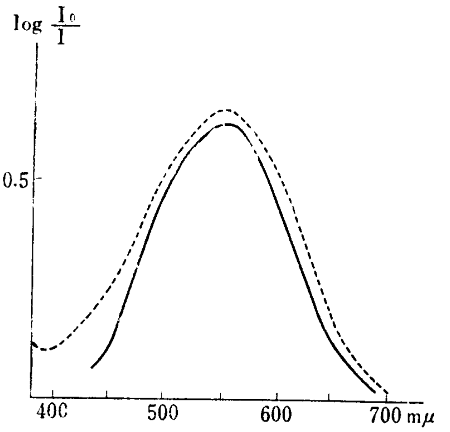


Fig. 1. Absorption Spectra for Developed Color of Glucose with 3,4-Dinitrophthalic Acid and 3-Nitro-4-hydroxylaminophthalic Acid

—— 0.1 ml. of glucose solution (5%) was heated with 5 ml. of 0.1% 3,4-dinitrophthalic acid dissolved in 5% Na_2CO_3 for 2 min., and diluted to 200 ml. with 5% Na_2CO_3 which contained 2% of $NaPO_3$.

------ 11 mg. of 3-nitro-4-hydroxylaminophthalic acid was dissolved in 250 ml. of 5% Na_2CO_3 which contained 2% of $NaPO_3$. UV : λmax 550 mμ (log ε 3.59).

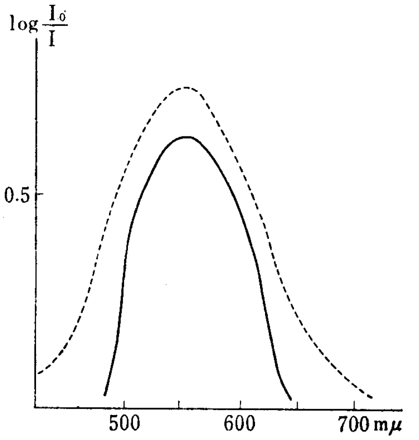


Fig. 2. Absorption Spectra for Developed Color of Glucose with 4,5-Dinitrophthalic Acid and 4-Hydroxylamino-5-nitrophthalic Acid

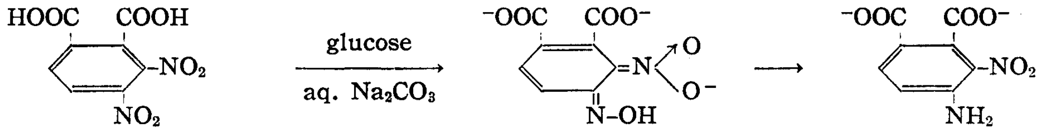
—— 0.1 ml. of glucose solution (5%) was heated with 5 ml. of 0.1% 4,5-dinitrophthalic acid dissolved in 5% Na_2CO_3 for 2 min., and diluted to 200 ml. with 5% Na_2CO_3 which contained 2% of $NaPO_3$.

------ 5 mg. of 4-hydroxylamino-5-nitrophthalic acid was dissolved in 200 ml. of 5% Na_2CO_3 which contained 2% of $NaPO_3$. UV : λmax 552 mμ (log ε 3.84).

TABLE I. Limit of Detection of Glucose with Dinitrophthalic Acids and Developed Color

Position of NO_2 groups in phthalic acid	3, 6	3, 4	4, 5	3, 5
Limit of detection of glucose (μg.)	0.2	5	5	15
Color	deep red ↓ orange red	violet	violet	yellow

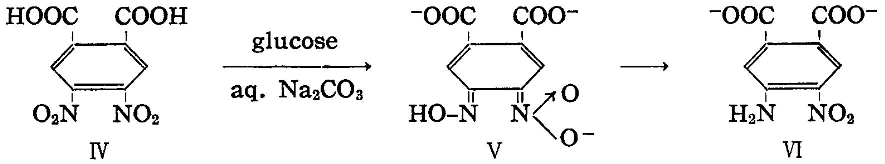
sodium carbonate yielded 3-nitro-4-aminophthalic acid (III), m.p. 234°, yellow prisms, in which the position of amino group was proved by the usual method of deamination to 3-nitrophthalic acid. Therefore, the coloring matter of the reaction was 3-nitro-4-hydroxylaminophthalic acid (II). The same compound was also obtained when 3,4-dinitrophthalic acid was reduced with ferrous sulfate or sodium hydrosulfite.



I II III

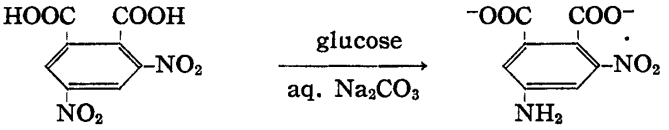
(2) 4,5-Dinitrophthalic acid : The developed color of glucose with 4,5-dinitrophthalic acid (IV) was also unstable, and its absorption curve measured by adding sodium metaphosphate was shown in Fig. 2-A. The isolation of the coloring matter was carried out in the same way as previous isomer, and the yielded compound, m.p. 167°, yellow needles, gave an unstable violet color in aqueous sodium carbonate. The absorption spectrum (Fig. 2-B) of the color measured in the presence of sodium metaphosphate coincided with the developed color in the maximum and shape of the curve, indicating that the compound was the main coloring matter of the reaction.

The dye had the formura $C_8H_6O_7N_2$, and reduced an ammoniacal solution of silver nitrate. Reduction of the dye by glucose in aqueous sodium carbonate gave yellow prisms, m.p. 238°, which were identified as 4-amino-5-nitrophthalic acid[8] (VI). These data revealed that the coloring matter of the reaction was 4-hydroxylamino-5-nitrophthalic acid (V).



IV V VI

(3) 3,5-Dinitrophthalic acid : The developed yellow color of glucose with 3,5-dinitrophthalic acid (VII) was rather weak, and its absorption curve had no maximum in the visible light region. The coloring matter seemed to be a mixture of several reduced substances, and 3-nitro-5-aminophthalic acid (VIII), m.p. over 300°, yellow prisms, was separated when the reaction was carried out for much longer time. The structure of VIII was proved by its deamination to 3-nitrophthalic acid.



VII VIII

Discussion

Now the mechanism of the color reactions of reducing sugars with all isomers of dinitrophthalic acid was wholly clarified. The *p*- and *o*-dinitro isomers revealed, respectively, a sensitive coloration by their reduction products, monohydroxylamino compounds, which might dissociate in quinoidal ion in alkaline medium. The *m*-isomer could not dissociate in the ion, and hence it showed a weak response. It is important to note

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8) H. Goldstein, J. Merminod : Helv. Chim. Acta, 35, 1476 (1952).

that the *p*-isomer, 3,6-dinitrophthalic acid, only gave the stable and hyperchromic reduction product, 3,3′-azobis-(6-nitrophthalic acid), in a proper condition. This fact proved that it was quite reasonable to use the acid in the determination of reducing sugars.[9]

Experimental

3,4-Dinitrophthalic Acid[8]——First method. 10 g. of 5,6-dinitro-1,2,3,4-tetrahydronaphthalene was boiled for 20 hr. with 70 g. of conc. HNO_3, filtered, and the filtrate was evaporated to dryness. The remaining substance was dissolved in a small amount of H_2O, filtered, and the filtrate was again evaporated to dryness. Recrystallization from Me_2CO-benzene gave pale yellow prisms, m.p. 213°. Yield, 3 g. *Anal.* Calcd. for $C_8H_4O_8N_2$: C, 37.51; H, 1.57; N, 10.94. Found : C, 37.94; H, 1.67; N, 10.84.

Second method. 0.5 g. of 3,4-dinitro-*o*-xylene was mixed with 10 g. of HNO_3 (S. G. 1.15) in a sealed glass tube and heated for 5 hr. at 140°. After cooling, the mixture was filtered, and the filtrate was evaporated to dryness. The remained substance was extracted with hot benzene to remove soluble substance, and recrystallized as above. Yield, 0.1 g.

3-Nitro-4-hydroxylaminophthalic Acid——1 g. of 3,4-dinitrophthalic acid, 1.5 g. of Na_2CO_3 and 2 g. of glucose were successively dissolved in 50 ml. of H_2O, and heated in a boiling water bath for 2 min. The mixture was immediately cooled in an ice bath, acidified with H_3PO_4 and quickly extracted with Et_2O to which a small amount of SO_2 was added. The solvent was evaporated in a reduced pressure with a oscillo·evaporator,[10] and the remained substance was recrystallized from AcOEt to yellow prisms, m.p. 169°. Yield, 0.1 g. *Anal.* Calcd. for $C_8H_6O_7N_2$: C, 39.68; H, 2.49; N, 11.56. Found: C, 39.35; H, 2.58; N, 11.29.

The same compound was obtained when 1 g. of the starting material was reduced with 5 g. of $FeSO_4 \cdot 7H_2O$ or 1 g. of $Na_2S_2O_4 \cdot 2H_2O$ in 20 ml. of H_2O at a room temperature.

3-Nitro-4-aminophthalic Acid——0.4 g. of 3-nitro-4-hydroxylaminophthalic acid, 1 g. of glucose and 1 g. of Na_2CO_3 were successively dissolved in 20 ml. of H_2O, heated in a boiling water bath for 10 min., acidified with 6*N* HCl, and extracted with Et_2O. After evaporation of the solvent, the remained substance was recrystallized from H_2O to yellow prisms, m.p. 234°. Yield, 0.1 g. *Anal.* Calcd. for $C_8H_6O_6N_2$: C, 42.49; H, 2.67; N, 12.39. Found : C, 42.29; H, 2.79; N, 12.22.

Deamination of 3-Nitro-4-aminophthalic Acid——To a solution of 0.3 g. of 3-nitro-4-aminophthalic acid in 10 ml. of H_2O, 5 ml. of HCl was added, and diazotized with 10% $NaNO_2$ at 5°. The separated diazonium salt was collected, and boiled with 10 ml. of 50% EtOH for 30 min. The mixture was then evaporated to dryness, and remained substance was recrystallized from H_2O to prisms, m.p. 220°. Yield, 0.1 g. This compound was identified as 3-nitrophthalic acid by the melting point of admixture and IR spectrum.

4-Hydroxylamino-5-nitrophthalic Acid——1 g. of 4,5-dinitrophthalic acid, 2 g. of glucose, and 1.5 g. of Na_2CO_3 were treated in the same way as the 3,4-isomer. The extracted substance was recrystallized from Me_2CO-benzene to yellow needles, m.p. 167°. Yield, 0.1 g. *Anal.* Calcd. for $C_8H_6O_7N_2$: C, 39.68; H, 2.49; N, 11.56. Found : C, 39.49; H, 2.70; N, 11.30.

The same compound was also obtained by the reduction of 4,5-dinitrophthalic acid with $FeSO_4 \cdot 7H_2O$ or $Na_2S_2O_4 \cdot 2H_2O$ at a room temperature.

4-Amino-5-nitrophthalic Acid——0.4 g. of 4-hydroxylamino-5-nitrophthalic acid, 1 g. of glucose, and 1 g. of Na_2CO_3 were treated in the same way as the 3,4-isomer, and the extracted crystals were recrystallized from H_2O to yellow needles, m.p. 238°. This substance was identified as 4-amino-5-nitrophthalic acid[8] by the melting point of admixture and IR spectrum. Its melting point of the literature, 203°, should be corrected as above.

3-Nitro-5-aminophthalic Acid——2 g. of 3,5-dinitrophthalic acid, 4 g. of glucose, and 5 g. of Na_2CO_3 were successively dissolved in 100 ml. of H_2O, heated in a boiling water bath for 1 hr., cooled, and acidified with H_3PO_4. The mixture is extracted with Et_2O, and the solvent was evaporated. The remained substance was recrystallized from H_2O to yellow prisms, m.p. over 300°. Yield, 0.3 g. *Anal.* Calcd. for $C_8H_6O_6N_2$: C, 42.49; H, 2.67; N, 12.39. Found : C, 42.13; H, 2.80; N, 12.05.

Deamination of 3-Nitro-5-aminophthalic Acid——0.3 g. of 3-nitro-5-aminophthalic acid was treated in the same way as the 3,4-isomer, and the obtained compound, m.p. 220°, was identified as 3-nitrophthalic acid.

Absorption Spectra——Visible light absorption spectra were measured with a Beckmann DK-2 ratio recording spectrophotometer with glass cells of 10 mm. optical length.

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9) T. Momose, A. Inaba, Y. Mukai, M. Watanabe : Talanta, **4**, 33 (1960); T. Momose, Y. Mukai, M. Watanabe : *Ibid.*, **5**, 275 (1960).

10) T. Momose : Yakugaku Zasshi, **83**, 653 (1963).

The authors express their gratitude to Messrs. M. Shido and M. Shirozu and Mrs. S. Matsuba for the microanalyses, and to Messrs. H. Matsui and K. Hikita, and Miss Y. Soeda for the spectral measurement.

Summary

3,4-Dinitrophthalic acid gave a sensitive violet coloration with glucose when heated in aqueous sodium carbonate. In this color reaction, the acid was reduced to 3-nitro-4-hydroxylaminophthalic acid. 4,5-Dinitrophthalic acid behaved in the same way as the 3,4-isomer, and was reduced to 4-hydroxylamino-5-nitrophthalic acid. 3,5-Dinitrophthalic acid was less sensitive in the reaction, showing a yellow coloration by its reduced substances down to 3-amino-5-nitrophthalic acid.

(Received September 4, 1963)

Chem. Pharm. Bull. 12 (1) 18 ~ 22

UDC 547.387 : 547.831.1

4. **Tetsuzo Kato and Hiroshi Yamanaka** : Studies on Ketene and its Derivatives. V.[1] Reaction of Diketene with Quinoline N-Oxide.

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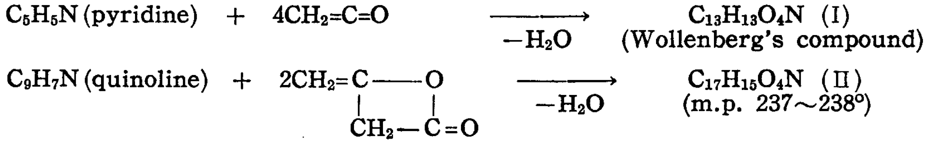
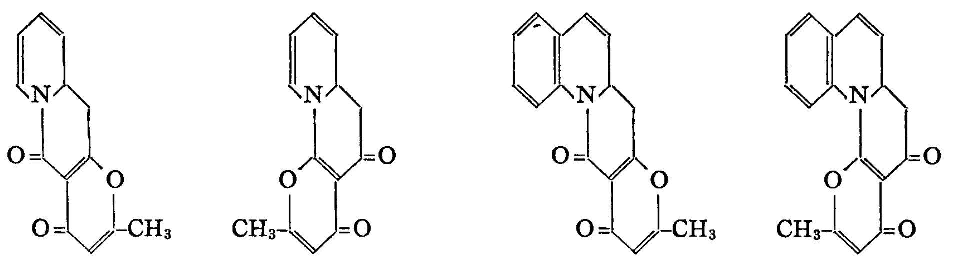
In a previous paper[2] of this series we have reported that diketene reacts with quinoline readily to give a yellow crystal of m.p. 237~238° (decomp.) which has a quite similar structure with one of socalled Wollenberg's compound[3,4] isolated from the reaction of ketene with pyridine, and that the compound thus obtained is represented as either II-a or II-b.



$$C_5H_5N\text{ (pyridine)} + 4CH_2{=}C{=}O \xrightarrow[-H_2O]{} C_{13}H_{13}O_4N\text{ (I) (Wollenberg's compound)}$$

$$C_9H_7N\text{ (quinoline)} + 2\,\begin{matrix}CH_2{=}C-O\\ | \quad\;\; | \\ CH_2-C{=}O\end{matrix} \xrightarrow[-H_2O]{} C_{17}H_{15}O_4N\text{ (II) (m.p. 237}\sim238^\circ)$$



I-a I-b II-a II-b

Chart I.

[*1] Kita-4, Sendai, Miyagi-Ken (加藤鉄三, 山中 宏).

1) Part IV. T. Kato, H. Yamanaka, F. Hamaguchi : Yakugaku Zasshi, **83**. 741 (1963).

2) T. Kato, T. Kitagawa, Y. Yamamoto : *Ibid.*, **83**, 267 (1963).

3) O. Wollenberg : Ber., **67**, 1675 (1934).

4) J. Berson, W. Jones : J. Am. Chem. Soc. **78**, 1625 (1956).