

## 4-Amino-3-hydrazino-5-mercapto-1,2,4,-triazole (PURPALD) in organic chemicals analysis: Distinguishing aldehydes and ketones

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Received on: 12-Jun-2023, Accepted and Published on: 20-Aug-2023

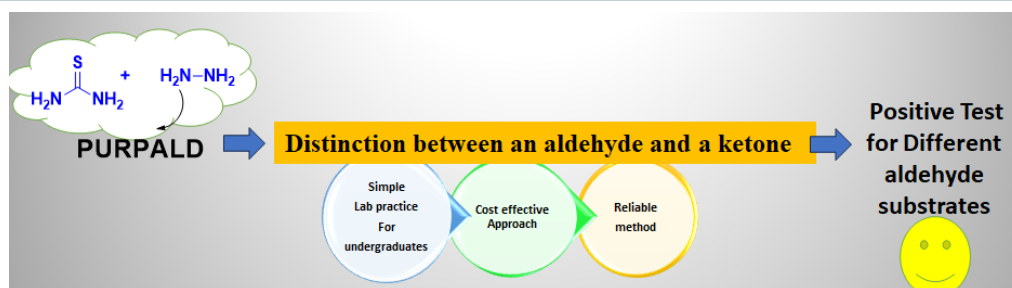
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### ABSTRACT

4-Amino-3-hydrazino-5-mercapto-1,2,4-triazole (AHMT) has emerged as a valuable reagent in organic analysis, particularly for distinguishing aldehydes and ketones. The application of AHMT in the differentiation of aldehydes and

ketones in organic compounds. The reaction mechanism between AHMT and carbonyl compounds is explored, highlighting the selective and sensitive nature of AHMT towards aldehydes and ketones. Various analytical techniques, including spectroscopy, and chemical methods, employed in conjunction with AHMT for analysis are discussed. This abstract serves as a valuable resource for researchers and analysts working in the field of organic analysis seeking to utilize AHMT as a reliable tool for distinguishing aldehydes and ketones.

**Keywords:** Aldehyde, Ketone, Aromatic carbonyls, Sugar, PURPALD



### INTRODUCTION

The carbonyl group, due to its wide range of reactivity and synthetic uses, is a significant functional group frequently referred to as the basis of organic synthesis. Aldehydes and ketones are two prominent groups of compounds in organic chemistry. A typical and widely used test for the identification of carbonyl groups is the reaction of aldehydes and ketones with 2,4-dinitrophenylhydrazine, which results in a coloured precipitate that is frequently orange or red. Different tests are typically used to distinguish between aldehydes and ketones. These are: (i) Fehling's test, (ii) Benedict's test, (iii) Tollen's test, (iv) Chromium trioxide-sulphuric acid test (Jones's test), (v) Baeyer's test, and (vi) Fuchsin aldehyde test (Schiff's test). The first four tests are based on the fact that an aldehyde group is readily oxidizable. In addition, it is well known that the Schiff's test is a non-oxidative test.<sup>1</sup>

The identification and distinction of aldehydes from ketones is greatly aided by spectroscopic methods. Both these classes of compounds display the carbon-oxygen double bond ( $\text{C}=\text{O}$ ) stretching frequency (IR) at about  $1725\text{ cm}^{-1}$ . In addition, the carbon hydrogen stretching frequency of the formyl group ( $\text{C}-\text{H}$ ) appears in the range  $2695\text{--}2730\text{ cm}^{-1}$ . Very often two bands near  $2720$  and  $2920\text{ cm}^{-1}$  are observed (Fermi resonance). The proton magnetic resonance spectrum shows a downfield aldehyde proton signal in the range of  $9.5\text{--}10$  ppm. The spectroscopic methods have largely supplemented the traditional chemical tests. However, the classical chemical tests have considerable educative value and will continue to be used in qualitative analysis.

Wayback in 1970, Dickinson and Jacobsen<sup>2</sup> described a test for the detection of aldehydes using the reagent 4-amino-3-hydrazino-5-mercapto-1,2,4-triazole (I). (Aldrich Chemical Company, USA markets the reagent under the trade name PURPALD). The procedure calls for reaction between an aldehyde and the reagent under alkaline conditions followed by aerial oxidation (aeration). An intense purple to magenta colour develops within minutes (1-5 min). The test is said to be negative with ketones, esters, amides, uric acid, and formic acid. The test is claimed to be very sensitive and specific for aldehydes.

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Cite as: *J. Integr. Sci. Technol.*, 2024, 12(1), 709.  
URN:NBN:sciencein.jist.2024.v12.709



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<http://pubs.thesciencein.org/jist>

The aqueous solution is used for the test mentioned above as originally published. Aldehydes (apart from those with low molecular weights) are not soluble in alkaline solution, which presents a challenge in the technique. Durst and Gokel<sup>3</sup> modified the test based on PTC technique (solid reagent + toluene solution of tri-*n*-caprylyl methyl ammonium chloride + 10% NaOH). Formation of deep rust colour in toluene layer is considered as a positive test. Several reports have appeared describing the use of (I) for qualitative and quantitative analysis of formaldehyde and other aldehydes.<sup>4</sup>

Del Nozal and coworkers (1993)<sup>5</sup> working with carbohydrates observed that oxidizing agents like Hydrogen Peroxide, Potassium periodate and Potassium persulphate can be used instead of molecular oxygen for colour development.<sup>5</sup> Considering the bio applicability of *N*-heterocycles, at present, several researchers are working on the *N*-containing bioactive heterocycles such as quinoline, indole & oxindole for analysis.<sup>6-11</sup>

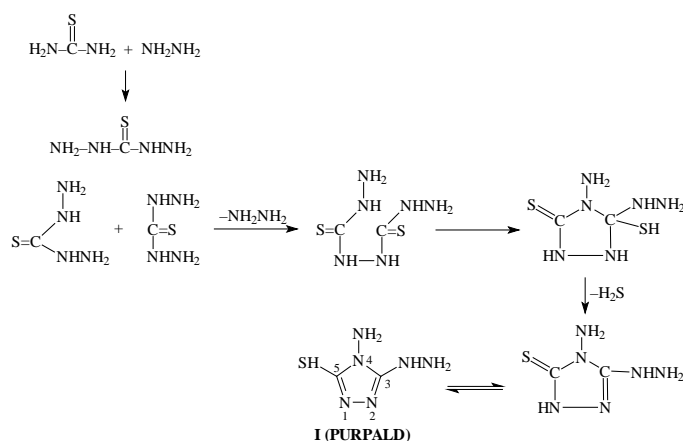
The purpose of this study is to bring this new test based on PURPALD to the notice of teachers and students of chemistry. Secondly, much of the information on PURPALD reagent is not accessible to most people. Finally, evaluation of the reagent and modifications in the test procedures is in order so that it could serve as a good alternative to the other known procedures mentioned earlier. Moreover, many applications of this reagent PURPALD are reported. It can be used for the synthesis of green light photo electro-catalysis and also for solid phase extraction.<sup>12,13</sup>

**Materials and Chemicals required:** All chemicals are commercially available and were used without further purification. The organic compounds used were Thiourea, hydrazine hydrate, sodium hydroxide, 30% peroxide, various aldehyde and ketone substrates (as mentioned below in the experimental part).

## EXPERIMENTAL

**Preparation of PURPALD (I):** The reagent can be made according to the procedure reported by Dickinson and Jacobsen from the readily available thiourea and hydrazine hydrate. We observed the yields of the product range between 20-30% depending upon the quality of hydrazine hydrate. PURPALD is commercially available. It can easily be prepared in the laboratory. The preparation itself involves very interesting chemistry.<sup>2</sup>

**Preparation:** 0.5 g thiourea was mixed with 99-100% hydrazine hydrate (5 ml) in a small flask fitted with a reflux condenser. The contents were heated over a steam bath for 2 hrs. The solution was cooled in an ice-bath, filtered from insoluble products, if necessary, and then acidified to pH 6-7 with dil. HCl. The triazole derivatives that get precipitated was collected, washed with water and ethanol. Solvents like ethers, ethyl acetate and many others can be used to remove contaminating impurities (PURPALD is sparingly soluble in these solvents). The product was recrystallized from boiling water (600-700 parts). Colourless needles m.p. 231-233 °C. The yield ranges 20-30%. The triazole is sparingly soluble in water and insoluble in a wide range of solvents. It is readily soluble in aqueous alkali solution (pH 10) and in aqueous acid (pH 1). The details of its preparation are given at the end. The formation of the triazole is explained as indicated below:



**Scheme 1.** Synthesis of PURPALD

## RESULTS AND DISCUSSION

**Reagent and test procedure:** The solid reagent is a stable white solid, soluble both in aqueous sodium hydroxide and aqueous HCl. Dickinson and Jacobsen reported the addition of solid reagent (100-200 mg) to aldehyde in 1 M sodium hydroxide. Since many aldehydes are not soluble in aq. medium, a suitable solvent is desirable. We tested a few solvents commonly available in most laboratories. **Table-1** indicates the results.

Evidently, the LR grade solvents are likely to contain aldehyde impurities. This is a noteworthy observation. However, ethanol specially prepared to make it free from aldehyde (acetaldehyde) contamination can be used as a solvent. We observed that stock solution of reagent prepared in pure ethanolic sodium hydroxide can be used as reagent solution but again such solution developed faint pink colour after 30 min. It is imperative therefore to prepare small volumes of reagent at a time and also avoid preparing dilute solutions; 2-5% in 1 M NaOH appeared to be a good concentration.

**Table 1:** PURPALD colour test on common solvent\*

S.No.	Solvent	Time required for +ve test (min.)
1.	Ethanol <sup>a</sup>	2-3 min.
2.	Ethanol (Aldehyde free)	1 hour (very faint pink)
3.	2 Methoxyethanol <sup>a</sup>	2 min.
4.	Dioxane <sup>b</sup>	2-3 min.
5.	THF <sup>b</sup>	3-4 min.
6.	DMF <sup>a</sup>	2 min.
7.	DMF <sup>b</sup>	4-5 min.
8.	CH <sub>3</sub> OH <sup>a</sup>	5 min. (at 60°-70°C)
9.	Pyridine <sup>b</sup>	-ve

\*Procedure as described by Dickinson and Jacobsen (air oxidation).

<sup>a</sup>Laboratory reagent grade. <sup>b</sup> Purified as described in Ref. [14]

The observations shown in **Table-2** indicate that colour test is positive and colour development (purple) is fast with aliphatic aldehydes.

### Modified Test for aromatic aldehydes

In general, aromatic aldehydes are slow in giving a colour,

**Table-2:** PURPALD test on selected Aliphatic aldehydes\*

S.No.	Aldehyde	Colour	Time (min)
1.	HCHO	Purple	< 1
2.	CH <sub>3</sub> CHO	Purple	< 1
3.	Heptaldehyde	Magenta	1
4.	Citral	Purple	1
5.	Cinnamaldehyde	Wine red	

\* Solid reagent is added to alkaline aldehyde followed by vigorous air oxidation.

**Table 3:** PURPALD test on selected aromatic aldehydes\*

S.No.	Aldehyde	Colour	Time
1.	Benzaldehyde	Red	4-5 min.
2.	p-Tolualdehyde	Red brown	5 min.
3.	Napthaldehyde	Red orange	5 min.
4.	3-Nitrobenzaldehyde	Red	1 min.
5.	Anisaldehyde	Red brown	2 min.
6.	4-chlorobenzaldehyde	Yellow red-brown	5 min.
7.	Piperonal (methylene dioxy benzaldehyde)	Reddish deep red	5 min.
8.	4-Dimethylamino benzaldehyde	Wine red	5 min.
9.	3-Hydroxybenzaldehyde	Orange red	7 min.

\* A mixture of aldehyde, sodium hydroxide solution, PURPALD (solid) was vigorously shaken at room temperature. Then warmed it to 70-80°.

particularly so with deactivated aldehydes. We developed a modification of the test procedure which will ensure rapid colour formation. This modification consists in adding one or two drops of 30% H<sub>2</sub>O<sub>2</sub> to a warm solution of aryl aldehydes in aqueous NaOH and the PURPALD reagent. Under these conditions all aryl aldehydes gave red colour instantly.

#### Sugars and related polyhydric alcohols

Carbohydrates are broadly classified as reducing and non-reducing.<sup>15</sup> The reducing sugars are further divided into aldoses and ketoses. Both contain  $\alpha$ -hydroxycarbonyl function which is responsible for their reducing properties.

**Table-4** shows the results on a few reducing sugars,<sup>16</sup> non-reducing sugars and related sugar polyols. Both glucose, galactose, fructose, xylose and cactose gave no colour under air oxidation conditions. A deep red orange colour develops after addition of 2-3 drops of H<sub>2</sub>O<sub>2</sub> and warming.<sup>17</sup> That fructose a keto sugar gives a positive test is understandable because under the alkaline conditions it partly rearranges to glucose (aldohexose), (Lobry de Bruyn-van Ekenstein rearrangement).<sup>18</sup> Thus, it is evident that reducing sugars requires an oxidising agent and gentle warming. These observations confirm the earlier results of Del Nozal *et al.*<sup>5</sup>

#### PURPALD test on Ketones and other non-aldehyde compounds

The results are as follows: Acetone – No colour; Cyclohexanone – No colour; 2-Butanone – No colour; Benzophenone – No colour; Benzoin – Faint yellow; Benzyl alcohol – Very light pink after 2 min. intensifies after warming. The observation is explainable

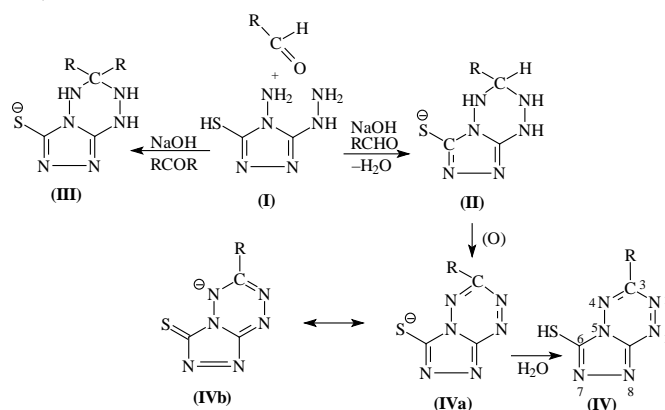
**Table 4:** PURPALD test on carbohydrates

S.No.	Carbohydrate	Observation
1.	Glucose	+ve
2.	Galactose	+ve
3.	Fructose	+ve
4.	Xylose	+ve
5.	Lactose	+ve
6.	Sucrose	-ve
7.	Mannitol	-ve
8.	Inositol (Hexa hydroxycyclohexane)	-ve
9.	Glucurono lactose	-ve
10.	Arbutin (hydroquinone monoglucoside)	-ve

based on the presence of traces of benzaldehyde or partial oxidation; Cyclohexanol – Faint yellow; Mandelic acid – No colour; Anisaldehyde oxime – No colour; Benzylidene aniline; (PhCH=NPh) – No colour.

#### Chemistry of colour test

Dickinson and Jacobsen proposed a scheme to explain the formation of purple colour in this test. The triazole reagent (I) reacts with aldehydes and ketones and forms the cyclic aminals (II) and (III) respectively. Only the aminal from aldehyde readily undergoes air oxidation to form 6-mercapto-3-substituted-5-triazolo-[4,3,b]-s-tetrazine (IV). The anion of this compound is highly conjugated bicyclic system and hence the colour (see IVa, IVb).

**Scheme 2.** Chemistry of colour test

Dickinson and Jacobsen condensed (I) with isobutyraldehyde and benzaldehyde on a preparative scale and isolated the tetrazines (IV R=CH<sub>3</sub>CH(CH<sub>3</sub>)-, R=Ph) as crystalline solids (146-147°, 192-193° respectively). The actual colours produced are dependent upon the aldehyde (R group). Aliphatic aldehydes give magenta, aromatic aldehyde purple or deep red and other mixed type purple, brown. The visible absorption maxima of (IV) for different aldehydes range from 520-550 nm<sup>1</sup>.

**Sensitivity:** The test appears to be highly sensitive. Many workers have observed aldehydes at a concentration of 1 x 10<sup>-4</sup> M can be detected. The sensitivity in the case of formaldehyde is 1

nmoles. The deactivated aldehyde such as 4-methoxybenzaldehyde may not give colour at room temperature but does so on heating in a hot water bath for about 2 min. We had no opportunity to test highly hindered aldehydes.

**Specificity:** The test is highly specific and sensitive for aldehydes under the conditions specified. Ketones, carboxylic acids (includes pure acid), esters, amides, quinones, aminophenols, hydroxyl amine, hydrazine, benzoin, benzyl alcohols, polyols, do not give a positive. Reducing sugars, ketoses and all compounds possessing  $-\text{COCH}_2\text{OH}$  or can lead to such a group ( $-\text{COCH}_2\text{Br}$ ) also give a positive test. Vinyl ethers derived from aldehydes ( $-\text{CH}=\text{C}-\text{OR}$ ) are stable in alkaline medium. So, they do not respond to the test. However, after acid hydrolysis, positive tests are common. Vinyl esters ( $-\text{CH}=\text{CH}-\text{O}-\text{COCH}_3$ ) give a positive test. Acetal do not show colouration but after generating the aldehyde (hydrolysis) positive test is shown.

#### False positive tests

Because of the high sensitivity of the test, aldehydic impurities present in solvent and test compounds give a positive test, for example ethanol, acetic acid and others. Misleading observations can be made on compounds which may undergo oxidation to aldehydic products, i.e. certain alcohols. Therefore, a blank test must be run carefully before any conclusion can be drawn. It is necessary to use pure solvents and reagents.

### CONCLUSION

Distinction between an aldehyde and a ketone is an important step in laboratory practise. The test mentioned earlier in this paper are useful when carefully applied. However, many other type of compounds interfere (quinols, aminophenols,  $\alpha$ -hydroxycarbonyl compounds) hydrazine and others. In view of the high specificity and sensitivity Brenal proposed the usefulness of this test in teaching laboratory.<sup>4</sup> Our own evaluation of the test reinforces this suggestion. We propose the following test procedure for routine work: Add the solid reagent I (20-40 mg) to an alkaline solution (1 M, about 1 ml) of the compound to be tested, shake vigorously and note a colour development. Now the solution is heated in a water bath (60-80°C) and one or two drops of 30%  $\text{H}_2\text{O}_2$  is added. Observe the formation of red to magenta colour. This will be regarded as a positive test for aldehydes.

### ACKNOWLEDGEMENTS

The authors gratefully acknowledge DBT, India for providing support to research under DBT star college scheme. The authors also thankful to Dr. J. M. Khurana for valuable discussion and to group of UG students, Shyamlal College, University of Delhi who participated in the preparation aspects of the reagents.

### CONFLICTS OF INTEREST

There are no conflicts of interest to declare.

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