

CHANGE OF 2,4-DINITROPHENYLHYDRAZONES ON ALUMINA AND SILICA GEL CHROMATOGRAPHIC COLUMNS*

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In an investigation of the "cardboard" flavour in skim milk (Forss, Pont, and Stark 1955a, 1955b), the compounds responsible for this defect were found to be the C_{5-11} 2-enals and the C_{6-11} 2,4-dienals. These unsaturated aldehydes were separated by passing their 2,4-dinitrophenylhydrazones through chromatographic columns. While these derivatives passed unchanged through alumina and silica gel columns when developed with ether and light petroleum, some of the 2,4-dinitrophenylhydrazones of the aliphatic saturated aldehydes and ketones which were also present were observed to change. Acetaldehyde 2,4-dinitrophenylhydrazone was the main product of these changes but formaldehyde and other 2,4-dinitrophenylhydrazones were also obtained. The proportion changed was greater when relatively small amounts of the 2,4-dinitrophenylhydrazone were applied to the column, and the effect was much more marked on silica gel than on alumina.

Since alumina and silica gel columns have been widely used for the fractionation of 2,4-dinitrophenylhydrazones (Gordon *et al.* 1951) and since further work on mixtures of aliphatic carbonyl compounds was contemplated in this laboratory, a study was made of the factors responsible for these changes.

Use of ethanol in place of ether, use of other brands of silica gel, washing the gel with hydrochloric acid to remove iron, and extensive washing with water to remove hydrochloric acid, all failed to suppress the changes.

It is possible that 2,4-dinitrophenylhydrazones and carbonyl compounds present in the solvents enter into an exchange reaction, but then the use of carbonyl-free solvents should suppress the changes, but this was not so.

There exists the possibility that carbonyl compounds are produced during the passage of the solvent through the column and, while the reason for this

* Manuscript received June 13, 1957.

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has so far eluded us, the conclusion nevertheless is that silica gel adsorption columns should not be used for the fractionation of mixtures of the 2,4-dinitrophenylhydrazones of saturated aliphatic aldehydes and ketones. There is much less risk in using alumina columns. Good separation without change can be obtained on cellulose partition columns with methanol and light petroleum (b.p. 100–120 °C) as solvents.

Experimental

Glass columns 30 by 1 cm were used for amounts of 2,4-dinitrophenylhydrazones up to 2 mg and columns 50 by 2 cm for amounts from 2 to 50 mg.

(a) *Identification of Compounds.*—All compounds obtained from chromatographic columns were characterized by measurement of their light absorption maxima in ethanol and their R_F values on paper with methanol and light petroleum (Meigh 1952). Because formaldehyde and acetaldehyde 2,4-dinitrophenylhydrazones occurred most frequently further support for their identity was sought. These derivatives sublime, which makes their melting point determination more difficult. When samples of authentic and of isolated formaldehyde 2,4-dinitrophenylhydrazone were placed between two microscope coverslips and placed on a Fisher type micro-melting point apparatus at 150 °C, both samples sublimed rapidly and the freshly formed crystals melted at 162–163 °C and 162–164 °C respectively. The melting behaviour of acetaldehyde 2,4-dinitrophenylhydrazone is further complicated by the fact that it exists in a number of forms with different melting points. It melted at 147 °C, which indicated that it was a mixture of the "metastable" form which melts at 157 °C and the "stable" form which melts at 168 °C (Huntress and Mulliken 1946).

Infra-red spectra of "Nujol" mulls of the formaldehyde and acetaldehyde 2,4-dinitrophenylhydrazones were determined by Dr. J. B. Willis, Division of Industrial Chemistry, C.S.I.R.O. The spectra of the isolated and authentic samples of the formaldehyde derivative were identical. The infra-red spectrum of the isolated acetaldehyde 2,4-dinitrophenylhydrazone differed from the authentic sample submitted but reference to the work of Ross (1953) showed that the isolated compound contained about 95% of the lower melting crystalline form and about 5% of the higher melting crystalline form of acetaldehyde 2,4-dinitrophenylhydrazone, while the authentic sample submitted was the higher melting form.

(b) *Alumina Columns.*—Acetone 2,4-dinitrophenylhydrazone was the only compound observed to change on an alumina column. When it was developed with 2% ether in light petroleum, about 75% of the acetaldehyde 2,4-dinitrophenylhydrazone and about 25% of a 2,4-dinitrophenylhydrazone with R_F value and light absorption maximum of butanone-2, were obtained. The 2,4-dinitrophenylhydrazones of *n*-pentanal, *n*-hexanal, and octanone-2 passed unchanged through an alumina column.

(c) *Silica Gel Columns.*—Change on a silica gel column was first noticed when *n*-hexanal 2,4-dinitrophenylhydrazone, isolated from an alumina column, was rechromatographed on a silica gel column with ether and light petroleum. There was complete conversion to the acetaldehyde derivative. When the synthetically prepared *n*-hexanal derivative was developed on a similar column, the same effect was noted. With ethanol in place of ether similar results were obtained.

Acetone 2,4-dinitrophenylhydrazone, when developed on silica gel with ether and light petroleum, was mainly converted to the acetaldehyde derivative, but the formaldehyde 2,4-dinitrophenylhydrazone and a 2,4-dinitrophenylhydrazone with R_F value and light absorption maximum of *n*-hexanal were also obtained. When ethanol and light petroleum were used as developers, the effect was similar. The addition of one part of celite (Johns Manville "Celite 545") to two parts of silica gel did not prevent the change nor did the use of columns packed with Mallinckrodt A.R. "Silicic Acid" or Davison "Selective Adsorption Silica Gel 950".

Propionaldehyde 2,4-dinitrophenylhydrazone on silica gel with ether and light petroleum as solvent partly changed to the acetaldehyde derivative.

The changes on silica gel columns became appreciable only after 24 hr. When a mixture of the 2,4-dinitrophenylhydrazones of acetone, butanone-2, hexanone-2, and undecanone-2 was developed on a silica gel column with ether and light petroleum for 2 days, the bulk of the 2,4-dinitrophenylhydrazones of hexanone-2 and undecanone-2 passed through unchanged, but the slower moving hydrazones of acetone and butanone-2 could not be recovered.

Up to this stage, the solvents, which were of analytical reagent quality, had not been treated to remove traces of carbonyl compounds. Treatment to remove carbonyl compounds did not affect the exchange. Preliminary passage of carbonyl-free solvent through a column containing 2,4-dinitrophenylhydrazine, before adding acetone 2,4-dinitrophenylhydrazone and passing it through a second column, brought about no improvement. Avoiding exposure of the column to light had no effect. When the silica gel, from a column in which acetone 2,4-dinitrophenylhydrazone had been developed with partial breakdown, was thoroughly washed with ether and light petroleum, dried for 12 hr at 110 °C, and repacked into a column, acetone 2,4-dinitrophenylhydrazone again broke down.

The possibility that traces of HCl in the silica gel might be catalysing the change was eliminated when it was found that after prolonged washing of the silica gel with water, the characteristic rearrangement still occurred. The silica gel was prepared by the method of Fairbairn and Harpur (1951), and contained iron. When the silica gel was repeatedly washed with 1 \times N HCl to remove the iron, alteration of acetone and *n*-hexanal 2,4-dinitrophenylhydrazones still took place.

The authors are indebted to Dr. R. B. Seligman, Philip Morris Inc., Richmond, Va., U.S.A., for the sample of "Mallinckrodt Analytic Reagent Silicic Acid"; to Mr. A. E. Burhenn, The Davison Chemical Corporation, Baltimore, Md., U.S.A., for several samples of silica gel; to Mr. W. Stark for technical assistance; and to Dr. H. H. Hatt and Mr. G. Loftus Hills for helpful criticism.

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