

2:4-DINITROPHENYLHYDRAZINE, A SUITABLE REAGENT FOR THE COLORIMETRIC DETERMINATION OF CARBONYL COMPOUNDS

BY MAURICE PESEZ

From the Research Laboratories, Roussel-Uclaf, Paris

Received March 2, 1959

Carbonyl compounds are determined by a method based on the stable orange colour of 2:4-dinitrophenylhydrazones in acidic medium. Under such conditions the blank test is negligible. The method proved useful for the determination of aliphatic, aromatic and steroid compounds.

2:4-DINITROPHENYLHYDRAZONES are highly coloured compounds; however they have but rarely been used for the colorimetric determination of small amounts of aldehydes or ketones, because the interference from excess reagent causes trouble and requires chromatographic separations¹ or extractions with various solvents²⁻⁵. Addition of alkali to the dinitrophenylhydrazones produces a red colour⁶⁻¹¹ which differs from that of the reagent, but the instability of the resulting colour limits this application.

According to our experiments the 2:4-dinitrophenylhydrazones can be obtained at room temperature in acetic-hydrochloric acid medium. Under these conditions the colour of the blank is only light yellow, whereas the condensation of the reagent with the carbonyl compounds yields a stable orange colour with an absorption maximum at 4125 Å. The intensity of the colour follows Beer's law; the determination of aldehydes and ketones can therefore be applied to samples ranging from 10 to 100 µg.

The intensity of the colour varies according to the conjugation of the carbonyl compound involved (Table I); differentiation is thus possible, especially between aliphatic and aromatic compounds. Benzophenone, camphor and sugars react rather slowly at room temperature, and require 15 minutes' heating on the boiling water bath, to ensure condensation, the absorption maximum being slightly shifted to 4325 Å. It should be noted

TABLE I
MOLECULAR ABSORPTION AFTER REACTION OF ALDEHYDES, KETONES OR SUGARS WITH
2:4-DINITROPHENYLHYDRAZINE

Aldehydes	ε	Ketones	ε
Acetaldehyde	4,225	Acetone	2,320
Benzaldehyde	8,480	Acetophenone	6,240
Cinnamaldehyde	22,970	Acetylacetone	null
Citral	9,120	Benzophenone (*)	765
Diphenylacrolein	27,450	Camphor (*)	80
Formaldehyde	2,790	cycloHexanone	1,765
Furfural	11,800	cycloPentanone	2,690
p-Hydroxybenzaldehyde	17,570	Ethylacetoacetate	1,690
Piperonal	18,000	Ethylmethylketone	2,090
Propionaldehyde	4,870		
Salicylaldehyde	14,275	SUGARS	
Vanillin	19,600	Glucose (*)	3,780
Veratraldehyde	19,420	Ribose (*)	4,500

(*) Heating 15 minutes at 100° C.

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that acetylacetone does not develop any colour even after heating, because its hydrazone undergoes cyclization to the slightly coloured 1-(2':4'-dinitrophenyl)3:5-dimethylpyrazole¹².

In the steroid series the condensations are made at 100°. The colour differences can be enhanced (Table II) for the 3-ketonic compounds, by adding an ethanolic solution of potassium acetate. A conjugated double bond in the C4(5) position enhances the molecular absorption whereas an additional C1(2)-double bond decreases it. Of all carbonyl compounds tested, only 11-keto steroids did not react.

TABLE II
CONDENSATION BETWEEN KETOSTEROIDS AND 2:4-DINITROPHENYLHYDRAZINE

Name of compound	Position of carbonyl	Position of double bonds	Molecular absorption	
			without AcOK	with AcOK
3 α -Acetoxy 11-keto etiocholanone ..	11		null	null
Cortisone	3, 11 and 20	4	2,600	6,000
3:17-Diketo androsta-4-ene	3 and 17	4	3,600	9,600
3:17-Diketo androsta-1:4-diene	3 and 17	1 and 4	700	1,700
Dehydro <i>epi</i> androsterone	17	5	735	735
3:12-Dihydroxy-7-keto cholanolic acid ..	7		560	560
3:20-Diketo pregnane	3, 20		2,400	3,250
Estrone	17	1, 3, 5	725	725
Hydrocortisone	3, 20	4	2,400	8,000
17 β -Hydroxy-3-keto androstane	3		450	1,500
3 β -Hydroxy-20-keto pregnane	20		1,650	1,650
3-Hydroxy-12-keto cholanolic acid	12		280	280
Prednisone	3, 11 and 20	1 and 4	1,200	2,550
Prednisolone	3, 20	1 and 4	1,400	2,000
Testosterone	3	4	2,250	7,300

The determination with 2:4-dinitrophenylhydrazine has been applied to pharmaceutical preparations after extraction of the carbonyl compound. Aqueous or oily solutions can be directly determined after suitable dilution with pure acetic acid.

EXPERIMENTAL*

Reagent. 0.1 per cent solution of pure 2:4-dinitrophenylhydrazine in acetic acid containing 0.5 per cent of concentrated hydrochloric acid.

General procedure. The sample is dissolved in 1 ml. of acetic acid; 5 ml. of the reagent is added. After mixing the solution is allowed to remain in the dark for 1 hour at room temperature.

For steroid compounds, the reaction is carried out by 15 minutes' heating on a boiling water bath. After cooling to 20°, 0.5 ml. of a molar ethanolic solution of potassium acetate is added.

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* With the co-operation of Miss G. Clement.