ml. of toluene was added over a period of 2 hr. and the reaction mixture was refluxed for an additional 5 hr. The hot toluene solution was decanted to a beaker, evaporated to one-fourth volume by a stream of air, and filtered. This solid was combined with any residue in the reaction flask, recrystallized from the appropriate solvent, after clarification with activated charcoal (Norit A or Darco G), and dried in a vacuum oven at 100° for 12 to 24 hr.

Preparation of 2,4-diamino-6-sulfanilylhydrazido-s-triazines from 2,4-diamino-6-acetylsulfanilylhydrazido-s-triazines. The 2,4-diamino-6-acetylsulfanilylhydrazido-s-triazine (0.01 mol.) was dissolved in 30 ml. of ethanol, to which 5 ml. of concentrated hydrochloric acid (0.05 mol.) had been added, in a 100-ml. flask equipped with a reflux condenser. The solution was refluxed on a steam bath for 1 to 2 hr., cooled, and made basic to phenolphthalein with 2*N* sodium hydroxide. The crude product was precipitated by flooding the solution with 300 ml. of water and was collected by filtration, washed with water, and recrystallized from the appropriate solvent, after clarification with activated charcoal (Norit A or Darco G), and dried in a vacuum oven at 100° for 12 to 24 hr.

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Preparation of 2,4-Dinitrophenylhydrazine Derivatives of Highly Oxygenated Carbonyl Compounds

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The reaction of α-hydroxycarbonyl compounds with 2,4-dinitrophenylhydrazine in boiling aqueous ethanol (90%) or in 2*N* hydrochloric acid (supersaturated with the reagent) at 0° has been shown to proceed to hydrazone formation without oxidation of the hydroxyl group. Chromatographic purification of reaction products demonstrated that other experimental conditions can lead to osazone formation accompanied by reduction of the terminal hydroxymethyl group to methyl. The reaction of triose reductone with 2,4-dinitrophenylhydrazine has been shown to proceed with oxidation. The 1,2-bis(2,4-dinitrophenylhydrazone) of mesoxaldehyde has been synthesized by a definitive method and converted to the tris derivative. The absorption spectrum of mesoxaldehyde 1,2-bis(2,4-dinitrophenylhydrazone) was markedly affected by the solvent medium. An explanation for this behavior is proposed.

In connection with the ignition decomposition of cellulose nitrate,[2] we became interested in 2,4-dinitrophenylhydrazine derivatives of short carbon chain (two and three carbon atoms) sugars and nonfragmented oxidation products thereof. These derivatives, and in some cases the parent carbonyl compounds as well, were little or not known. In addition, when literature was available on these 2,4-dinitrophenylhydrazine derivatives, it was often contradictory. The reactivity of α-hydroxycarbonyl compounds toward 2,4-dinitrophenylhydrazine is a case in point since some workers[3,4] have reported the sole formation of 2,4-dinitrophenylosazones whereas other workers[5–7] have been able to prepare the 2,4-dinitrophenylhydrazones. In the work herein reported, the reaction of glycolaldehyde, acetol (CH_3—CO—CH_2OH), dihydroxyacetone, and DL-glycerose (glyceraldehyde) with 2,4-dinitrophenylhydrazine in boiling ethanol, a method of preparing 2,4-dinitrophenylhydrazine derivatives introduced by Brady and Elsmie[8] and used by Reich and Samuels[7] to prepare the 2,4-dinitrophenylhydrazones of α-hydroxycarbonyl compounds, was shown to proceed without oxidation and, in the case of dihydroxyacetone and DL-glycerose, without hydroxymethyl group reduction[9] as well. These facts were established by isolative column chromatography[10] of the reaction products. By means of the same chromatographic method, it was shown that the use of a supersaturated solution of 2,4-dinitrophenylhydrazine in 2*N* hydrochloric acid at 0°, a reagent solution used by Collatz and Neuberg[5] to prepare glycolaldehyde 2,4-dinitrophenylhydrazone, to form a derivative of DL-glycerose resulted in no oxidation or reduction.[9]

Since dihydroxyacetone and glycerose are known to be converted to methylglyoxal in the presence of

(1) Fellow of The Monsanto Chemical Co. (1956–57).

(2) M. L. Wolfrom and G. P. Arsenault, *J. Am. Chem. Soc.*, in press.

(3) W. H. Linnell and I. Roushdi, *Quart. J. Pharm. Pharmacol.*, **12**, 252 (1939).

(4) T. Banks, C. Vaughn, and L. M. Marshall, *Anal. Chem.*, **27**, 1348 (1955).

(5) H. Collatz and Irene S. Neuberg, *Biochem. Z.*, **255**, 27 (1932).

(6) D. B. Sprinson and E. Chargaff, *J. Biol. Chem.*, **164**, 417 (1946).

(7) H. Reich and Barbara K. Samuels, *J. Org. Chem.*, **21**, 68 (1956).

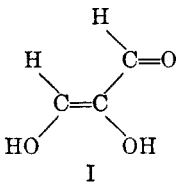
(8) O. L. Brady and Gladys V. Elsmie, *Analyst*, **51**, 77 (1926).

(9) C. L. Bernier and W. L. Evans, *J. Am. Chem. Soc.*, **60**, 1381 (1938).

(10) M. L. Wolfrom and G. P. Arsenault, *Anal. Chem.*, in press.

an acid catalyst,[9] reports of the preparation, starting from either of these trioses and using an acid catalyst, of hydroxypyruvaldehyde bis(2,4-dinitrophenylhydrazone) should be viewed with suspicion unless some evidence is presented to establish the absence of methylglyoxal bis(2,4-dinitrophenylhydrazone) in the product. No such evidence was shown by Reich and Samuels[7] and we have established the presence of methylglyoxal bis(2,4-dinitrophenylhydrazone) in the product obtained following their procedure. However, their product afforded hydroxypyruvaldehyde bis(2,4-dinitrophenylhydrazone) after chromatographic purification.

The reaction of mesoxaldehyde with an excess of 2,4-dinitrophenylhydrazine in hot 2*N* hydrochloric acid afforded a product which after chromatographic purification was shown to be the tris derivative. Triose reductone (I)



I

also afforded mesoxaldehyde tris(2,4-dinitrophenylhydrazone) when reacted at room temperature with an excess of reagent in 30% perchloric acid according to the procedure of Neuberg and coworkers.[11] In addition, several attempts were made to prepare a mono and a bis(2,4-dinitrophenylhydrazone) of triose reductone. The products thus obtained always contained a considerable amount of polymeric material and the only well defined constituents of these products were derivatives of mesoxaldehyde. The ease with which triose reductone was oxidized to mesoxaldehyde by 2,4-dinitrophenyhydrazine was not unexpected since reductones are strong reducing agents.[12] In addition, triose reductone had previously been shown to afford a mesoxaldehyde derivative when reacted with phenylhydrazine at room temperature.[13]

The periodate oxidation of D-*arabino*-hexose (D-glucose) 2,4-dinitrophenylosazone, carried out in *N*,*N*-dimethylformamide–water, afforded mesoxaldehyde 1,2-bis(2,4-dinitrophenylhydrazone). This derivative was identical with that obtained by reacting equimolar amounts of mesoxaldehyde and 2,4-dinitrophenylhydrazine in ethanol at room temperature. No method was found by which mesoxaldehyde 1,3-bis(2,4-dinitrophenylhydrazone) could be obtained by the action of 2,4-dinitrophenylhydrazine with mesoxaldehyde. The conversion of mesoxaldehyde 1,2-bis(2,4-dinitrophenylhydrazone) into the tris derivative was accomplished in dimethyl sulfoxide–water.

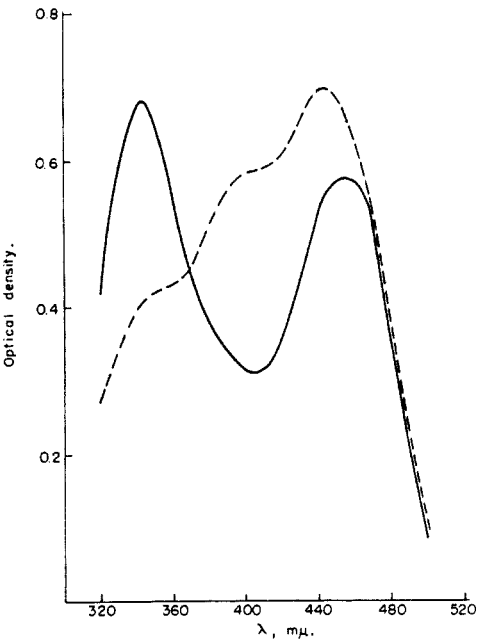
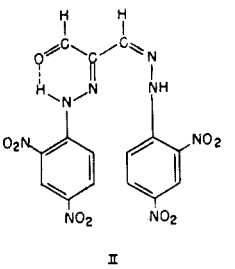
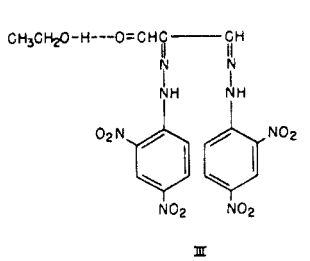


Fig. 1. Absorption spectrum of mesoxaldehyde 1,2-bis(2,4-dinitrophenylhydrazone); ———, in ethyl acetate; - - - - - - - -, in ethanol (96%); *c* 0.0007

The absorption spectra of mesoxaldehyde 1,2-bis(2,4-dinitrophenylhydrazone) in ethanol (96%) and in ethyl acetate are shown in Fig 1. The difference between these two spectra is very marked and no solvent effect of this kind has heretofore been reported in the case of derivatives of 2,4-dinitrophenylhydrazine. It is suggested that this difference may be due to hydrogen bonding, since the hydrogen bonded species II may predominate in ethyl acetate, whereas the hydrogen bonded species III may predominate in ethanol (96%).



II



III

The bis- and tris(2,4-dinitrophenylhydrazones) which we prepared could not be obtained in a chromatographically pure form by recrystallization. The extreme insolubility of these compounds makes chromatographic treatment quite difficult. However, it was possible to purify these compounds by silicic acid chromatography using a method which bears some resemblance to the method of

(11) C. Neuberg, A. Grauer, and B. V. Pisha, *Anal. Chim. Acta*, **7**, 238 (1952).

(12) H. v. Euler and H. Hasselquist, *Reduktone. Ihre chemischen Eigenschaften und biochemischen Wirkungen*, 1st Ed., F. Enke, Stuttgart, Germany, 1950, p. 1.

(13) H. v. Euler, H. Hasselquist, and L. Lööv, *Arkiv Kemi, Mineral. Geol.*, 26A, No. 17 (1948).

frontal analysis of Tiselius, Claesson and collaborators.[14]

EXPERIMENTAL[15]

Hydroxypyruvaldehyde bis(2,4-dinitrophenylhydrazone). The method of preparation described by Reich and Samuels[7] was followed. Dihydroxyacetone (0.6 g.) was added to a solution of 4 g. of 2,4-dinitrophenylhydrazine in 1 l. of 2*N* hydrochloric acid. After being stirred to insure the complete solution of dihydroxyacetone, the preparation was allowed to stand for 3 days at 25°. The precipitate was filtered, and washed with 2*N* hydrochloric acid and water; yield 2.9 g. (97%). This product was extracted with chloroform and recrystallized from nitrobenzene to constant melting point; m.p. 280–284° (dec.), intermediate between those of hydroxypyruvaldehyde bis(2,4-dinitrophenylhydrazone) (see below) and methylglyoxal bis(2,4-dinitrophenylhydrazone) [m.p. 304.5–305.5° (dec.)]. Exploratory chromatograms, developed with benzene, of the crude reaction product and of the recrystallized material on silicic acid–Celite (5:1; 8% water)[10] revealed in each case the presence therein of two constituents, the less adsorbed of which had chromatographic properties identical with those of methylglyoxal bis(2,4-dinitrophenylhydrazone). Thus, hydroxypyruvaldehyde bis(2,4-dinitrophenylhydrazone) could not be obtained directly in a pure state by the procedure of Reich and Samuels.[7]

Methylglyoxal, a substance found in aged dihydroxyacetone,[16] was not present in the sample of dihydroxyacetone used in the above preparation. This fact was shown by refluxing a mixture of 0.99 g. of the dihydroxyacetone and 1.98 g. of 2,4-dinitrophenylhydrazine in 20 ml. of absolute ethanol for 3 hr. A clear orange solution resulted, indicating the absence of methylglyoxal bis(2,4-dinitrophenylhydrazone) in the reaction product since this substance is highly insoluble in ethanol.

The crude reaction product (500 mg.) obtained following the procedure of Reich and Samuels[7] was dissolved in 250 ml. of warm nitrobenzene. The solution was diluted with 1 l. of benzene and immediately adsorbed on a column (5.4 cm., diam., × 10 cm.) of silicic acid-Celite (5:1). Prior to use this adsorbent was dried overnight at 200°[17, 18] and will be referred to as (5:1; 0% water). The chromatogram was developed with 3.5 l. of benzene. The material obtained by elution with acetone of the orange-red zone located 2–45 mm. from the top of the column was rechromatographed and recrystallized from nitrobenzene to constant melting point; yield 35 mg., red needles, m.p. 267–268° (dec.) (lit.,[7] m.p. 278°); maxima in ethyl acetate at 400 and 437 mμ. This material was chromatographically pure and contained no methylglyoxal bis(2,4-dinitrophenylhydrazone).

Anal. Calcd. for $C_{15}H_{12}N_8O_9$: C, 40.18; H, 2.70; N, 25.00. Found: C, 40.19; H, 2.78; N, 24.94.

A second crop of chromatographically pure hydroxypyruvaldehyde bis(2,4-dinitrophenylhydrazone) was obtained from the nitrobenzene mother liquors; yield 60 mg., m.p. 268–272° (dec.).

The nitrobenzene-benzene effluent (400 ml.) from the above chromatogram was adsorbed on a column (5.4 cm., diam., × 20 cm.) of silicic acid–Celite (5:1; 0% water) and developed with 2 l. of benzene. The material in the orange zone located 20–100 mm. from the column top was extracted with boiling ethanol and identified as methylglyoxal bis-(2,4-dinitrophenylhydrazone) by infrared spectrum.

Mesoxaldehyde tris(2,4-dinitrophenylhydrazone). (a) Triose reductone, prepared as described by Bauer and Teed,[19] was oxidized to mesoxaldehyde with selenium dioxide following the procedure of Holker.[20] To a solution of 196 mg. of mesoxaldehyde in 10 ml. of water was added a solution of 2 g. of 2,4-dinitrophenylhydrazine in 250 ml. of 2*N* hydrochloric acid. The reaction mixture was heated at 90° for 3 hr. The material that separated was collected, and washed with 2*N* hydrochloric acid and water; yield 1.22 g. (86%), m.p. 286–288° (dec.). An exploratory chromatogram, developed with benzene, on silicic acid–Celite (5:1; 8% water) revealed the presence of three constituents in this material. The major constituent, least adsorbed, could not be obtained in a chromatographically pure form by recrystallization of the crude material from nitrobenzene.

An amount (500 mg.) of the 2,4-dinitrophenylhydrazine derivative of mesoxaldehyde was dissolved in 500 ml. of warm nitrobenzene. The solution was diluted with 2 l. of benzene and immediately adsorbed on a column (5.4 cm., diam., × 6 cm.) of silicic acid-Celite (5:1; 0% water). The chromatogram was developed with 870 ml. of nitrobenzene-benzene (1:4 by vol.) and 100 ml. of benzene. The red material obtained, on solvent removal under reduced pressure, from the column effluent was extracted with two 40-ml. portions of boiling chloroform and recrystallized from nitrobenzene to constant melting point; yield 93 mg., m.p. 306–308° (dec.); maxima in ethyl acetate at 402 and 465 mμ. This product, microscopic red needles, was chromatographically pure and no carbonyl absorption band was found in its infrared spectrum.

Anal. Calcd. for $C_{21}H_{14}N_{12}O_{12}$: C, 40.26; H, 2.25; N, 26.84. Found: C, 40.41; H, 2.39; N, 26.82.

A second crop of chromatographically pure mesoxaldehyde tris(2,4-dinitrophenylhydrazone) was obtained from the nitrobenzene mother liquors; yield 35 mg.

(b) An amount of 50 mg. of triose reductone,[19] dissolved in 5 ml. of water, was added to 1.2 g. of 2,4-dinitrophenylhydrazine in 50 ml. of 30% perchloric acid.[11] The mixture was allowed to stand at room temperature, in the dark, under nitrogen, for 48 hr. The precipitate was filtered and washed with water; yield 360 mg. (106%). The brick red product (200 mg.) was dissolved in 200 ml. of warm nitrobenzene. The solution was diluted with 800 ml. of benzene and immediately adsorbed on a column (3.5 cm., diam., × 6 cm.) of silicic acid-Celite (5:1; 0% water). The chromatogram was developed with 75 ml. of benzene. A red solid slowly precipitated out of the column effluent which stood at 4° for 5 days. The precipitate was filtered, and washed with nitrobenzene-benzene (1:4) and ether; yield 61 mg., red micro needles, m.p. 300–302° (dec.) undepressed on admixture with authentic mesoxaldehyde tris(2,4-dinitrophenylhydrazone). The identity of this precipitate with a specimen of mesoxaldehyde tris(2,4-dinitrophenylhydrazone) was further demonstrated by comparison of their chromatographic properties and infrared spectra.

Several attempts were made to prepare a mono and a bis(2,4-dinitrophenylhydrazone) of triose reductone. In all cases, even when the preparations were carried out in the absence of an acid catalyst, the products obtained contained a considerable amount of polymeric material and the only well defined constituents of these products were derivatives of mesoxaldehyde.

(14) E. Lederer and M. Lederer, *Chromatography. A Review of Principles and Applications*, 1st Ed., Elsevier Publishing Co., New York, N. Y., 1953, p. 3.

(15) All melting points were taken on a Kofler micro hot stage and are corrected. The ultraviolet absorption spectra, in the range 320–600 mμ, were taken in a Beckman spectrophotometer, Model DU. The infrared spectra were obtained with a Perkin-Elmer spectrophotometer Model 21 using the potassium bromide pellet technique.

(16) P. A. Levene and A. Walti, *J. Biol. Chem.*, **78**, 23 (1928).

(17) K. N. Trueblood and E. W. Malmberg, *Anal. Chem.*, **21**, 1055 (1949).

(18) K. N. Trueblood and E. W. Malmberg, *J Am. Chem. Soc.*, **72**, 4112 (1950).

(19) H. F. Bauer and Carol Teed, *Can. J. Chem.*, **33**, 1824 (1955).

(20) J. R. Holker, *J. Chem. Soc.*, 579 (1955).

D-*Arabino-Hexose* (D-*glucose*) *2,4-dinitrophenylosazone.* The procedure of Neuberg and Strauss[21] was followed. α-D-Glucose monohydrate (5 g.), dissolved in 50 ml. of water, was added to a hot solution of 14.9 g. of 2,4-dinitrophenylhydrazine in 900 ml. of 2*N* hydrochloric acid to which 9 ml. of ethanol (96%) had been added. The mixture was heated on a steam bath for 20 hr. The hot suspension was filtered, and the residue was washed with 2*N* hydrochloric acid and water; yield 13.0 g. (96%). The product was reddish brown and partially crystalline. It melted below 245° over a very wide range in temperature and contained much tar. Thus, the claim made by Neuberg and Strauss[21] for the preparation of crystalline D-glucose 2,4-dinitrophenylosazone is misleading. However, the product obtained by their method of preparation was readily purified since 2,4-dinitrophenylhydrazine tars were found to be very soluble in nitrobenzene. Thus, the crude product (8 g.) was extracted repeatedly with portions of nitrobenzene. On repeated extraction, the color of the nitrobenzene extracts changed from black to orange. The extraction residue was recrystallized once from nitrobenzene; yield 2.3 g., reddish orange microscopic needles, m.p. 263–267° (dec.) [lit.,[22] m.p. 256–257° (dec.)] unchanged by further recrystallization from nitrobenzene.

Anal. Calcd. for $C_{18}H_{18}N_8O_{12}$: C, 40.15; H, 3.37; N, 20.81. Found: C, 40.22; H, 3.51; N, 20.56.

Mesoxaldehyde 1,2-bis(2,4-dinitrophenylhydrazone). (a) An amount of 5 g. of D-*arabino*-hexose (D-glucose) 2,4-dinitrophenylosazone was dissolved in 500 ml. of *N,N*-dimethylformamide. While the solution was stirred and cooled in ice, 10.65 g. of paraperiodic acid (H_5IO_6), dissolved in 125 ml. of water, was added slowly. Stirring was continued for 30 min. The precipitate was filtered, and washed with a little *N,N*-dimethylformamide, water and acetone; yield 1.90 g. (46%), m.p. 250–253° (dec.). An exploratory chromatogram, developed with benzene, on silicic acid–Celite (5:1; 8% water)[10] revealed the presence of two constituents in this material. The major constituent, less adsorbed, could not be obtained in a chromatographically pure form by recrystallization from nitrobenzene (150°) since it was somewhat sensitive to heat.

The crude preparation (1 g.) was dissolved in 500 ml. of warm nitrobenzene. The solution was diluted with 2 l. of benzene and immediately adsorbed on a column (5.4 cm., diam., 5.5 × cm.) of silicic acid–Celite (5:1; 0% water). The chromatogram was developed with 160 ml. of nitrobenzene-benzene (1:4) and 200 ml. of benzene. A reddish orange crystalline (fine needles) precipitate formed in the column effluent when it stood at 4° for 24 hr.; yield 567 mg., m.p. 262–269° (dec.), infrared band at 6.0μ. This mesoxaldehyde 1,2-bis(2,4-dinitrophenylhydrazone) was chromatographically pure and its absorption spectrum is shown in Fig. 1.

Anal. Calcd. for $C_{15}H_{10}N_8O_9$: C, 40.36; H, 2.26; N, 25.11. Found: C, 40.51; H, 2.28; N, 25.11.

(b) A solution of 100 mg. of mesoxaldehyde[20] in 2 ml. of water was added to a suspension of 230 mg. of 2,4-dinitrophenylhydrazine in 48 ml. of absolute ethanol. The suspension was shaken at room temperature for 28 hr., filtered, and washed with ethanol (96%) and acetone; yield 66 mg. (25%). The product (60 mg.) was purified using the chromatographic method described in section (a) above; yield 35 mg., fine reddish orange needles, m.p. 263–269° (dec.) undepressed on admixture with authentic mesoxaldehyde 1,2-bis(2,4-dinitrophenylhydrazone). The chromatographic properties, the infrared absorption spectra, and the ultraviolet and visible absorption spectra of this material and of authentic mesoxaldehyde 1,2-bis(2,4-dinitrophenylhydrazone) were identical.

Conversion of mesoxaldehyde 1,2-bis(2,4-dinitrophenylhydrazone) into mesoxaldehyde tris(2,4-dinitrophenylhydrazone). To an amount of 100 mg. of mesoxaldehyde 1,2-bis(2,4-dinitrophenylhydrazone), dissolved in 100 ml. of warm dimethyl sulfoxide, was added 53 mg. of 2,4-dinitrophenylhydrazine and one drop of concentrated hydrochloric acid. The mixture was allowed to stand at room temperature for 3 days, during which time a total of 20 ml. of water was added to it in small aliquots at regular intervals. The preparation was centrifuged, and the residue was washed with dimethyl sulfoxide–water (4:1), water and acetone; yield 139 mg. (99%), red microscopic needles, m.p. 295–297° (dec.). After one recrystallization from nitrobenzene, a product of m.p. (and mixed melting point with authentic material) 306–308° (dec.) was obtained. The identity of this product with mesoxaldehyde tris(2,4-dinitrophenylhydrazone) was further shown by comparative chromatograms and infrared spectra.

Attempted preparation of mesoxaldehyde 1,3-bis(2,4-dinitrophenylhydrazone). Some eight separate and unsuccessful attempts were made to prepare mesoxaldehyde 1,3-bis(2,4-dinitrophenylhydrazone) by reacting mesoxaldehyde[20] with 2,4-dinitrophenylhydrazine in different solvent media. In five cases, precipitates were obtained and these precipitates were shown to contain no mesoxaldehyde derivative other than mesoxaldehyde 1,2-bis(2,4-dinitrophenylhydrazone) or mesoxaldehyde tris (2,4-dinitrophenylhydrazone) or both.

Reaction of 2,4-dinitrophenylhydrazine with α-hydroxycarbonyl compounds. (a) To an amount of 20 mmol. (1.8 g.) of DL-glycerose dimer dissolved in 10 ml. of hot water was added a suspension of 60 mmol. (11.9 g.) of 2,4-dinitrophenylhydrazine in 100 ml. of absolute ethanol. After refluxing for 16 hr., the suspension was filtered while still hot. The filtration residue, the evaporation residue obtained on solvent removal under reduced pressure from the filtrate, and a synthetic mixture of derivatives of 2,4-dinitrophenylhydrazine were chromatographed simultaneously, on separate columns, on silicic acid–Celite (5:1; 8% water) using successively benzene, ether-benzene (1:19) and ether-benzene (1:4) as developer, according to the method of Wolfrom and Arsenault.[10] The synthetic mixture contained DL-glycerose 2,4-dinitrophenylhydrazone, hydroxypyruvaldehyde bis(2,4-dinitrophenylhydrazone), 2,4-dinitrophenylhydrazine, methylglyoxal bis(2,4-dinitrophenylhydrazone) and 2,4-dinitroaniline. By means of this method of chromatographic comparison, it was shown that the filtration residue contained only 2,4-dinitrophenylhydrazine, and that the evaporation residue contained 2,4-dinitrophenylhydrazine and DL-glycerose 2,4-dinitrophenylhydrazone.

A method identical to that just described was used to show that the reaction of glycolaldehyde, acetal, and dihydroxyacetone with 2,4-dinitrophenylhydrazine, in refluxing ethanol, takes place without any oxidation of the hydroxyl group and, in the case of dihydroxyacetone, without rearrangement[9] as well. In these three cases, a carbonyl compound-reagent molar ratio of 1.0:0.9 was used and the preparations were refluxed for 3 hr.

(b) A solution of 4.0 g. (44 mmol.) of DL-glycerose dimer in 25 ml. of water at 0° was added to a supersaturated solution of 8.0 g. (40 mmol.) of 2,4-dinitrophenylhydrazine in 480 ml. of 2*N* hydrochloric acid at 0°. After the preparation stood at 0° for 6 hr., the precipitate was filtered, and washed with 2*N* hydrochloric acid and water; yield 10.0 g. (92%). The crude preparation was shown by the method of comparative chromatography described in section (a) above to contain only DL-glycerose 2,4-dinitrophenylhydrazone.

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(22) E. Glaser and N. Zuckermann, *Z. physiol. Chem.*, 167, 37 (1927).