Communications to the Editor

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Colorimetric Determination of Carboxylic Acids with 2-Nitrophenylhydrazine Hydrochloride

Aliphatic and aromatic acids and 2-nitrophenylhydrazine hydrochloride were coupled in aqueous ethanol with dicyclohexylcarbodiimide as a coupling agent in the presence of pyridine and thiourea. The resultant hydrazide solution was made alkaline to develop a violet color, and heated at 60° to decolorize the brown blank color. This colorimetric method of determination is suitable for aqueous and aqueous ethanol samples of carboxylic acids.

Keywords——carboxylic acids; aliphatic acids; aromatic acids; 2-nitrophenylhydrazine hydrochloride; 2-nitrophenylhydrazides; colorimetry

2-Nitrophenylhydrazides of carboxylic acids are an acid–base indicator, and give an intense violet color in an alkaline medium. Legradi[1] first utilized the hydrazides as a spot test of carboxylic acids by converting an acid to acid chloride and reacting with 2-nitrophenylhydrazine. Munson[2] applied the reagent to the colorimetric determination of carboxylic acid anhydrides and chlorides.

Recently, Munson and Bilous[3] reported a colorimetric method for determination of aliphatic acids by a direct coupling reaction of an acid with 2-nitrophenylhydrazine using dicyclohexylcarbodiimide as a coupling agent, which had been successfully used for the formation of hydroxamic acids by coupling carboxylic acids with hydroxylamine.[4] In the reported method, the reaction was carried out in a non-aqueous mixed solvent with free 2-nitrophenylhydrazine, and the resultant hydrazide was extracted with aqueous sodium hydroxide solution to measure the absorbance. This method may be cumbersome for aqueous samples, and not be applied to aromatic acids which do not react by the proposed method.

I had studied the coupling reaction of carboxylic acids with 2-nitrophenylhydrazine in the presence of dicyclohexylcarbodiimide, and found that the hydrochloride of 2-nitrophenylhydrazine reacted sensitively with aliphatic and aromatic acids in aqueous ethanol by the addition of a small amount of pyridine, though the free base gave only a weak response with aliphatic acids in the same conditions. The blank test showed a brown color to obstract the measurement of absorbance, but it was decolorized to a faint orange when the alkaline solution of the hydrazide was heated at 60° for 10—15 minutes.

Now, the absorbance of blank color became to an acceptable range, yet a slight turbidity appeared in the solution. Further studies revealed that the turbidity was eliminated by the addition of a small amount of thiourea in the reaction mixture. Thus, a new method for determination of aliphatic and aromatic acids was developed by the combination of above reagents. A suggestible procedure for general use is as follows.

0.02 M 2-Nitrophenylhydrazine Hydrochloride Solution: Dissolve 0.380 g of the reagent in 100 ml of 99.5% ethanol by gentle warming.

Thiourea Solution: Dissolve 2.0 g of the reagent in 100 ml of 99.5% ethanol.

Working 2-Nitrophenylhydrzine Hydrochloride Solution: Mix the same volume of above two reagent solutions before use.

Pyridine Solution: Mix 7.0 ml of pyridine with sufficient 99.5% ethanol to measure 100 ml.

1) L. Legradi, *Microchem. J.*, **16**, 1 (1971).
2) J.W. Munson, *J. Pharm. Sci.*, **63**, 252 (1974).
3) J.W. Munson and R. Bilous, *J. Pharm. Sci.*, **66**, 1403 (1977).
4) Y. Kasai, T. Tanimura, and Z. Tamura, *Anal. Chem.*, **47**, 34 (1975).

0.25 M Dicyclohexylcarbodiimide Solution: Dissolve 5.16 g of the reagent in sufficient 99.5% ethanol to measure 100 ml.

Potassium Hydroxide Solution: Dissolve 5 g of potassium hydroxide in 5 ml of water, and add sufficient methanol to measure 50 ml.

Pipette 0.50 ml of an aqueous or aqueous ethanol sample in a reaction tube of about 6 ml capacity with a screw cap,[5] and add successively 2.0 ml of working 2-nitrophenylhydrazine hydrochloride solution, 1.0 ml of pyridine solution and 1.0 ml of 0.25 M dicyclohexylcarbodiimide solution. Incubate at 25° for 2 hours with the reagent blank. Add 0.50 ml of potassium hydroxide solution, cap tightly, and immerse in a water bath of 60° for 15 minutes. Cool to 25°, and read the absorbance against the reagent blank at proper wavelength, for example, at 550 nm for acetic acid and at 565 nm for benzoic acid.

Alternatively, the coupling reaction can be carried out at 37° for 1 hour, or at 60° for 20 minutes, though the absorbance is decreased with increasing temperature by the probable formation of unreactive esters. The calibration curves were linear in the range of 0.1 to 1 micromoles of acetic, stearic (in 70% ethanol) and benzoic acids, and 0.05 to 0.5 micromoles of citric acid. Most amino acids showed negative reactions in the procedure with a few exceptions.

This method of coloration of carboxylic acids may be used for the spot test and thin-layer or high performance chromatography. Details of these experiments will be published in the near future.

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5) The reaction tubes for "Rapid Blood Analyser," (Chugai Seiyaku Kabushiki Kaisha, Iwamotocho, Chiyoda-ku, Tokyo) were used in this work.