Paper Chromatography of 2,4-dinitrophenylhydrazones of Saturated Aliphatic Aldehydes[1]

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THE OXIDATION OF A FAT leads to the scission and degradation of the molecules into short-chain aldehydes and ketones. Some of the volatile decomposition products that have been identified from the autoxidation of ethyl linoleate are methyl ethyl ketone, acetaldehyde, propanal, pentanal, hexanal, crotonal, 2-pentenal, and 2-heptanal (3, 6). Kawahara and Dutton (7) have identified acetaldehyde, propionaldehyde, α-pentenal, and hexanal from the volatile cleavage products of autoxidized soybean oil by separation of their diphenylhydrazones on silica gel column. Kawahara *et al.* (8) have also identified acetaldehyde, propionaldehyde, and α-pentenal from the volatile products of autoxidizing methyl linolenate by following the same technique. In a recent study butterfat, corn oil, and pure methyl esters were subjected to thermal oxidative polymerization, and the volatile compounds were collected. The amount of volatile carbonyl compounds obtained was sometimes so small that column chromatography could not be applied for the separation and identification of the carbonyls. It was felt that paper chromatography of the 2,4-dinitrophenylhydrazine derivatives of the carbonyl compounds could be conveniently used for their identification. A number of methods have been suggested for the separation of 2,4-dinitrophenylhydrazones (1,2,5,9,10,12,13,14,15). Most of these methods were found to be unsatisfactory because of poor resolution or streaking, and some were time-consuming. The method suggested by Ellis *et al.* (4) involves the use of two systems and was found to be good for a mixture containing a few compounds but was found to be unsatisfactory because of a lack of resolution of a complex mixture of carbonyls in our studies. Silberstein (16) was able to separate the 2,4-dinitrophenylhydrazones of C_1 to C_6 aldehydes on filter paper impregnated with 20% propylene glycol with heptane-propylene glycol as a flowing solvent by descending chromatography. This method however cannot be used to separate aldehydes greater than six carbon atoms in chain length. The method developed in our laboratory is a modification of Silberstein's method, and it has been found possible to separate a more complex mixture of 2,4-dinitrophenylhydrazones of higher aliphatic aldehydes than was possible by the method of Silberstein. The new technique involved the use of filter paper impregnated with ethylene glycol, and the chromatogram was developed by a descending system with methanol saturated n-heptane.

Solvents and Reagents

Absolute Methanol. The absolute methanol was acidified with a few drops of hydrochloric acid and was refluxed for 2 hrs. over 2,4-dinitrophenylhydrazine and distilled through an air condenser loosely packed with glass wool. This methanol was redistilled. n-Heptane, Phillips 66, Ethylene glycol, U.S.P. grade, redistilled.

Materials and Equipment

Whatman No. 1 filter paper cut into strips 40 × 5 cms. Chromatographic jar 45 × 15 cms., with stainless steel rack and cradle, glass solvent trough, anchor rod, and two anti-syphon rods.

Experimental

Normal saturated aldehydes from C_1 to C_9, acetone, methyl ethyl ketone, methyl propyl ketone, and 4-heptanone were obtained from commercial sources. Two-tenth g. of the carbonyl compound was dissolved in 10 ml. of ethyl alcohol, and the solution was added dropwise, with continuous stirring, to 100 ml. of a saturated solution of 2,4-dinitrophenylhydrazine in 2N hydrochloric acid. The precipitate was allowed to digest at 0°C. for 1 hour. It was then filtered through a Buchner funnel with suction, and the precipitate was washed with 2N hydrochloric acid and finally with cold water till free from acid. The precipitate was dried in vacuum over sulfuric acid. The hydrazones were further purified by recrystallization from methanol. Stock solutions of the hydrazones in absolute methanol were prepared so as to contain 25 γ per ml. Aliquots of these solutions were used for preparation of mixtures for spotting the chromatograms.

The descending chromatographic system was followed for the separation of a mixture of hydrazones of C_1 to C_9 aldehydes. The chromatographic jar was saturated with the solvent over-night at room temperature by pouring about 200 ml. of n-heptane containing 1 ml. of methanol into the jar. The filter paper strips were drawn through a solution containing 20% ethylene glycol and 80% methanol (by volume) and air-dried at room temperature for 1 hr. in a forced draft hood. The paper strips were spotted with a mixture of hydrazones and dried in a draft of air. The paper strips were then suspended from the solvent trough with the aid of an anchor rod in the jar and left for 30 min. to attain equilibrium. Fifteen ml. of n-heptane saturated with methanol were then poured into the solvent trough, and the top was closed with a glass plate and sealed with the help of grease. The strips were removed from the jar at the end of 2½ hrs. In this period of time the solvent front had moved about 30 cm. The paper strips were dried in a current of air, and the Rf values of the different separated hydrazones were calculated. Throughout the course of this study, paper chromatography was conducted at room temperature.

Individual dinitrophenylhydrazones were also run simultaneously to determine the variation in Rf values. Separated spots from the mixture were extracted and run singly to determine the completion of resolution. The yellow spots of the hydrazones were found to be separated out clearly and could be made

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more distinct by spraying with a solution of 0.25N potassium hydroxide in ethyl alcohol, which impart a brown color to the hydrazones. The brown color of the hydrazones of the higher aldehydes was found to fade quickly.

Results and Discussion

The melting points of the 2,4-dinitrophenylhydrazones of the aldehydes prepared in the laboratory are shown in Table I. The melting points of the hydrazones were found to agree closely with those reported in the literature (11).

TABLE I
Melting Points of 2,4-Dinitrophenylhydrazones

2,4-Dinitrophenylhydrazone	Melting point (°C.)	
	Found	Literature(11)
Methanal	164	166
Ethanal	162	167.5
Propanal	153	155
n-Butanal	118	119
n-Pentanal	107	106
n-Hexanal	104	105
n-Heptanal	105	106
n-Octanal	106	105.5
n-Nonanal	105	106.5

The separation of 2,4-dinitrophenylhydrazones of the aliphatic aldehydes from C_1 to C_9 is shown in Figure 1. It was observed that the various hydrazones separate out in distinct spots on the filter paper treated with ethylene glycol and could be identified from their Rf values. The distance between the two adjacent spots was found to decrease with an increase in the chain length of the aldehydes. The Rf values of the 2,4-dinitrophenylhydrazones of the aliphatic aldehydes from C_1 to C_9 are reported in Table II. It was found that the Rf values of the individual hydrazones were identical with or slightly lower than those of the corresponding hydrazones separated from the mixture. The Rf values of the extracted spots however agreed closely with those of the corresponding hydrazones run singly.

TABLE II
Separation of 2,4-Dinitrophenylhydrazones of Aliphatic Aldehydes on Filter Paper Impregnated with 20% Ethylene Glycol in Methanol and Developed with Heptane-Methanol at Room Temperature

2,4-Dinitrophenylhydrazones of aliphatic aldehydes	Rf values[a]		
	Mixture	Individual	Spots extracted from the mixture
Methanal	0.23	.23	.23
Ethanal	.38	.38	.38
Propanal	.58	.56	.56
n-Butanal	.71	.69	.69
n-Pentanal	.80	.78	.79
n-Hexanal	.86	.85	.85
n-Heptanal	.90	.89	.89
n-Octanal	.94	.92	.93
n-Nonanal	.97	.95	.96

[a] Based on averages of 6 paper strips.

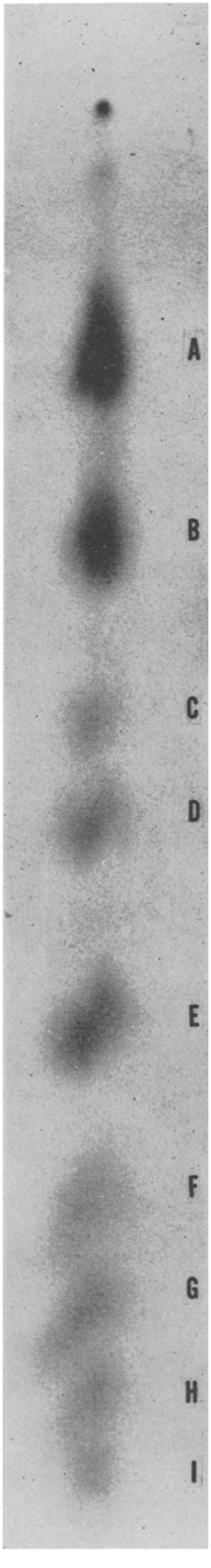

FIG. 1. Separation of 2,4-dinitrophenylhydrazones of (A) methanal, (B) ethanal, (C) propanal, (D) n-butanal, (E) n-pentanal, (F) n-hexanal, (G) n-heptanal, (H) n-Octanal, and (I) n-nonanal on filter paper impregnated with 20% ethylene glycol in methyl alcohol.

A mixture of 2,4-dinitrophenylhydrazones of acetone, methyl ethyl ketone, methyl propyl ketone, and 4-heptanone was also run on the filter paper impregnated with 20% ethylene glycol in methanol. The different hydrazones were found to separate out in clear spots. It will be noted from Table III that the Rf values of the hydrazones separated from the mixture agreed closely with those of the individual compounds run simultaneously. This method is thus found to be useful in the separation of 2,4-dinitrophenylhydrazones of the ketones also.

Attempts were made to impregnate the paper with 20% propylene glycol in methanol. Good separation of the hydrazones was obtained. However a day-to-day variation in the Rf values of the hydrazones was observed. The effect of varying the drying time of the filter paper after impregnating with ethylene glycol is shown in Table IV. There was practically no difference in the Rf values of the hydrazones separated.

TABLE III
Separation of 2,4-Dinitrophenylhydrazones of Some Ketones on Filter Paper Impregnated with 20% Ethylene Glycol in Methanol and Developed with Heptane-Methanol at Room Temperature

2,4-Dinitrophenylhydrazones of ketones	Rf values[a]		
	Mixture	Individual	Spots extracted from the mixture
Acetone	0.63	0.62	0.62
Methyl ethyl ketone	0.77	0.76	0.77
Methyl propyl ketone	0.85	0.84	0.84
4-Heptanone	0.93	0.92	0.92

[a] Based on averages of 6 paper strips.

The effect of variation in the amount of impregnating agent in the paper on the Rf values is shown in Table V. With an increase in the amount of ethylene glycol in the paper, there was a lowering in the Rf values. The results seem to indicate that the separation of the 2,4-dinitrophenylhydrazine derivative of aldehydes higher than C_9 could be achieved by increasing the amount of impregnating agent.

It will be noted from Table II that the differences in the Rf values of the higher hydrazones is very small. It is therefore advisable to use comparatively small amounts for application on the paper to achieve good separation of the 2,4-dinitrophenylhydrazones of C_7, C_8, and C_9 aldehydes.

TABLE IV
Effect of Variation in Time of Drying of Paper Strips After Impregnating with 20% Ethylene Glycol in Methanol

2,4-Dinitrophenylhydrazones	Rf values[a]		
	Time of drying in hours		
	1	2	3
Methanal	0.23	0.23	0.24
Ethanal	0.37	0.38	0.38
n-Butanal	0.70	0.71	0.73

[a] Based on averages of 3 paper strips.

In order to obtain reproducible results from day to day, it is extremely important that the chromatographic jar be emptied once every two or three days in order to avoid contamination of the chamber with moisture. It is further necessary to prepare the impregnating solvent mixture and the heptane-methanol solution fresh before use. The ethylene glycol and the methyl alcohol should be free from moisture, otherwise the spots have a tendency to streak. In the separation and identification of unknown compounds, a mixture of known compounds should be run simultaneously on the same paper. The sensitivity of this chromatographic system is shown by efficient separation of compounds having close Rf values.

TABLE V
Effect of Variation in Amount of Impregnating Agent on Rf Values

Mixture of 2,4-dinitrophenylhydrazones	Rf values[a]		
	Ethylene glycol, % in methanol		
	10	20	30
Methanal	0.30	0.22	0.18
Ethanal	0.45	0.38	0.35
n-Butanal	0.75	0.70	0.67

[a] Based on averages of 3 paper strips.

Summary

A rapid method of separating the 2,4-dinitrophenylhydrazones of C_1 to C_9 aldehydes by descending chromatographic system has been described. Whatman No. 1 filter paper was impregnated with ethylene glycol-methanol (1:4, v/v), and n-heptane saturated with methanol was used as a developing solvent. Complete separation was achieved in 3 hrs. It has been shown that the method can also be used for the separation of 2,4-dinitrophenylhydrazone derivatives of ketones.

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The Reaction of Methyl Vinyl Ketone with alpha- and beta-Eleostearic Acids

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IN CONTINUING the exploration of the scope of the Diels-Alder reaction of *alpha*- and *beta*-eleostearic (9,11,13-octadecatrienoic) acids (1, 2, 3), we have now investigated the suitability of methyl vinyl ketone as a dienophile for these conjugated acids. The prior literature appears to contain only a single reference to the use of methyl vinyl ketone as a dienophile for conjugated fatty acids, in which a cursory examination was made of its reaction with *trans,trans*-9,11-octadecadienoic acid (4). It seemed of interest therefore to evaluate the dienophilic activity of methyl vinyl ketone with both *alpha*- and *beta*-eleostearic acids.

This communication presents an account of the reaction of methyl vinyl ketone with the eleostearic acids as well as certain considerations regarding the structures of the resultant adducts.

Experimental

Starting Materials

Methyl Vinyl Ketone. This material, supplied by Chas. Pfizer and Company, was used without further purification.

beta-Eleostearic Acid. Pure *beta*-eleostearic acid was prepared by the low-temperature fractional crystallization of the mixed fatty acids obtained from isomerized tung oil (5). The acid, m.p. 71–72°C., had an absorptivity, (a) = 201.8 at 269.0 millimicrons in cyclohexane.

The Methyl Esters of Tung Oil Fatty Acids. These esters were prepared by methanolysis of *alpha*-tung oil, employing sodium methylate catalyst as described in a previous publication (6). This material, boiling range 152–155°C./5 microns, contained 82% *alpha*- and 3% *beta*-methyl eleostearates.[2]

Preparation of Methyl Vinyl Ketone-Methyl alpha-Eleostearate Adduct. Approximately 100 g. of the methyl esters of tung oil fatty acids (*ca.* 0.25 mole methyl *alpha*-eleostearate), 50 g. of methyl vinyl ketone (*ca.* 0.70 mole), and 100 ml. of benzene were heated at 150°C. in a 325-ml., sealed stainless steel bomb for 16 hrs. Benzene and the excess methyl vinyl ketone were removed by vacuum-stripping on a steam bath, employing a stream of carbon dioxide as a sweep gas. Approximately 130 g. of the crude product were obtained, which had only 2.7% of unreacted eleostearates. High-vacuum distillation of this

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[2] The amount of eleostearates present was determined by the method of Hoffmann *et al.* (5).