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Chromatographic Separation of Aliphatic 2,4-Dinitrophenylhydrazones

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Chromatographic adsorption on bentonite from ether and/or hexane has been used to separate many aliphatic dinitrophenylhydrazone mixtures. Twenty-two pairs of derivatives representing twelve aliphatic aldehydes and ketones were examined. Eighteen were separated satisfactorily; four pairs were incompletely separated.

IN 1935 Strain (8) proposed chromatographic adsorption for the separation of 2,4-dinitrophenylhydrazones. He described the separation of the β -ionone and camphor derivatives and of the feronic acid and levulinic acid derivatives. He used talc as the adsorbent, and suggested alumina, aluminum phosphate, magnesium phosphate, and fuller's earth as other adsorbents. Lucas, Prater, and Morris (6) separated acetaldehyde dinitro-

phenylhydrazone from propionaldehyde dinitrophenylhydrazone on alumina; later Buchman, Schlatter, and Reims (3) used chromatographic separation on alumina to separate two cyclobutylcyclobutanone derivatives. Roberts and Green (7) have reported the chromatographic separation on silicic acid-Super Cel of any mixtures of the 2,4-dinitrophenylhydrazones of acetaldehyde, acetone, propionaldehyde, and methyl ethyl ketone except

acetone-propionaldehyde. Johnston (5) has recently described the separation of the dinitrophenylhydrazones of certain androgens on an alumina column.

During a study of the volatile components of apple juice, it became necessary to separate and identify small quantities of aliphatic carbonyl compounds. It was found that 325-mesh Volclay bentonite strongly adsorbed and gave excellent separation of many dinitrophenylhydrazones. Results of adsorption of 22 pairs of derivatives involving 12 aldehydes and ketones are reported here. (Three other bentonites were found unsatisfactory. Magnesium oxide and silicic acid did not appear to be as useful as the adsorbent selected. The first decomposed the derivatives and the last showed considerably less strength of adsorption with more diffuse bands than did the Volclay bentonite.)

EXPERIMENTAL

Materials. The adsorbent mixture consisted of three parts by weight of the bentonite and one part of a diatomaceous filter aid, well mixed. No special activation was necessary with the batch used unless the mixture had been exposed to the atmosphere for several weeks. Heating for 2 hours in a vacuum at 70° C. was sufficient to restore the adsorbent. Columns were packed dry, with vacuum applied to the lower end of the column. Solvents were not specially purified. (The ether was a chemically pure diethyl ether. The label carried the statement "Ether conforming to this specification normally contains about 2% of alcohol and about 0.5% of water." It was not further purified before use. The hexane was a petroleum ether boiling at 63° to 70° C.)

Procedure. Samples of 10 to 20 mg. of each derivative were mixed, dissolved in 5 to 20 ml. of the appropriate solvent (Table I), and adsorbed on a 13 × 200 mm. column. It was developed by washing with 30 to 200 ml. of the appropriate solvent, which was chosen to give maximum spread of the adsorbed zones. Improper choice of developing solvent resulted in either failure of the bands to move down the column or very weak adsorption without separation. Air pressure (30 cm. of mercury) was used to increase filtration rates. Development was continued until complete separation of the zones was noted, or if this was not attained, until more than one zone showed. In some cases two adsorptions were necessary.

When convenient, the lower zone was washed through the column. Otherwise both zones were removed and eluted with methanol, ethanol, or ether-ethanol (in order of decreasing strength of adsorption of the 2,4-dinitrophenylhydrazones). The solutions were filtered through Selas crucibles to remove traces of adsorbent, evaporated dry on a steam bath, and weighed. Melting points (uncorrected) were determined on the evaporated residues rather than after recrystallization, in order to avoid any fractionating effect of crystallization. When derivatives melting at the same point were separated, mixed melting points with known compounds were used to identify them.

Example. Eighteen milligrams of formaldehyde 2,4-dinitrophenylhydrazone (melting point 165° C.) and 20 mg. of acetone 2,4-dinitrophenylhydrazone (melting point 124° C.) were adsorbed from ether and developed with 5% acetone in ether until the following zones appeared:

40 mm.	Colorless
55 mm.	Orange
15 mm.	Colorless
18 mm.	Bright yellow

The zones were removed, eluted with methanol-ether, and evaporated to dryness. The upper zone yielded 19 mg. of acetone 2,4-dinitrophenylhydrazone, melting point 124° C., and the lower zone yielded 17 mg. of formaldehyde 2,4-dinitrophenylhydrazone, melting point 166° C.

Application. As an application of the separation procedure, a crude preparation of dinitrophenylhydrazones of volatile carbonyl compounds from the chromic acid oxidation of technical 2-ethylbutanol was adsorbed from hexane and developed with 75% hexane in ether. Six bands were observed. The two predominating zones were removed. The upper band yielded diethyl ketone 2,4-dinitrophenylhydrazone, melting point 153.5–154.5° C.; the lower band, after readsorption to remove traces of unidentified compounds, yielded 2-ethylbutyraldehyde 2,4-dinitrophenylhydrazone, melting point 136–136.5° C. [94.5–95° (2); 129–130° (4)].

Table I. Chromatographic Separation of the 2,4-Dinitrophenylhydrazones of Twelve Aldehydes and Ketones

Upper Zone	Lower Zone	Developing Solvent
A. Completely Separated		
2,4-Dinitrophenylhydrazone	Acetone	1% acetone in ether
Acetone	Formaldehyde	5% acetone in ether
Acetone ^a	Acetaldehyde	Ether
Acetone	Propionaldehyde	Ether
Acetone	Methyl ethyl ketone	Ether
Acetaldehyde	Methyl ethyl ketone	Ether
Acetaldehyde	Propionaldehyde	Ether
Methyl ethyl ketone	Methyl <i>n</i> -propyl ketone	25% hexane in ether
Methyl ethyl ketone ^a	<i>n</i> -Butyraldehyde	25% hexane in ether
Propionaldehyde ^a	<i>n</i> -Butyraldehyde	50% hexane in ether
Methyl <i>n</i> -propyl ketone	<i>n</i> -Butyraldehyde	67% hexane in ether
Methyl <i>n</i> -propyl ketone	<i>n</i> -Valeraldehyde	50% hexane in ether
<i>n</i> -Butyraldehyde	<i>n</i> -Valeraldehyde	50% hexane in ether
<i>n</i> -Valeraldehyde ^a	Isobutyraldehyde	50% hexane in ether
Methyl <i>n</i> -butyl ketone	Isovaleraldehyde	50% hexane in ether
<i>n</i> -Butyraldehyde ^a	<i>n</i> -Valeraldehyde	67% hexane in ether
Methyl <i>n</i> -propyl ketone ^a	<i>n</i> -Valeraldehyde	67% hexane in ether
<i>n</i> -Valeraldehyde	Methyl <i>n</i> -butyl ketone	67% hexane in ether
	Isobutyraldehyde	67% hexane in ether
B. Incompletely Separated ^b		
Formaldehyde	Acetaldehyde	
Propionaldehyde	Methyl ethyl ketone	
Methyl <i>n</i> -butyl ketone	<i>n</i> -Butyraldehyde	
<i>n</i> -Valeraldehyde	<i>n</i> -Hexaldehyde	

^a Two adsorptions necessary.

^b In all cases, sections of zone showed different melting points.

Diethyl ketone dinitrophenylhydrazone C₁₁H₁₄O₄N₄ requires 21.05% N; found 21.01% N.

2-Ethylbutyraldehyde dinitrophenylhydrazone C₁₂H₁₆O₄N₄ requires 19.99% N; found 20.18% N.

RESULTS AND DISCUSSION

Table I shows the 2,4-dinitrophenylhydrazone pairs studied and the developing solvent. The first named of each pair was the more strongly adsorbed. In some cases two adsorptions were necessary for satisfactory separation. In all pairs listed as incompletely separated only one zone was observed. Material from the upper and lower parts of the zone showed different melting points, indicating that a mixture was present, inasmuch as a single compound shows the same melting point throughout the zone. These pairs were not completely separated by any mixture of ether and hexane, the mixtures being chosen to cover the entire range from strong adsorption to virtual elution.

The pairs were chosen to include all those that might be expected to be difficult to separate. By examination of Table I, the separability of other pairs or of mixtures of more than two dinitrophenylhydrazones can be ascertained. For example, as the dinitrophenylhydrazones of acetone and of methyl ethyl ketone are separated and those of methyl ethyl ketone and methyl *n*-propyl ketone are separated (in each case the first-named being more strongly adsorbed), it is obvious that acetone dinitrophenylhydrazones can be separated from methyl *n*-propyl ketone dinitrophenylhydrazones. Similarly, a mixture of the first four methyl ketone dinitrophenylhydrazones can be separated by proper development. In making such a separation, the mixture should be adsorbed from hexane and washed successively with 67% hexane-ether, 25% hexane-ether, and ether. These separations have been made.

When an unknown mixture is being examined, it is often of value to adsorb from ether and develop with ether, thus dividing the mixture into two fractions, those that remain adsorbed and can be separated by ether development, and those that are eluted with ether and must be readsorbed from hexane and developed with hexane-ether solution.

Use of the correct solvent mixture for development results in clear, definite zones with sharp lower boundaries, which permit observation of faint zones near the more prominent bands. Occasionally a single derivative will give rise to two adjacent bands that cannot be separated by continued washing. Elution and evaporation yield apparently identical compounds. In all such

cases, the upper of the two bands is an orange color and the lower may be buff or yellow. To avoid confusion, a mixture or unknown should be developed until the bands actually separate.

Variability of Adsorbent. The work reported herein was done with a single lot of bentonite which had been in open storage in the laboratory for approximately 4 years. No activation or drying was necessary for good results. A sample from current production was obtained which also gave satisfactory results; however, it was necessary to heat the material at 110° in a vacuum oven for 6 hours before mixing with the filter aid. A new Volclay product, BC dust Volclay bentonite, also gave excellent separation but had a slower filtration rate than the 325-mesh Volclay bentonite. The former should show greater uniformity from lot to lot than the other product. The addition of more filter aid (to a 1 to 1 ratio) increased the flow rate satisfactorily. Some indication was noted that the BC dust might require drying of the solvents for best results.

Mixed Crystals. Brandstätter (1), in a study of mixed crystal formation with the 2,4-dinitrophenylhydrazones has stated that certain errors may be made in the estimation of their purity or identity. In some cases the presence of a homologous impurity may raise the melting point of a dinitrophenylhydrazone. She also observed that certain mixed crystal systems may be mistaken for pure compounds. She found eight dinitrophenyl-

hydrazones which showed thirteen complete series of mixed crystals. Of these thirteen pairs, six were studied by the author's procedure in this laboratory, and all were chromatographically separable.

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