Summary

Solid nickel peroxide has been shown to be capable of oxidizing aromatic primary amines to the corresponding azo-compounds in benzene solution. Benzylamine and its derivatives bearing a substituent in the benzene ring could be easily oxidized by the same oxidant to give the corresponding nitriles in good yields regardless of the position and the variety of a substituent. Alkyl amines also underwent similar oxidation. In addition, the mechanisms of these oxidations were discussed.

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50. Tsutomu Momose, Yosuke Ohkura, and Kazuya Kohashi: Organic Analysis. XXXIX.*1 Improved Method of Detecting Active Methylene Compounds with Trinitrobenzene or Picric Acid.

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Trinitrobenzene or picric acid gave a red or orange coloration with active methylene compounds in alkaline medium, and was used for their detection[1] and estimation of both cardiac glycosides[2] and creatinine.[3] According to the original method of detection, however, a blank test showed a fairly colored solution, and was sometimes undistinguishable from the developed coloration with naked eye, especially in the case of trinitrobenzene.

In the previous paper[4] of this series, it was shown that the addition of sodium dihydrogen phosphate in the reaction mixture intensified the developed coloration by picric acid with creatinine, which was made possible to determine the latter compound in blood in a micro scale. The new method may be generally applicable to the detection of active methylene compounds, when the blank color of picric acid or trinitrobenzene diminishes or fades away, and then the developed coloration will be evidently increased even when observed with naked eye. Thus, a new sensitive spot test for the compounds has been established by selecting suitable reaction conditions.

Experimental

Reagents

0.1% 1,3,5-Trinitrobenzene solution: 100 mg. of trinitrobenzene, recrystallized from EtOH, m.p. 121°, is dissolved in 30 ml. of dimethylformamide, mentioned below, and diluted with H_2O to measure 100 ml., and stored in a light resistant container.

0.3% picric acid solution: 300 mg. of picric acid, JIS 1st grade, is dissolved in 100 ml. of H_2O.

2% and 10% NaOH solution: are prepared by the usual method, respectively.

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10% NaH_2PO_4 solution : 10 g. of $NaH_2PO_4 \cdot 2H_2O$, JIS 1st grade, is dissolved in H_2O and made up to 100 ml.

Dimethylformamide : JIS 1st grade, is dried over KOH, distilled *in vacuo*, and stored in a light resistant bottle.

Procedure

By Trinitrobenzene——To 1 drop of an aqueous or dimethylformamide solution of a sample, 1 drop of 0.1% trinitrobenzene solution and 1 drop of 2% NaOH are added successively, and allowed to stand for about 5 min. At the end of the time, if the test gives only a weak response, it is advisable to warm the mixture at 30~40° for a short period of time. Then 1~2 drops of 10% NaH_2PO_4 solution is added to the mixture, and shaken thoroughly. An orange, red, or violet color is developed in the presence of active methylene compounds. A blank test is almost colorless.

By Picric Acid——To 1 drop of an aqueous or dimethylformamide solution of a sample, 1 drop of 0.3% picric acid solution and 1 drop of 10% NaOH are added successively. After red or brown color about 5 min., 3 drops of NaH_2PO_4 solution is added in the mixture, shaken thoroughly, and an orange, appears. A blank test remains light yellow.

Absorption Spectra

They were measured with a Hitachi EPU-2 spectrophotometer or a Beckman DK-2 ratio-recording spectrophotometer in a glass cell of 10 mm. optical length.

Results and Discussion

A blank test solution of trinitrobenzene gives a red coloration by sodium hydroxide as its absorption spectrum is shown in Fig. 1 B_1. Therefore, the developed coloration of an active methylene compound (Fig. 1 A_1) can scarcely be observed in such a low concentration of the sample. When sodium dihydrogen phosphate is added to both solutions, the blank color is almost decolorized (Fig. 1 B_2), and the developed coloration increases, in general, more than 2~3 times as much in its intensity (Fig. 1 A_2). This change is caused by the lowering effect of pH by the salt formation, and the optimum pH of the resulting mixture seems to be in the range of 6.5~7 giving the maximum intensity, as the color becomes unstable with an increase of hydrogen ion concentration. Other acidic substances which have a buffer effect with alkali, may be applicable

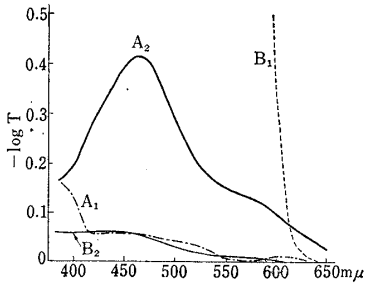

Fig. 1. Effect of Sodium Dihydrogen Phosphate on Developed Coloration of Orcinol with Trinitrobenzene

A_1 : To 1 ml. of aqueous orcinol solution (30γ/ml.), 1 ml. of 0.1% trinitrobenzene solution and 1 ml. of 2% NaOH were added successively, maintained for 5 min. at 20°, diluted with 2 ml. of H_2O, and measured against the reagent blank B_1.

A_2 : 1 ml. of the same orcinol solution was treated as above, diluted with 2 ml. of 15% $NaH_2PO_4 \cdot 2H_2O$ instead of H_2O, and measured against the reagent blank B_2.

B_1 : The blank test solution for A_1.

B_2 : The blank test solution for A_2.

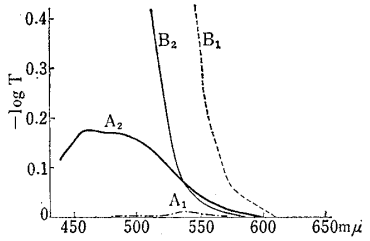

Fig. 2. Effect of Sodium Dihydrogen Phosphate on Developed Coloration of 4′-Aminoacetophenone with Picric Acid

A_1 : To 1 ml. of 4′-aminoacetophenone solution in dimethylformamide (100 γ/ml.), 1 ml. of 0.3% picric acid solution and 1 ml. of 10% NaOH were added successively, maintained for 5 min. at 20°, diluted with 3 ml. of H_2O, and measured against the reagent blank B_1

A_2 : 1 ml. of the same 4′-aminoacetophenone solution was treated as above, diluted with 3 ml. of 10% $NaH_2PO_4 \cdot 2H_2O$ instead of H_2O, and measured against the reagent blank B_2.

B_1 : The blank test solution for A_1.

B_2 : The blank test solution for A_2.

TABLE I. Color and Limit of Detection of Active Methylene Compounds in One Drop of Sample Solution

Compound \ Reagent	Trinitrobenzene				Picric acid			
	Color[a]	Increase of intensity[b]	Stability[c]	Limit of detection (γ/drop)	Color[a]	Increase of intensity[b]	Stability[c]	Limit of detection (γ/drop)
Acetone	R V	++	S	0.5	R B	++	F	4
2-Butanone	O R	++	S	0.1	O R	++	F	2
Acetaldehyde	R V	++	S	0.05	O	++	S	0.5
Propionaldehyde	O B	++	S	1	O	++	S	5
Crotonaldehyde	O B	++	S	0.05	O	++	F	0.5
Methylglyoxal	R V	+	S	0.1	O Y	+	S	5
Acetylacetone	R V	++	US	0.1	O R	±	US	1
Pyruvic acid	R V	++	F	0.5	O R	++	S	5
Ascorbic acid	O B	++	US	0.6	O	++	US	4
Ethyl acetoacetate	R B	++	US	0.1	O R	+	US	0.5
Ethyl cyanoacetate	R V	++	US	0.05	O	++	US	0.1
Ethyl malonate	R V	++	US	0.05	O	++	US	0.5
Acetophenone	R V	++	S	0.1	O R	++	S	0.5
2′- or 4′-Hydroxyacetophenone	R V	++	S	0.1	O R	++	S	0.5
4′-Aminoacetophenone	R V	++	S	0.1	O R	++	S	0.5
4′-Methylacetophenone	R V	++	S	0.2	O	++	S	0.5
Cyanoacetophenone	R V	++	US	0.1	O	+	US	0.1
2-Acetyl-1-naphthol	B	+	S	10	O	+	S	50
2-Propionyl-1-naphthol	B	+	S	5	O	+	S	10
p-Methoxybenzoyl propionic acid	R V	++	S	0.2	O R	++	S	1
2,5-Dimethoxybenzoyl propionic acid	R V	++	S	0.2	O R	++	S	1
Benzoyl valeric acid	R V	++	S	0.2	O	++	S	1
Resorciol	R B	++	S	0.01	O R	++	S	0.08
Orcinol	R B	++	S	0.01	O	++	S	0.08
Phloroglucinol	R B	++	F	0.05	Y	±	F	0.1
Pyrogallol	R B	++	S	0.01	O	++	S	0.05
Cyclohexanone	R V	++	S	0.1	O R	++	S	0.5
2-Acetoxycyclohexanone	R B	++	S	0.1	O R	++	S	0.5
α-Tetralone	R V	++	F	0.1	O R	++	F	0.5
7-Hydroxy-1-tetralone	R V	++	F	0.01	O	++	F	0.05
7-Methoxy-1-tetralone	R V	++	F	0.01	O R	++	F	0.05
5-Nitro-1-tetralone	R V	++	F	0.01	O R	++	F	0.05
5,8-Diacetoxy-1-tetralone	R B	++	F	0.01	O	++	F	0.05
6-Hydroxy-1-indanone	R V	++	F	0.01	O R	++	F	0.05
5,6-Dimethoxy-1-indanone	R V	++	F	0.01	O R	++	F	0.05
Creatinine	R V	++	S	0.01	O R	++	S	0.05
Pyrrole	R B	++	S	1	O	++	S	5
Indole	R V	++	S	0.006	O R	++	S	0.01
Quinaldine	O	+	F	1	Y	+	F	10
Digitoxin	R V	+	F	0.5	O R	+	F	2
Oleandrin	R V	+	F	0.5	O R	+	F	2
Ouabain	R V	+	F	0.5	O R	+	F	2
Androsterone	R B	+	F	0.5	O	++	F	1

a) R : red O : orange B : brown V : violet Y : yellow.
b) When NaH_2PO_4 solution is added, the intensity of color is ± : unchanged, + : increased and ++ : much increased.
c) S : stable F : fade gradually US : unstable

instead of sodium dihydrogen phosphate. Those involve acetic, tartaric, citric, and succinic acids and also ammonium chloride.

An alkaline solution of picric acid has an orange color (Fig. 2 B_1), and its developed coloration with an active methylene compound is shown in Fig. 2 A_1. The addition of sodium dihydrogen phosphate turns the blank color to light yellow (Fig. 2 B_2), and increases the developed coloration in its intensity as shown in Fig. 2 A_2.

In this test, purified dimethylformamide is successfully used as the solvent for trinitrobenzene and water-insoluble samples. Trinitrobenzene solution under the procedure is almost colorless, and stable enough for a long storage of more than four months when stored in a brown bottle, though it turns yellow in the day light with decomposition.[5] Methanol and ethanol, on the other hand, are unsuitable for the solvent in the test, as they give a positive result in a blank test within three days after their purification. Other solvents such as isopropanol and dioxane are so difficult to remove their impurities perfectly that they sometimes give an erroneous result.

Active methylene compounds seem to require individually an appropriate concentration of alkali to develop their maximum intensity with trinitrobenzene. For example, acetone gives the most intensive coloration with about 2% sodium hydroxide, whereas digitoxin, ouabain and oleandrin are detected with sodium carbonate solution more sensitively. For the purpose of spot test, however, it is in general preferable to use 2% sodium hydroxide solution. In the case of picric acid, 10% sodium hydroxide solution proved to be sufficient to develop the coloration.

Reaction temperature and time also affect the color development in the test. A maximum color is generally obtained when the alkaline reaction mixture is maintained at 30~40° for 20~30 minutes, but it gives satisfactory results in a routine detection to carry out the reaction at room temperature for about five minutes.

The results obtained by the procedures are shown in Table I. Most active methylene compounds give positive test in an amount less than 0.5 γ per drop with trinitrobenzene. Other substances tested give a positive result in an amount of 1~10 γ per drop by both reagents. These involve glucose, fructose, xylose, furfural, urea, thiourea, anthrone and carbazole.

Negative results are obtained with the following compounds, such as malonic acid, succinic ethyl ester, phenylacetic acid and camphor. Other substances than active methylene compounds give no coloration in the test.

The present method with trinitrobenzene as the reagent is most sensitive in the detection of active methylene compounds when compared with other methods which are commonly used for the same purpose. For example, acetone is detected at 5 γ with sodium nitroprusside,[6] at 10 γ with sodium 1,2-naphthoquinone-4-sulfonate,[7,8] and at 5 γ with *p*-dimethylaminobenzaldehyde,[7] respectively. All of these three methods need about ten times as much of a sample as the present method for the detection of methylglyoxal, pyruvic acid, ascorbic acid, acetophenone, 4′-aminoacetophenone, resorcinol, ethyl malonate, creatinine, indole, cyclohexanone, and 7-methoxytetralone. Therefore, this new method may generally be preferable to the micro detection of active methylene compounds.

The authors express their gratitude to Mr. K. Hikita for the spectral measurements.

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Summary

A sensitive spot test for active methylene compounds was established by adding sodium dihydrogen phosphate in the alkaline reaction mixture of either trinitrobenzene or picric acid. A blank color faded or diminished in this method, and the developed coloration increased wtih a few exceptions. The color and a limit of detection of many active methylene compounds were tabulated.

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51. Zen-ichi Horii, Toyoshi Katagi, Yasumitsu Tamura, Teiji Tanaka, and Yasuhiko Yamawaki : Synthetic Studies on Sorigenins. IV.[1] Synthesis of γ-Lactone of 3-Hydroxymethyl-4-methoxy-2-naphthoic Acid.

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In continuation of the previous work[1] on the preparation of 3-hydroxymethyl-4-methoxy-2-naphthoic acid γ-lactone (VI), two new routes leading to VI from ethyl 4-oxo-1,2,3,4-tetrahydro-2-naphthoate (I)[2] were established, which involved aromatization and successive reduction of the 3-hydroxymethylene and the 3-ethoxalyl derivatives of I, respectively, as shown in Charts 1 and 2.

Ethyl 3-hydroxymethylene-4-oxo-1,2,3,4-tetrahydro-2-naphthoate (II), which was obtained by condensation[3] of I with ethyl formate in benzene in the presence of sodium ethoxide, was brominated with bromine in chloroform to ethyl 3-bromo-3-formyl-4-oxo-1,2,3,4-tetrahydro-2-naphthoate (III). Dehydrobromination of III by heating with N,N-dimethylaniline in a water bath for 2 hours gave ethyl 3-formyl-4-hydroxy-

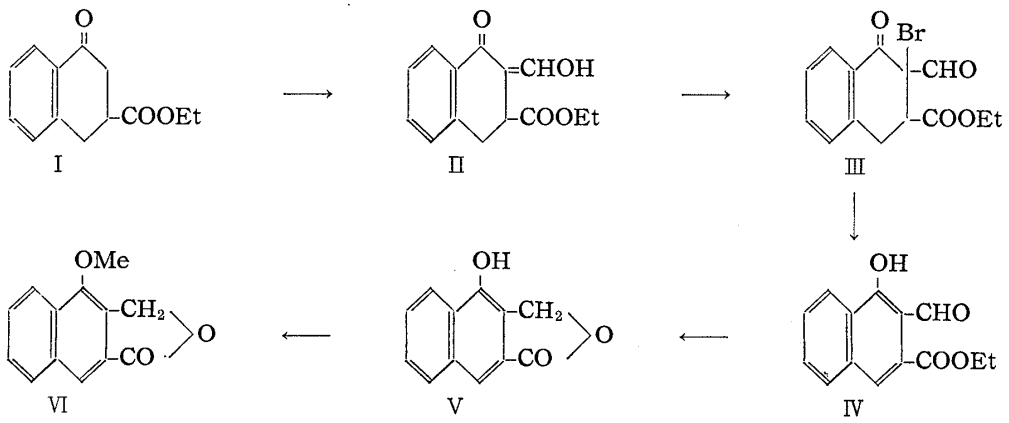

Chart 1.

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