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Note

The reaction of some carbohydrates and related compounds with 4-amino-3-hydrazino-5-mercapto-1,2,4-triazole

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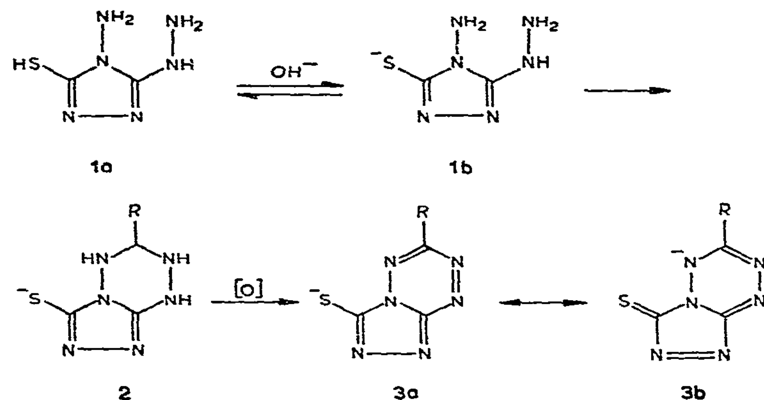
The reaction of 4-amino-3-hydrazino-5-mercapto-1,2,4-triazole (**1**) with aldehydes and ketones has been suggested as a method for specifically determining aldehydes in the presence of ketones[1]. We have examined the reaction with some carbohydrates. Although the method is not as specific for aldehydes as claimed, it turned out to be very useful for analyzing complex mixtures that are otherwise very difficult to determine, such as glyceraldehyde–1,3-dihydroxy-2-propanone–pyruvaldehyde. At the same time, it was found possible to determine the number of carbon atoms of simple aldoses, and to distinguish between aldoses and ketoses.

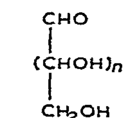


R = Aliphatic or aromatic substituents having additional functional groups.

The reaction between aliphatic or aromatic aldehydes and compound **1** produces 1,2,3,4-tetrahydro-6-mercapto-3-monosubstituted-*s*-triazolo[4,3-*b*]-*s*-tetrazine (**2**), which is unstable; upon oxidation, 6-mercapto-3-monosubstituted-*s*-triazolo[4,3-*b*]-*s*-tetrazine (**3**) is formed[1]. The latter compound is evidently stable, because constant absorbance readings are obtained after oxidation of the tetrahydrotetrazine derivative (**2**).

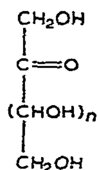
The aldoses and ketoses studied (listed in Table I) gave mercaptotetrazine derivatives that are violet-colored and absorb at 520–555 nm. All of the compounds examined followed Beer's law within the range of concentration (10^{-5}–10^{-4}M) observed.

Aldoses (*series* **a**). — In the aldose series, compounds **6** and **8** have about the same molar absorptivity (a_M) at 540 nm, as can be observed in Table I. As the chain-length increases, there is a linear decrease of the absorptivities going from compounds **8**, **11**, and **12**, up to **13** (Table I). This linear decrease occurs without significant change in the absorption maximum. In the aldohexose series, compounds **14** and **15** present essentially the same absorptivities as **13**.



Series a

n = 0, 1, 2, 3, or 4



Series b

n = 0 or 3

A decrease in the absorptivities of compounds that display similar spectra, with bands absorbing at about the same wavelengths, has been observed in certain biological molecules (such as nucleic acids) and has been ascribed to the transition from a disordered to an ordered state[3]. The phenomenon has been termed hypochromism.

In the present instance, a more-ordered state may be envisaged in the series through intramolecular hydrogen-bonding of the hydroxyl groups and N-2 of the mercapto-tetrazine derivative **3**. Compounds **6** and **8** have about the same possibility for hydrogen bonding. As the number of carbon atoms in the chain increases, the possibility for hydrogen bonding between the hydroxyl groups and the tetrazine ring also increases, and the molecules present a more-ordered state because of the decrease in number of rotational conformers.

Ketoses (*series* **b**). — The method not so specific for aldehydes as claimed[1]. Whereas compound **9** has an absorptivity about one-half that of **8** (Table I), compound **13** and some of its stereoisomers display absorptivities about one tenth those of some ketohexoses such as **16** and **17**. These ketohexoses have the same structure, and the same absorptivity would be expected for each.

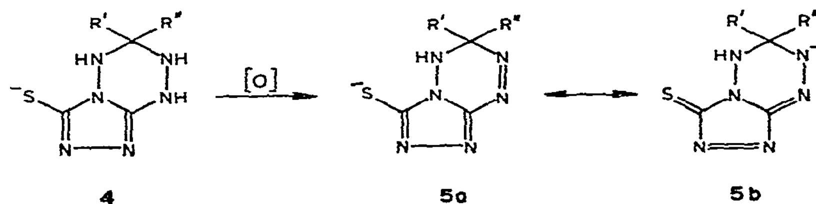


TABLE I

ABSORPTIVITIES FOR SOME CARBOHYDRATES AND RELATED COMPOUNDS[a]

Compound	$10^{-3}\ a_M$
Glycolaldehyde (6)	31.40
Glyoxal (7)	0.60
DL-Glyceraldehyde (8)	32.80
1,3-Dihydroxy-2-propanone (9)	15.25
Pyruvaldehyde (10)	0.40
D-Erythrose (11)	22.50
D-Ribose (12)	12.20
D-Glucose (13)	0.48
D-Galactose (14)	1.46
D-Mannose (15)	0.83
D-Fructose (16)	7.30
L-Sorbose (17)	3.47

[a]Molar absorptivities at 540 nm, 25.0°, in liter.mol^{-1}.cm^{-1}.

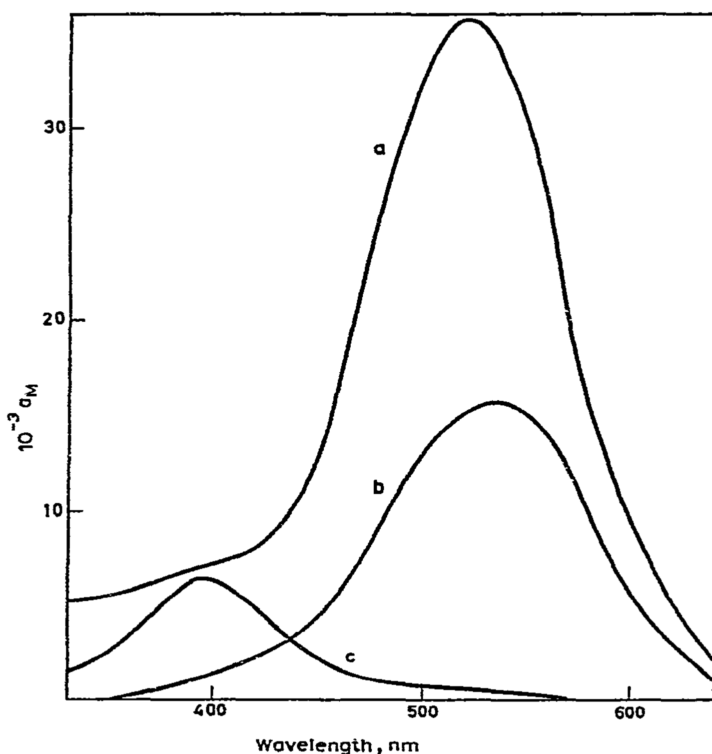


Fig. 1. Spectra of derivatives of (*a*) glyceraldehyde, (*b*) 1,3-dihydroxy-2-propanone, and (*c*) pyruvaldehyde with 1 after oxidation with hydrogen peroxide.

The reaction of compounds of series **b** with **1** should lead to a 2,4-dihydro-6-mercapto-3-disubstituted-*s*-triazole-[4,3-*b*]-*s*-tetrazine (**4**), oxidation of which would give the disubstituted tetrazine **5**. Again, in this system, the chain-length of the two substituent groups appear to decrease the absorptivity. For compound **9** it is about twice that of **16** or **17** (Table I), although too few compounds of the series have been examined to observe any systematic change.

The tetrazine derivative formed from compound **8**, for example, is stable in the presence of hydrogen peroxide, as is the product formed from **10**.

However, the derivative from **9** is unstable and the absorbance decreases with time. Constant readings are obtained when oxygen is bubbled through the sample instead of using hydrogen peroxide as oxidant. This method might be convenient for distinguishing between compounds of series **a** and **b**. At the same time, this instability might explain the differences found in the absorptivities of the ketohexoses.

Dicarbonyl compounds. — Vicinal dicarbonyl compounds present some important differences as compared with compounds of series **a** and **b**. Derivatives of **7** and **10** have a band showing a maximum at 400 nm, which might be due to the decreased conjugation associated with formation of the seven-membered heterocyclic ring. For compound **10**, the molar absorptivity of this band is 6.3×10^3. There is a large difference of a_M for **6** and **7**, and **8** and **10** at 540 nm (Table I). The structure of the derivative obtained from these dicarbonyl compounds and **1** is not known.

The difference in spectra and absorptivities of **8**, **9**, and **10** suggests that they may be determined in admixture by this reaction (Fig. 1).

EXPERIMENTAL

Materials. — Compounds **6**, **7**, **9**, **12**–**17**, (see Table I) D-erythrose 4-phosphate, and alkaline phosphatase from *Escherichia coli*, were purchased from Sigma Chemical Co. D-Glucose was analyzed by a Glucostat Kit from Worthington Biochemical Co. Compounds **1** ("Purpald"), **8**, and pyruvaldehyde dimethyl acetal were obtained from Aldrich Chemical Co. The acetal was distilled *in vacuo* (b.p. 127°–128° at 1.5 torr) and hydrolyzed in 0.87M hydrochloric acid for 3 min at 100°. The extent of the reaction was monitored by g.l.c. with a Perkin–Elmer Model 800 chromatograph, using a column of 1,4-butanediol succinate polyester at 65°.

The stock solution of **11** was prepared by hydrolyzing D-erythrose 4-phosphate, with alkaline phosphatase from *E. coli* at pH 8.0, using hydrogen carbonate as buffer. The total concentration of orthophosphate was measured by the Fiske–Subbarow method[2]. The concentration of D-erythrose 4-phosphate was calculated by assuming that the only contaminant was D-glucose 6-phosphate.

Determination. — For the determination a modification of the method described[1] by Dickinson and Jacobsen was used. A stock solution of **1** was prepared just before the analysis and kept under nitrogen. The stock solution contained 1.25 g of the reagent in 25 ml of M sodium hydroxide. The assay was performed in matched cells of 1-cm path by adding, to 2.6 ml of the reagent solution, 0.2 ml of the unknown

(for the blank, the same amount of water was used instead), and 0.2 ml of 30 vol hydrogen peroxide. The solution was kept for 2 h at 25.0° and the absorbance was read at 540 nm in a Gilford automatic spectrophotometer (model 2400). The apparatus was calibrated at 550 nm with absorbance standards.

ACKNOWLEDGMENT

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