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Characteristic Detection and Determination of Aliphatic Aldehydes[1]

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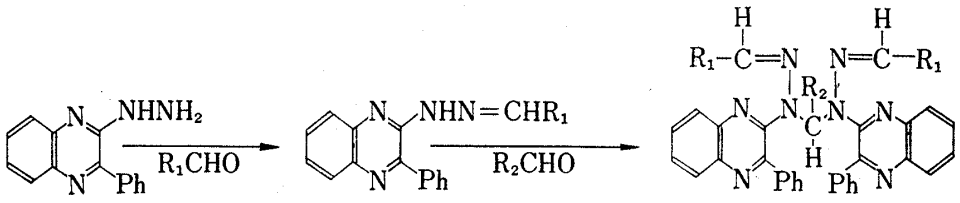
Aliphatic aldehydes can be detected and determined by reaction with propionaldehyde (3-phenyl-2-quinoxalinyl)hydrazone in ethanolic solution. The red color is produced within 20 min at 40°, then faded gradually. Detectable responses are obtained with aliphatic aldehydes but not with aromatic aldehydes, glucose, and ketones. With acetaldehyde, propionaldehyde, butyraldehyde, and iso-butyraldehyde, absorbance of the reaction mixture measured at 465 nm is directly proportional to the concentration. Acetaldehyde can be determined in the concentration range of 5×10^{-5} to 1×10^{-3} M and other aldehydes in the range of 1×10^{-4} to 1×10^{-3} M.

There have been many reports published on the color reaction of aldehydes.[3] Most of the reactions are common to aliphatic and aromatic aldehydes, ketones, and reducing sugars. We have previously reported a color reaction specific to aliphatic aldehydes[4] and the structure of its reaction product. In short, aliphatic aldehydes react with 2-hydrazino-3-phenylquinoxaline (I) to produce a yellow hydrazone (II) which further reacts with aliphatic aldehyde to form a red dye (III). This dye (III) is not formed with aromatic aldehydes and ketones. From the ultraviolet (UV) spectra of II ($R_1=C_2H_5$) and III ($R_1=C_2H_5$, $R_2=C_2H_5$), which have been shown in the previous paper, it was found that both II and III have absorption maximum at 280 nm and the absorption at 386 nm in II shifts to 465 nm in III.



[Ph=phenyl]

These facts suggest that the reagent is specific to aliphatic aldehydes among various carbonyl compounds. This paper is an attempt to examine the possibility of qualitative and quantitative analyses of aliphatic aldehydes using this hydrazon.

Experimental

Synthesis of Propionaldehyde (3-phenyl-2-quinoxalinyl)hydrazone (II)——To a solution of 2.76 g (0.01 mole) of I in iso-PrOH (10 ml) which was cooled in ice-water, 0.58 g (0.01 mole) of propionaldehyde was added dropwise with stirring. The resulting hydrazone was filtered, washed with petroleum benzine and sucked as dry as possible. Recrystallization from petroleum benzine gave pale yellow needles, mp 124°.

1) The 94th Annual Meeting of Pharmaceutical Society of Japan, Sendai, April 1974.
2) Location: *Gofuku Toyama.*
3) W. Ried, *Angew. Chem.*, **64**, 391 (1952); W.C. Tobie, *Ind. Eng. Chem. Anal. Ed.* **14**, 404 (1942); H. Seiler and H. Schmid, *Helv. Chim. Acta*, **39**, 1 (1954).
4) S. Tagami and K. Sasayama, D. Shiho, *Chem. Pharm. Bull.* (Tokyo), **17**, 5 (1969).

Apparatus——Absorbance measurements were carried out with a Shimadzu Spectrophotometer Model QV-50. PH was measured with a Hitachi-Horiba pH meter Type M-4.

Reagent Solution——A reagent solution (1×10^{-3}M) was made fresh daily by dissolving 13.8 mg of II in 50 ml of 95% EtOH. This reagent solution cannot be used after a day because of decomposition.

Carbonyl Compounds Examined——The aldehydes and ketones used in the present work were obtained by redistillation of guaranteed grade reagents from various sources.

Detection Procedure——To 2 ml of 95% ethanolic solution of II (1×10^{-3}M) in a test tube, 1 or 2 drops of sample solution was added and the mixture was heated at 40°. After 20 min, a red color indicated the presence of aliphatic aldehyde (Table I). The blank was pale yellow.

Preparation of Calibration Curve——Ethanolic solution (10 ml) of an aliphatic aldehyde and ethanolic solution (10 ml) of II (1×10^{-2}M), which had been kept at 30° for 15 min beforehand, were mixed in a flask and kept at 30°. Aliquots were periodically withdrawn and absorbance of the reaction mixture was read at 465 nm against a reagent blank. When the absorbance was plotted as ordinate and time as abscissa, time-absorbance curve was obtained. Calibration curve was prepared from the plots of aldehyde concentration *vs.* the absorbance which was obtained from time-absorbance curve at 3 hr, and followed a straight line. It was found from these calibration curves that acetaldehyde can be determined in the concentration range of 5×10^{-5} to 1×10^{-3}M and other aldehydes except for octaldehyde in the range of 1×10^{-4} to 1×10^{-3}M.

Determination Procedure——To 10 ml of ethanolic solution of II was added 10 ml of ethanolic solution of a sample. After agitation, the reaction mixture was kept at 30° for 3 hr. The absorbance of the reaction mixture was then read at 465 nm against a reagent blank. Amount of the aldehyde in the sample was measured referring to the calibration curve.

Table I. Color-producing Reaction of Carbonyl Compound with Propionaldehyde (3-phenyl-2-quinoxalinyl)hydrazone at 40°

Carbonyl compound	Time (min)				
	5	10	20	30	40
Formaldehyde	OR	R	R	R	R
Acetaldehyde	R	R	R	R	R
Propionaldehyde	R	R	R	R	R
Butyraldehyde	OR	R	R	R	R
Isobutyraldehyde	OR	R	R	R	R
Isovarelaldehyde	OR	R	R	R	R
Capronaldehyde	OR	R	R	R	R
Octaldehyde	O	OR	R	R	R
Nonylaldehyde	O	OR	R	R	R
Benzaldehyde	OY	OY	OY	Y	Y
m-Bromobenzaldehyde	Y	Y	Y	Y	Y
p-Methylbenzaldehyde	Y	Y	Y	Y	Y
p-Methoxybenzaldehyde	Y	Y	Y	Y	Y
Acetone	Y	Y	Y	Y	Y
Methyl isobutyl ketone	Y	Y	Y	Y	Y
1,1-Dimethyl-3-butanone	Y	Y	Y	Y	Y
Glucose	Y	Y	Y	Y	Y
Blank	Y	Y	Y	Y	Y

R: red, O: orange, Y: yellow

Table II. Detection of Aliphatic Aldehyde

Aldehyde	Identification limit (μg/ml)
Acetaldehyde	40
Propionaldehyde	115
Butyraldehyde	140
Capronaldehyde	170
Octaldehyde	160

Results

Detection

As shown in Table I, all the aliphatic aldehydes examined developed red color at 40°, while aromatic aldehydes and ketones did not. The color grew deepest at 20 min after addition of aldehyde to the reagent solution, then faded gradually. The color developed quickly at 40° than 30°, but faded rapidly when the reaction temperature was raised. Consequently, 40° seemed to be the most suitable temperature for this color reaction. Limit of detection of aliphatic aldehydes by this reaction is listed in Table II.

Determination

Effect of pH——The reaction solution of propionaldehyde was adjusted to various pH by the addition of buffer solution, and time-absorbance curves thus obtained for these solutions are shown in Fig. 1. The reaction is accelerated in acid condition, but is inhibited in alkaline medium. At pH 3.0, decompositions of both reagent and dye are so fast. The dye decomposes slowly at pH 4.6 and is stable for more than 5 hr at pH 7.0, while the reaction is markedly slowed down at pH 9.4. Taking these results into account, we adopted the reaction in 95% ethanolic solution without adjusting pH with buffer solution.

Reaction Temperature——A mixture of 1×10^{-3}M propionaldehyde and 1×10^{-2}M hydrazone was kept at various temperatures and time-absorbance curves were plotted at 465 nm. As shown in Fig. 2, the absorbance reaches plateau in about 6 hr when reacted at 20° and remains constant for more than 9 hr thereafter. When reacted at 30°, the absorbance reaches an equilibrium in 2—3 hr and is stable for 4—5 hr thereafter. When reacted at 40°, the absorbance gets to maximum in 2—3 hr, then decreases gradually. The optimal reaction temperature, therefore, was chosen to be 30°. Time-absorbance curves were plotted at 30° and the curves for acetaldehyde, butyraldehyde, and isobutyraldehyde were similar to that for propionaldehyde in Fig. 2. In these cases, the reaction reached an equilibrium in 2—3 hr and color was stable for several hours thereafter. In the case of octaldehyde, however, the dye formed was unstable in 95% EtOH and the time-absorbance curve became convex to vertical axis, therefore, octaldehyde cannot be determined.

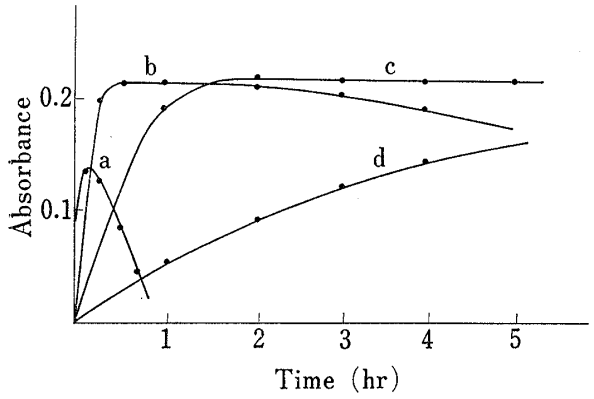


Fig. 1. Effect of pH on Time-Absorbance Curve for Propionaldehyde at 30°
(a) pH 3.0, (b) pH 4.6, (c) pH 7.0, (d) pH 9.4

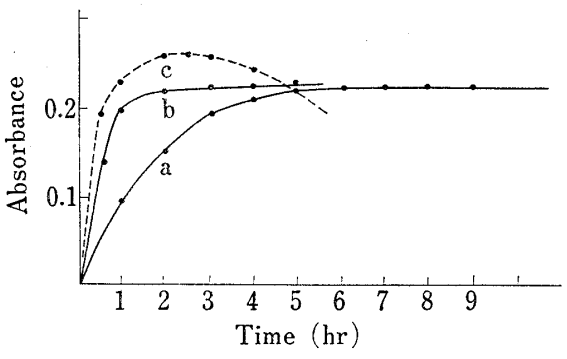


Fig. 2. Effect of Temperature on Time-Absorbance Curve for Propionaldehyde
(a) 20°, (b) 30°, (c) 40°

Discussion

The color is produced by the formation of a red dye from the reaction of II with aliphatic aldehydes. We have already reported the isolation of this dye and the determination of its

molecular weight, elementary analysis, and kinetic studies on the reaction rate of the dye formation, and have also explained the reason why the structure colors red.

The lone-pair electrons of the nitrogen in the side-chain –NH group in the hydrazone (II) first attacks the δ^+ carbon in the carbonyl group of the aldehyde to form an additive intermediate and another molecule of the hydrazone undergoes dehydrative condensation with this intermediate to form a dye. The reaction rate constants shown in the previous paper indicate that the larger the electron donation of the alkyl group to the carbonyl-carbon of the aldehyde, the slower the reaction. As shown in Table I, aliphatic aldehydes that show red color are those with a carbonyl-carbon with strong δ^+ nature among various carbonyl compounds.

The reason why aromatic aldehydes and ketones do not develop red color is found in their weak δ^+ nature due to the resonance of the carbonyl group and the benzene ring in aromatic aldehydes, and due to the electron-donating effect of the two alkyl groups bonded to the carbonyl-carbon in ketones. From these reasons, this reaction can be used for the detection of aliphatic aldehydes among various carbonyl compounds.

It was found possible to determine the small molecular weight aldehydes with this reagent because the colored products formed from them are comparatively stable, while impossible in the case of octaldehyde because the colored product from it is unstable and easily suffers decomposition. Moreover the reagent itself is also unstable and easily subjected to dehydrative cyclization to an *s*-triazole compound. For these reasons, the reagent must be prepared freshly every time for use.