

INTERNATIONAL UNION OF PURE  
AND APPLIED CHEMISTRY

ANALYTICAL CHEMISTRY DIVISION

COMMISSION ON ANALYTICAL REACTIONS  
AND REAGENTS

**COLORIMETRIC AND FLUORIMETRIC  
DETERMINATION OF ALDEHYDES AND  
KETONES**

Prepared for publication by  
J. BARTOS  
M. PESEZ

Roussel-Uclaf, Romainville,  
France

PERGAMON PRESS  
OXFORD · NEW YORK · PARIS · FRANKFURT

## COLORIMETRIC AND FLUORIMETRIC DETERMINATION OF ALDEHYDES AND KETONES

Jaroslav Bartos and Maurice Pesez

Roussel-Uclaf, F-93230 Romainville

Abstract - In this report are described 13 procedures allowing the colorimetric and fluorimetric determination of aldehydes and ketones. Eight of these methods make use of the reaction of the carbonyl group with the  $-NH_2$  group of an arylamine, a hydrazine or a hydrazone, giving rise to Schiff's bases, hydrazones or azines. In the two latter cases, further reactions, yielding a Meisenheimer type complex or a formazan derivative, allow very sensitive determinations. Two methods are based on the Hantzsch reaction, giving derivatives of dihydropyridine or decahydroacridine, which either absorb in the UV range or fluoresce. Through the Doebner-Miller reaction,  $\alpha$ -methylene aldehydes give fluorescent derivatives of 7-aminoquinoline-5-carboxylic acid, whereas  $\alpha$ -methylene ketones can be determined colorimetrically through the Zimmermann reaction.  $\beta$ -Diketones, reacting with o-phenylenediamine, give colored benzodiazepines.

### INTRODUCTION

It is quite obvious that numerous methods of colorimetric or fluorimetric determination of aldehydes and ketones have been described in the literature and that a definite reaction has very often given rise to various procedures, and it would certainly be worth mentioning a number of them. But we thought that a selection of methods which were checked in our laboratory, and which we consider as reliable, would be of greater value to the analyst than a mere compilation.

In the colorimetric determinations described, Beer's law is obeyed at least up to the absorbance value of 0.8. For the fluorimetric determinations, the linear relationship is observed within the limits given. Smaller amounts of compounds can however be sometimes determined, but then the linear relationship usually does not hold.

In all cases, the compounds mentioned under Results, as well as the compounds which are mentioned as reacting only weakly or not at all are the only compounds we tested. Therefore, no reaction should be considered as limited to the compounds listed. It is obvious that it was not possible for us to test the hundreds of compounds bearing a carbonyl group which are described in the literature. Many of them interest only a few specialists, and this is why we preferred to select only some simple compounds, in order to illustrate the sensitivity of the procedure.

All the reagents used were of analytical grade quality, and the solvents of reagent grade quality. By water is meant distilled water. For all reagents in solution, and unless otherwise stated, the concentration is always expressed in w/v if the solute is a solid, and in v/v if it is a liquid.

For the sake of brevity, the limits within which a given temperature or a given time should be observed were not specified for each of the methods.

Temperature : When it is written : "Heat at  $t^\circ C$ ", it means that the temperature should be maintained at  $t \pm 1^\circ C$ . "Room temperature" means that the temperature is within the range 18-24°C.

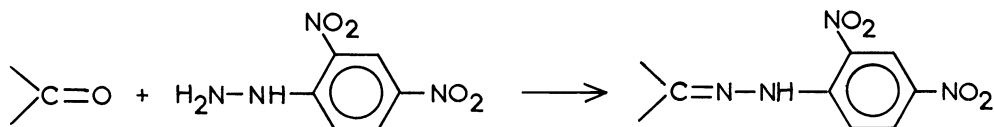
Time : When it is written : "Let stand for x min" or "Heat for x min", it means  $x \text{ min} \pm 5\%$ . When it is written : "Let stand for x min and read", it means : start with reading after  $x \text{ min} \pm 5\%$ .

The absorbance was always read against the blank of the reagents.

## A. ALDEHYDES and KETONES (Colorimetry)

1. Reaction with 2,4-dinitrophenylhydrazine (1)

Formation of the 2,4-dinitrophenylhydrazone in an acetic acid hydrochloric acid medium : yellow-orange color.



Chemicals. Glacial acetic acid, concentrated hydrochloric acid, and 2,4-dinitrophenylhydrazine.

Reagent solution. An 0.10% solution of 2,4-dinitrophenylhydrazine in glacial acetic acid containing 0.50% (v/v) of concentrated hydrochloric acid.

Method. To 1.0 ml of sample solution in glacial acetic acid, add 5.0 ml of reagent. Let stand in the dark for 1 h at room temperature, and read at 412 nm.

Results

Sample size ( $\mu\text{g}$ ) for A = 0.30  
(1 cm - cell)

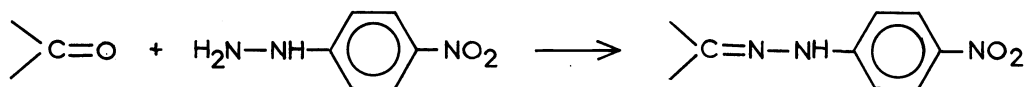
Acetaldehyde	20
Benzaldehyde	21
Cinnamaldehyde	11
Citral	30
3,3-Diphenylacrolein	14
Formaldehyde	20
Furfural	14
p-Hydroxybenzaldehyde	12
Piperonal	15
Propionaldehyde	21
Salicylaldehyde	15
Vanillin	14
Veratraldehyde	15
Acetone	45
Acetophenone	35
Cyclohexanone	100
Cyclopentanone	56
Ethyl acetoacetate	140
Methyl ethyl ketone	65

Camphor and benzophenone react but weakly. After 15 min at 100°C, A = 0.30 is given (at 432 nm) by 430  $\mu\text{g}$  benzophenone and 3500  $\mu\text{g}$  camphor. Ketosteroids can be determined upon the same principle.

The sensitivity and the wavelength of the maximum of absorption may vary slightly with the batch of acetic acid used, as well as with the purity of the 2,4-dinitrophenylhydrazine. Discrepancies are observed even between analytical grade reagents. The data given above were obtained with B.D.H. dinitrophenylhydrazine.

2. Reaction with p-nitrophenylhydrazine (2)

Formation of the p-nitrophenylhydrazone, and development of the color with benzyltrimethylammonium hydroxide in dimethylformamide : red to violet color.



The exact mechanism of the development of the color upon the action of the base is unknown.

**Chemicals.** Dimethylformamide, ethanol, concentrated hydrochloric acid, a 40% solution of benzyltrimethylammonium hydroxide in methanol, and *p*-nitrophenylhydrazine.

**Reagent solutions.** (a) An 0.040% solution of *p*-nitrophenylhydrazine in ethanol containing 0.10% (v/v) of concentrated hydrochloric acid. (b) Dilute 1.0 ml of 40% solution of benzyltrimethylammonium hydroxide in methanol to 100.0 ml with dimethylformamide. Prepare fresh just before use.

**Method.** To 0.50 ml of sample solution in ethanol, add 0.50 ml of reagent a, heat at 70°C for the given length of time, cool in a water bath, add 9.0 ml of reagent b, and read.

### Results

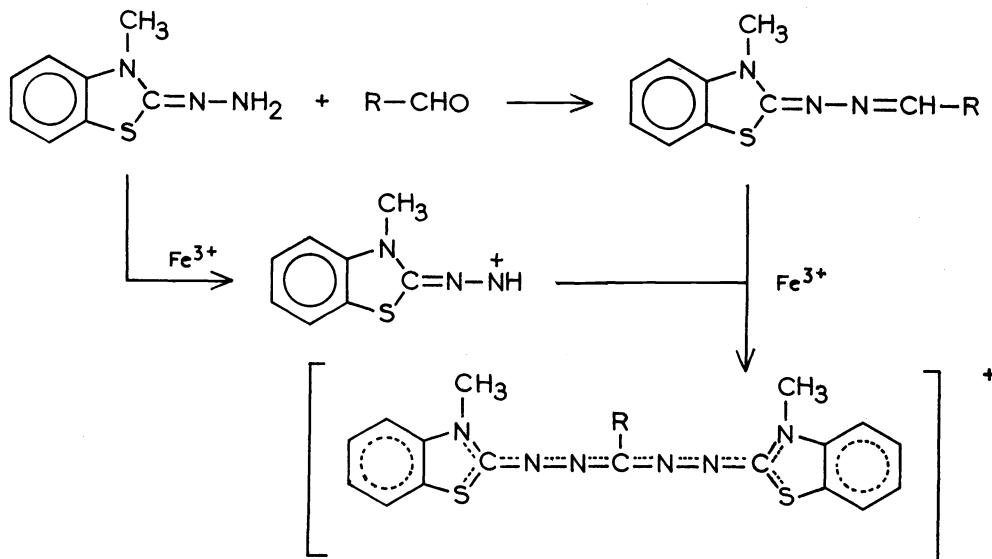
	Heating time (min)	$\lambda$ Max. (nm)	Sample size ( $\mu\text{g}$ ) for A = 0.30 (1 cm - cell)
Acetaldehyde	30	510	25.0
Formaldehyde	20	510	20.0
Glyoxal (monohydrate)	30	710	6.4
Piperonal	20	550	9.7
Propionaldehyde	30	520	28.0
Salicylaldehyde	20	580	8.8
Vanillin	20	590	9.3
Acetone	30	520	6.0
Acetophenone	30	560	31.0
Cyclohexanone	20	520	10.0
Cyclopentanone	30	520	13.0
Diacetyl	30	560	15.2
$\alpha$ -Ionone	30	550	20.0
Methyl ethyl ketone	30	520	13.0

Acetylacetone, benzophenone, camphor, ethyl acetoacetate, glucose and ribose give only a very faint color. The same procedure allows the colorimetry of ketosteroids.

### B. ALIPHATIC ALDEHYDES (Colorimetry)

**Reaction with 3-methylbenzothiazolin-2-one hydrazone (3)**

Condensation with 3-methylbenzothiazolin-2-one hydrazone, and conversion into formazan upon reaction with the diazonium salt generated in the reaction medium by oxidation of the excess reagent : blue-green to green color.



**Chemicals.** Isopropyl alcohol, ferric chloride hexahydrate, 3-methylbenzothiazolin-2-one hydrazone, and sulfamic acid.

**Reagent solutions.** (a) An 0.40% aqueous solution of 3-methylbenzothiazolin-2-one hydrazone. (b) Aqueous solution containing 1.0% of ferric chloride hexahydrate and 1.60% of sulfamic acid.

**Method.** To 1.0 ml of sample solution in water or isopropylalcohol, add 1.0 ml of reagent a and let stand for 20 min at room temperature. Add 1.0 ml of reagent b, mix and let stand for 10 min, then add 2.0 ml of water, mix and read.

### Results

	Solvent *	$\lambda$ Max. (nm)	Sample size ( $\mu$ g) for A = 0.30 (1 cm - cell)
Acetaldehyde	W	610	1.2
Butyraldehyde	W	615	2.0
Caprylic aldehyde	IA	635	4.6
Citral	IA	640	13.5
Citronellal	IA	640	11.6
Formaldehyde	W	635	0.9
Lauric aldehyde	IA	635	33.6
Propionaldehyde	W	620	1.7

\* W = water                      IA = Isopropyl alcohol

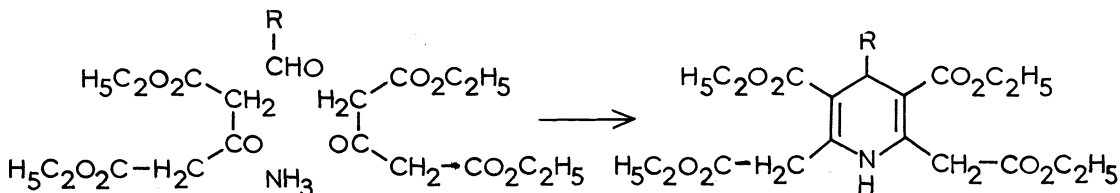
**Notes.** 1. This method is an improvement over a previously described one (4) which was less sensitive because it necessitated a dilution with acetone in order to eliminate a turbidity. This turbidity is avoided in the above procedure by adding sulfamic acid to the ferric chloride solution.

2. Glyoxal and acrolein do not give satisfactory results. However, a modification of the above procedure allows their determination: To 1.0 ml of sample solution in water, add 1.0 ml of reagent a and 1.0 ml of reagent b. Let stand at room temperature for 30 min, add 2.0 ml of water and read at 625 nm. Beer's law is then obeyed, and A = 0.30 is given by 2.73  $\mu$ g of glyoxal or 1.32  $\mu$ g of acrolein.

### C. ALIPHATIC ALDEHYDES (UV spectrophotometry)

#### Reaction with diethyl acetonedicarboxylate and ammonia (5)

Condensation based on the Hantzsch reaction. The formation of a 4-substituted 3,5-bis(ethoxycarbonyl)-1,4-dihydro-2,6-pyridinediacetic acid may be assumed: slightly yellow color, but the absorption maximum is located in the near-UV range.



**Chemicals.** Ethanol, glacial acetic acid, 2.0 M sodium hydroxide, ammonium acetate, and diethyl acetonedicarboxylate.

**Reagent solution.** Dissolve 8.0 g of ammonium acetate in 10.0 ml of water, add 8.0 ml of diethyl acetonedicarboxylate and dilute to 50.0 ml with ethanol.

**Method.** To 1.0 ml of sample solution in glacial acetic acid, add 2.0 ml of reagent. Heat at 60°C for 90 min, add 2.0 ml of 2.0 M sodium hydroxide and read at 344 nm.

Results

Sample size ( $\mu\text{g}$ ) for  $A = 0.30$   
(1 cm - cell)

Acetaldehyde	4.6
Cinnamaldehyde	14.0
Formaldehyde	16.0
Propionaldehyde *	6.2

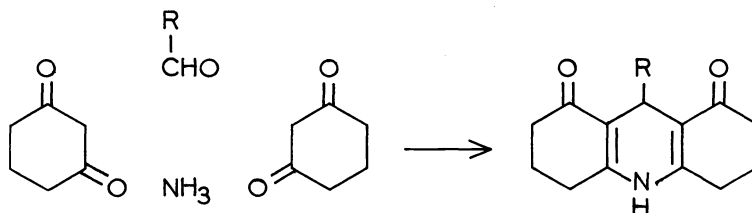
\* Read at 340 nm.

Furfural also reacts.  $A = 0.30$  for  $6.2 \mu\text{g}$  at 344 nm. Beer's law is not obeyed with citral and aromatic aldehydes. Ketones do not react.

## D. ALIPHATIC ALDEHYDES (Fluorimetry)

Reaction with cyclohexane-1,3-dione and ammonia (6)

Condensation based on the Hantzsch reaction. Formation of a 9-substituted decahydro-acridine-1,8-dione : blue fluorescence in acidic medium, yellow-green fluorescence in alkaline medium.



Chemicals. Glacial acetic acid, 10.0 M sodium hydroxide, ammonium acetate, and cyclohexane-1,3-dione.

Reagent solution. Dissolve 0.250 g of cyclohexane-1,3-dione, 10.0 g of ammonium acetate and 5.0 ml of glacial acetic acid in 80 ml of water and dilute to 100.0 ml with water.

Method. To 3.0 ml of sample solution in water, add 1.0 ml of reagent, heat at  $60^\circ\text{C}$  for 1 h, chill for 2 min in ice water, and add either 1.0 ml of water (blue fluorescence) or 1.0 ml of 10.0 M sodium hydroxide (yellow-green fluorescence). Read at exc : 366 nm ; em : 470 nm (acidic medium) or at exc : 436 nm ; em : 520 nm (alkaline medium).

Results

	Determination limits ( $\mu\text{g}$ )	
	Acidic medium	Alkaline medium
Acetaldehyde	0.20 - 1.00	0.20 - 1.00
Butyraldehyde	0.60 - 3.00	0.50 - 2.50
Formaldehyde	0.50 - 2.50	0.50 - 2.50
Propionaldehyde	0.30 - 1.50	0.20 - 1.00

Citral, glyoxal, glyoxylic acid and isobutyraldehyde react much more weakly. Benzaldehyde develops a fluorescence in acidic medium only, and can be determined in the range 2.0 - 10.0  $\mu\text{g}$ , at em : 455 nm. No fluorescence is displayed by vanillin.

## E. 2,3-UNSATURATED ALDEHYDES (UV spectrophotometry)

Reaction with m-phenylenediamine (7)

Formation of a Schiff base : yellow-brown color, but the absorption maximum is located in the near-UV range.

Chemicals. Ethanol and m-phenylenediamine oxalate.

Reagent solution. A 1.0% solution of m-phenylenediamine oxalate in a 4 : 1 mixture of ethanol and water.



This condensation is based on the Doebner-Miller synthesis of quinoline derivatives, the mechanism of which is not as yet completely elucidated.

Chemicals. Phosphoric acid,  $d = 1.62$ , and 3,5-diaminobenzoic acid dihydrochloride.

Reagent solution. To 2.0 g of 3,5-diaminobenzoic acid dihydrochloride, add 10.0 ml of phosphoric acid ( $d = 1.62$ ), and dilute to 20.0 ml with water.

Method. To 2.0 ml of sample solution in water, add 2.0 ml of reagent and heat at  $50^{\circ}\text{C}$  for 30 min, protected against bright light. Allow to cool to room temperature, add 2.0 ml of water, and read at exc : 405 nm ; em : 495 nm.

### Results

#### Determination limits ( $\mu\text{g}$ )

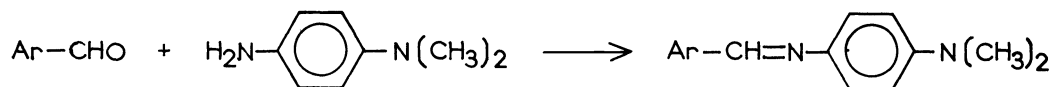
Acetaldehyde	2.0 - 10.0
Butyraldehyde	2.0 - 10.0
Propionaldehyde	2.0 - 10.0

With some modifications, the same reagent allows the determination of 2-deoxy sugars (6).

### H. AROMATIC ALDEHYDES (Colorimetry)

#### 1. Reaction with p-dimethylaminoaniline (9)

Formation of a Schiff base : yellow or orange color.



Chemicals. Glacial acetic acid and p-dimethylaminoaniline oxalate.

Reagent solution. A 2.0% solution of p-dimethylaminoaniline oxalate in glacial acetic acid.

Method. To 0.50 ml of sample solution in glacial acetic acid, add 2.0 ml of reagent. Let stand for 5 min in the dark, add 1.50 ml of acetic acid, and read.

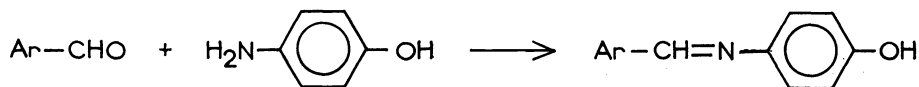
### Results

	$\lambda$ Max. (nm)	Sample size ( $\mu\text{g}$ ) for $A = 0.30$ (1 cm - cell)
o-Aminobenzaldehyde	480	16.0
p-Anisaldehyde	465	9.0
Benzaldehyde	465	19.0
2-Methoxy-6-naphthaldehyde	475	11.5
p-Nitrobenzaldehyde	450	33.0
Piperonal	465	10.0
Protocatechualdehyde	465	7.5
Salicylaldehyde	465	27.0
p-Tolualdehyde	460	12.0
Vanillin	460	9.0
Veratraldehyde	465	10.0

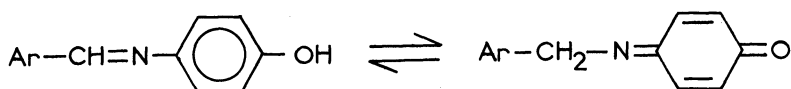
Furfural also reacts ( $A = 0.30$  for 9  $\mu\text{g}$  at 480 nm). Aliphatic aldehydes also react, but the colors obtained are less stable and Beer's law is not always obeyed. Simple ketones react but very weakly ( $A = 0.21$  is given by 700  $\mu\text{g}$  of acetone or 235  $\mu\text{g}$  of methyl ethyl ketone).

#### 2. Reaction with p-aminophenol (5)

Formation of a Schiff base : yellow color.







Chemicals. Glacial acetic acid and *p*-aminophenol.

Reagent solution. A 2.0% solution of *p*-aminophenol in glacial acetic acid.

Method. To 3.0 ml of sample solution in glacial acetic acid, add 2.0 ml of reagent. Let stand for 15 min, and read.

### Results

	$\lambda$ Max. (nm)	Sample size ( $\mu\text{g}$ ) for A = 0.30 (1 cm - cell)
Benzaldehyde	350	18.0
<i>p</i> -Dimethylaminobenzaldehyde	440	3.8
2-Methoxy-6-naphthaldehyde	410	10.8
Salicylaldehyde	350	12.0
Vanillin	390	7.6
Veratraldehyde	390	9.0

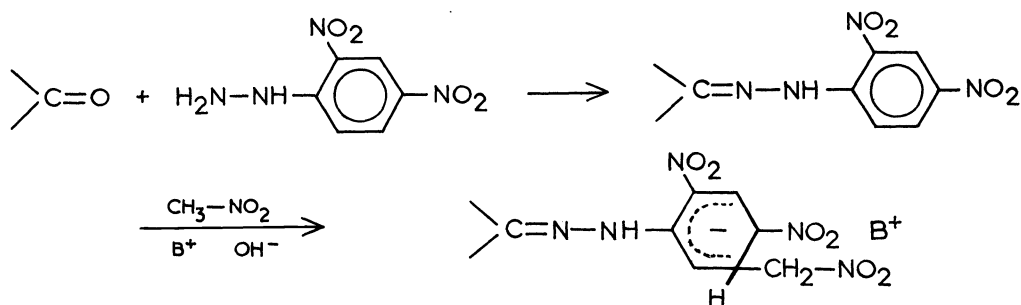
Furfural also reacts (A = 0.30 for 9  $\mu\text{g}$  at 375 nm).

This method may be compared with the method F for 2,3-unsaturated aldehydes. However, these ones react but very weakly under the above conditions.

### I. KETONES (Colorimetry)

Reaction with 2,4-dinitrophenylhydrazine and nitromethane (10)

Formation of a 2,4-dinitrophenylhydrazone, and development with nitromethane in alkaline medium (Janovsky reaction). The excess of reagent does not interfere: violaceous-pink color.



Chemicals. Dimethylformamide, ethanol, nitromethane, concentrated hydrochloric acid, a 40% solution of benzyltrimethylammonium hydroxide in methanol, and 2,4-dinitrophenylhydrazine.

Reagent solution. Dissolve 0.025 g of 2,4-dinitrophenylhydrazine in a mixture of 10.0 ml of ethanol and 0.50 ml of concentrated hydrochloric acid, and dilute to 50.0 ml with ethanol.

Method. Operate protected against light. To 0.50 ml of sample solution in ethanol, add 0.50 ml of reagent a and let stand at the given temperature for the given length of time. Cool to room temperature if necessary, add 1.50 ml of nitromethane, 1.50 ml of dimethylformamide and 0.30 ml of 40% solution of benzyltrimethylammonium hydroxide in methanol, shake for 30 seconds, and read at 565 nm.

### Results

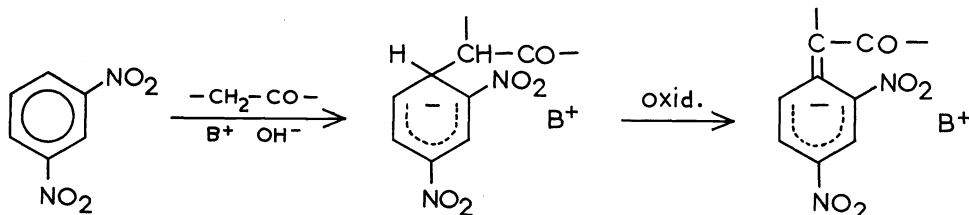
	Temperature ( $^{\circ}\text{C}$ )	Reaction time (min)	Sample size ( $\mu\text{g}$ ) for A = 0.30 (1 cm - cell)
Acetone	18 - 24	20	4.3
Cyclohexanone	18 - 24	10	7.4
Cyclopentanone	50	20	6.0
Methyl ethyl ketone	18 - 24	20	5.3

The sample weight giving the absorbance 0.30 is fairly inversely proportional to the molecular weight of the ketone.  $\beta$ -Diketones afford a very transient color making measurements impossible. Aldehydes react but very weakly.

#### J. $\alpha$ -METHYLENE KETONES (Colorimetry)

Reaction with *m*-dinitrobenzene in alkaline medium (5)

Formation of a Meisenheimer-like complex, which is then oxidized by the excess reagent (Zimmermann reaction) : violet color.



**Chemicals.** Pyridine, a 40% solution of benzyltrimethylammonium hydroxide in methanol, ammonium acetate, and *m*-dinitrobenzene.

**Reagent solutions.** (a) A 3.0% solution of *m*-dinitrobenzene in pyridine. (b) Dilute 2.50 ml of 40% solution of benzyltrimethylammonium hydroxide in methanol to 10.0 ml with pyridine. (c) Dilute 5.0 ml of 10.0% aqueous solution of ammonium acetate to 100.0 ml with pyridine.

**Method.** Operate protected against light. To 1.0 ml of sample solution in pyridine, add 0.50 ml of reagent a, chill to 0° in ice water, add 0.20 ml of reagent b and let stand at 0°C for 1 min. Add 3.0 ml of reagent c, mix, and read immediately at 570 nm.

#### Results

Sample size (μg) for A = 0.30  
(1 cm - cell)

Acetone	2.8
Acetophenone *	7.8
Cyclohexanone	3.2
Cyclopentanone	3.0
Methyl ethyl ketone	3.2

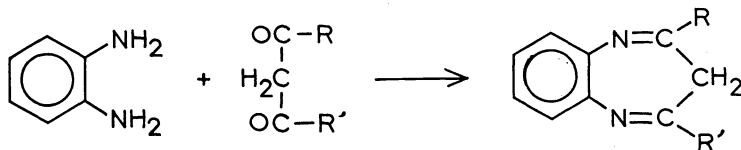
\* Operate at room temperature.

Aldehydes react but weakly. Absorbance 0.30 is given by 50 μg of propionaldehyde. Ketones with no methylene group in  $\alpha$  position to the carbonyl group, such as benzil and benzophenone, do not react.

#### K. $\beta$ -DIKETONES (Colorimetry)

Reaction with *o*-phenylenediamine (11)

Formation of a substituted benzodiazepine in acid medium : red color.



**Chemicals.** 1.65 M phosphoric acid, 1.20 M sulfuric acid, dipotassium phosphate, and *o*-phenylenediamine.

Reagent solution. Dissolve 0.40 g of *o*-phenylenediamine in a mixture of 4.35 g of dipotassium phosphate, 2.0 ml of 1.65 M phosphoric acid and 50 ml of 1.2 M sulfuric acid, and dilute to 100.0 ml with water. Prepare fresh before use.

Method. To 5.0 ml of sample solution in water, add 1.0 ml of reagent. Let stand for 30 min, and read at 500 nm.

#### Results

Sample size ( $\mu\text{g}$ ) for A = 0.30  
(1 cm - cell)

Acetylacetone	216
Heptane-3,5-dione	268
Hexane-2,4-dione	243

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