

Separation of 2,4-Dinitrophenylhydrazone Derivatives of Aliphatic Monocarbonyls into Classes on Magnesia

► A method is described for separating the 2,4-dinitrophenylhydrazone derivatives of aliphatic monocarbonyls into classes on a magnesia-Celite column. The classes elute in the sequence: methyl ketones, saturated aldehydes, 2-enals, and 2,4-dienals. The short chain members of each class behave anomalously, moving close to, or into, the class immediately following. Characteristic colors for each class are displayed on the adsorbent and aid in their identification. Methyl ketones are gray; saturated aldehydes are tan; 2-enals are rust-red; and 2,4-dienals are lavender.

SEPARATION of aliphatic monocarbonyls into classes (methyl ketones, saturated aldehydes, 2-enals, and 2,4-dienals) is a valuable aid in the characterization of carbonyls obtained as a mixture at the micromole level. Class separation of carbonyls as their 2,4-dinitrophenylhydrazones has been accomplished by Van Duin (8) on zinc carbonate and by Gaddis and Ellis (3) on filter paper strips. Applications of both methods have been described (1, 3, 9).

This report describes a method for performing the class separation on magnesia. The technique as used in our laboratory has proved extremely useful in studies on butterfat oxidation and has also aided in the detection and characterization of several classes of carbonyls other than those mentioned above. A preliminary report of the procedure has been presented (7), but since that time

modifications have been incorporated which have improved the method.

MATERIALS

Magnesia 2665 (iodine number 19 to 23), Fisher Scientific Co., Silver Spring, Md. Protect the contents of a once-opened container from undue exposure to moisture.

Celite 545, Johns Manville Co., dried 24 hours at 150° C. Chloroform, ACS grade. *n*-Hexane, Phillips high purity grade, redistilled. Chromatographic column, 3 cm. i.d. by 30 cm. long, with coarse fritted glass disk.

EXPERIMENTAL

Preparation of Column. Ten grams of magnesia and 10 grams of Celite are slurried together with about 90 ml. of a 15% solution of CHCl_3 in hexane and poured through a long-stemmed funnel into a chromatographic tube clamped at the outlet. Air bubbles are removed from the column by running a Burgess Vibra Graver up the tube until bubbles cease to break through the surface of the column. The column is then packed under light air or nitrogen pressure (1 to 2 p.s.i.) until the volume of solvent above the solid is reduced to a height of about 2 mm.

Application of Sample. The sample of 2,4-dinitrophenylhydrazones in a 5-ml. beaker is dissolved in 0.3 ml. of CHCl_3 , and 1.7 ml. of hexane is added. The solution is carefully applied to the column using a minimum volume of 15% CHCl_3 in hexane to effect a quantitative transfer. A satisfactory way to apply the solution without disturbing the top of the

column consists of permitting the solution to run evenly down the sides of the tube from a pipet rotated rapidly around the upper inner wall of the tube. After absorption of the hydrazones, the sides of the tube are carefully washed down with the solvent in the same manner. A small wad of glass wool is then placed just above the top of the bed, and development of the chromatogram is begun.

Development and Monitoring of Chromatogram. Separation of the four classes of 2,4-dinitrophenylhydrazones is effected by using the following sequence of solvents: 150 ml. of 15% CHCl_3 in hexane; 150 ml. of 30% CHCl_3 in hexane; 100 ml. of 60% CHCl_3 in hexane, and finally, CHCl_3 .

Separation of the classes can be followed visually because of the rather remarkable phenomenon of a more or less marked contrast in color exhibited by the different classes. Thus, methyl ketones are grey, saturated aldehydes are tan, 2-enals are rust-red, and 2,4-dienals are lavender. These colors are extremely useful as an aid to the characterization of an unknown.

In this laboratory, separation is monitored by a continuous flow analyzer (Canal Industrial Corp., Bethesda, Md.) using a 340- μ filter. The effluent is collected in 5-ml. fractions using an automatic fraction collector. The tubes comprising a given peak are pooled for further analyses. Separation

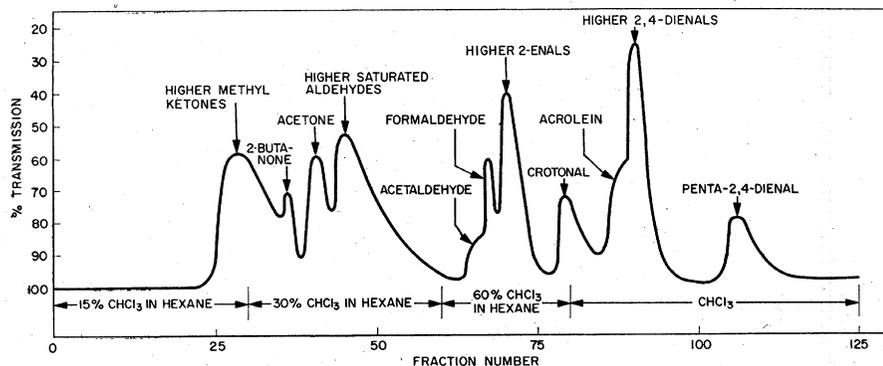


Figure 1. Separation of mixture of 2,4-dinitrophenylhydrazone derivatives of methyl ketones, saturated aldehydes, 2-enals, and 2,4-dienals into classes on magnesia. Time, 5.0 hours

Table I. Recovery of 2,4-Dinitrophenylhydrazones from Magnesia

Compound	Concentration, μM	Recovery, %
2-Nonadecanone	0.33	96
2-Nonanone	0.33	101
2-Pentanone	0.30	100
2-Butanone	0.31	100
Acetone	0.33	98
Octadecanal	0.31	96
Dodecanal	0.32	100
Octanal	0.33	99
Butanal	0.37	99
Acetaldehyde	0.39	101
Formaldehyde	0.41	97
Octadeca-2-enal	0.11	102
Undeca-2-enal	0.30	101
Octa-2-enal	0.34	100
Crotonal	0.34	100
Acrolein	0.30	95
Octadeca-2,4-dienal	0.32	97
Dodeca-2,4-dienal	0.31	100
Penta-2,4-dienal	0.30	99

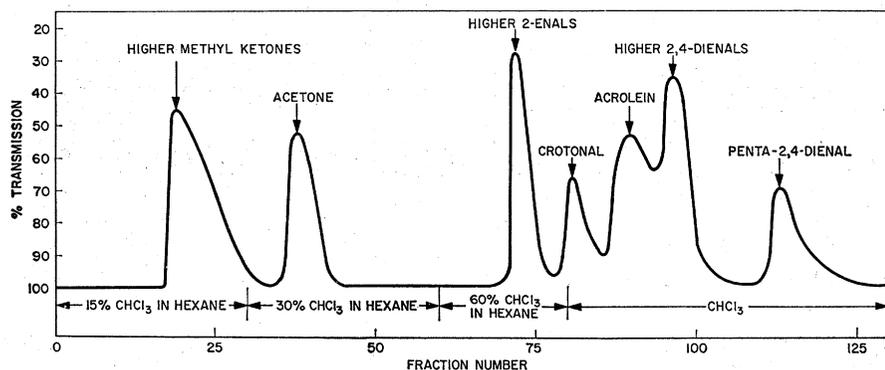


Figure 2. Separation of mixture of 2,4-dinitrophenylhydrazone derivatives of methyl ketones, 2-enals, and 2,4-dienals into classes on magnesia. Saturated aldehyde class omitted. Time, 5.0 hours

can be monitored manually by collecting small (preferably 5 ml. or less) fractions and reading their absorbance against the proper solvent blank.

RESULTS

A mixture of 2,4-dinitrophenylhydrazones representing the four classes and considered to be more complex and more difficult to separate than one which would ordinarily be encountered in practice was selected for study. Table I lists the constituents of the mixture and their respective concentrations.

Preliminary work indicated that separation of carbonyls of the same chain length—e.g., 2-pentanone, pentanal, penta-2-enal, and penta-2,4-dienal—could be easily accomplished, but that complete separation of a short chain hydrazone in the faster moving class from a long chain compound in the class immediately following would be more difficult to achieve. For that reason, the 2,4-dinitrophenylhydrazone derivatives of the C_{18} saturated aldehyde, 2-enal, and 2,4-dienal were synthesized and included in the study.

Figure 1 is a chromatogram depicting the separation of the compounds comprising the four classes in the concentration listed in Table I. The members comprising the individual peaks were identified by column partition chromatography using the acetonitrile-hexane system described by Corbin, Schwartz, and Keeney (2) and by paper chromatography (4, 5).

It will be noted in Figure 1 that acetone, acetaldehyde, formaldehyde, crotonal, acrolein, and penta-2,4-dienal behave anomalously, moving behind their respective classes. In a few instances when the main ketone zone is quite compact, 2-butanone will tend to lag slightly behind the higher ketones, but in the authors' experience this occurs rather rarely. All attempts to overcome the irregular behavior of the lower members have been unsuccessful. These attempts involved the use of several different lots of magnesia, vary-

ing the flow rate of the column, and use of other solvent combinations.

The abnormal movement of the lower member(s) of each class is not a serious shortcoming to the subsequent identification of the compounds comprising a given zone, since some type of chromatography will usually be employed to separate the individual members of an isolated class. The peak (in case of column partition chromatography) or the spot (paper) corresponding in position to the suspected anomalous hydrazone can be subjected to the class separation again to determine its purity. This class separation can be performed very rapidly on a short column, since members of the same or approximately the same chain length are readily separated from each other.

To determine whether the omission of an entire class would seriously affect the position of the other three classes, a chromatogram was run without the saturated aldehyde class. The concentration of the members of the three classes was the same as given in Table I. The separation achieved under these conditions is shown in Figure 2. Figures 1 and 2 are quite similar, the main difference being in the inclusion of butanone with the higher methyl ketones,

which, as mentioned above, usually occurs.

An additional class separation was performed in which the concentration of the constituents comprising the saturated aldehyde class was tripled while leaving the members of the other classes the same as listed in Table I. The chromatogram obtained under these conditions is shown in Figure 3. Separation of the classes is still very satisfactory, but the acetone peak has been almost completely enveloped by the saturated aldehyde class. It should be stated, however, that the acetone band in the concentration chromatographed, is easily seen moving down the column behind the main ketone zone but at the forward edge of the aldehyde zone. Another point of interest in Figure 3 is that formaldehyde has blended with the 2-enal fraction and has bridged the 2-enal fraction with crotonal. This is undoubtedly due to diffusion.

Stability and Recovery of 2,4-Dinitrophenylhydrazones. The stability of each hydrazone included in the study was examined by applying it to an individual column in the concentration listed in Table I. It was then eluted, its concentration determined spectrophotometrically, and the recovered compound was checked for decomposition, artifact formation, and any other alteration in the molecule by chromatographing it in the acetonitrile-hexane system of Corbin *et al.* (2).

Recoveries of the individual members are given in Table I. The chromatographic results indicated that all members are obtained pure.

DISCUSSION

The separation of the four most commonly encountered classes of aliphatic monocarbonyl 2,4-dinitrophenylhydrazones on magnesia has proved to be a very valuable asset in studying butterfat oxidation in the authors' laboratory. Mixtures as complex as the one presented have never been encountered, but other classes of carbonyls have

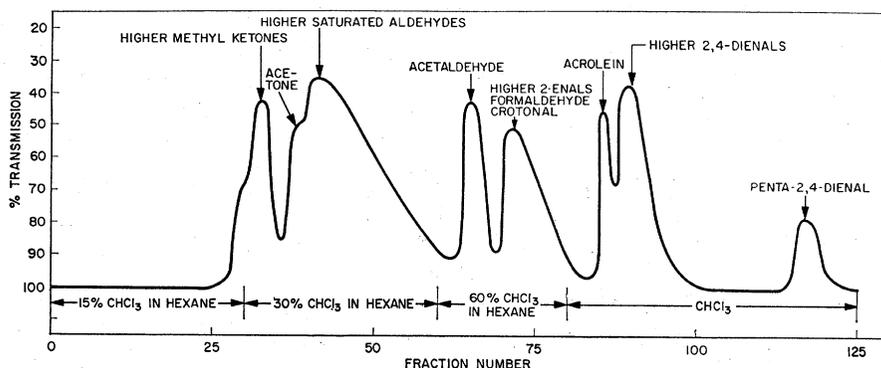


Figure 3. Separation of mixture of 2,4-dinitrophenylhydrazone derivatives of methyl ketones, saturated aldehydes, 2-enals, and 2,4-dienals into classes on magnesia. Time, 5.5 hours

been observed and isolated but as yet not identified unequivocally. Recognition of classes other than those included here has been facilitated by the phenomenon of color formation on contact of the hydrazones with magnesia. The chemistry involved in color formation on this adsorbent is not known, but probably involves the formation of a type of quinoidal anion similar to that discussed by Porter (6) for *m*-dinitro compounds in general under the proper alkaline conditions. The ability of the 2,4-dinitrophenylhydrazones of the various classes to give different colors on magnesia is a fortuitous circumstance and attests further to the utility of 2,4-dinitrophenylhydrazine as a reagent for carbonyls. Van Duin (8) recognized the value of color formation in his work involving class separation on zinc carbonate and was able to enhance the colors on that adsorbent by incorporating a basic constituent (pyridine) in his developing solvent.

In connection with the possibility of identifying a class by the fraction at which it peaks, it should be noted that there is some variation in the peak fraction of each class in the three chromatograms which have been presented in this study. This has occurred even though all three chromatograms have been obtained on the same lot of adsorbent and under conditions which were kept as nearly identical as possible. This is undoubtedly due to small variations in the manner in which the slurry packs—i.e., homogeneity of the Celite-magnesia slurry, as well as the

tightness of the pack. However, the sequence of elution has never been found to vary.

It should be expected that variations between different lots of magnesia will occur, and for this reason, if no other, fraction numbers could not be used as an absolute means of classifying a given peak. Inspection of the three chromatograms reveals, however, that acetone has peaked at fraction 41, 38, 39. It should, therefore, be possible to get an approximate standardization of a given lot of magnesia relative to acetone, by running the latter alone over several different columns.

The characteristic colors of the classes do not vary to any great extent unless a relatively high concentration of methyl ketones is on the column, in which case a grey-black or charcoal color may be observed for them.

Characterization of a class can be facilitated by checking the absorption maximum of the peak in chloroform or hexane. In this laboratory, the maxima used for the four classes in chloroform are methyl ketones, 365; saturated aldehydes, 355 (formaldehyde, 345); 2-enals, 373 (acrolein, 368); 2,4-dienals, 390. In hexane, the maxima used are those given by Corbin *et al.* (2). The presence of an anomalous hydrazone in another class—e.g., formaldehyde in the 2-enal class—lowers the absorption maximum of that class, the magnitude being dependent on the relative concentrations of the anomalous component and the class in which it has moved.

Class separation has also been accomplished on lots of magnesia stronger

(higher iodine number) than the one used in this study. The solvents used to separate the classes depends on the strength of the magnesia. With lots of magnesia having iodine numbers between 23 and 80, increasing concentrations of CHCl_3 in hexane will suffice to separate the classes satisfactorily. With lots of magnesia with iodine numbers over 80, combinations of CHCl_3 and methanol will accomplish the class separation.

LITERATURE CITED

- (1) Badings, H. T., *J. Am. Oil Chemists Soc.* **36**, 648 (1959).
- (2) Corbin, E. A., Schwartz, D. P., Keeney, M., *J. Chromatog.* **3**, 322 (1960).
- (3) Gaddis, A. M., Ellis, R., Currie, G. T., *J. Am. Oil Chemists' Soc.* **38**, 371 (1961).
- (4) Huelin, F. E., *Australian J. Sci. Research* **5B**, 328 (1952).
- (5) Klein, F., DeJong, K., *Rec. trav. chim.* **75**, 1285 (1956).
- (6) Porter, C. C., *ANAL. CHEM.* **27**, 805 (1955).
- (7) Schwartz, D. P., Parks, O. W., Keeney, M., Abstracts, p. 15B, 138th Meeting, ACS, New York, N. Y., September 1960.
- (8) Van Duin, H., Thesis, Free University, Amsterdam, May 1961.
- (9) Van Duin, H., *Neth. Milk Dairy J.* **12**, 74-81 (1958).

RECEIVED for review November 30, 1961. Accepted March 12, 1962. In part, Division of Analytical Chemistry, 138th Meeting, ACS, New York, N. Y., September 1960. Work done at a laboratory of the Eastern Utilization Research and Development Division, Agricultural Research Service, USDA, Washington 25, D. C. The use of trade names does not imply endorsement of the product or its manufacture by the U. S. Department of Agriculture.