

Paper Chromatography of Fatty Acids as Their Acetol Ester Derivatives

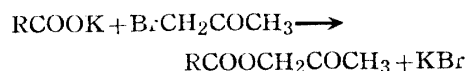
By

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Reversed-phase paper chromatography has recently been effectively applied to the separation and identification of higher fatty acids and their derivatives.¹⁻⁶⁾ However it has needed to improve the separability and sensitivity of this method for its practical application to the analysis of natural mixed fatty acids. A convenient reversed-phase technique for separating unsaturated fatty acids was reported in a previous paper,⁷⁾ and it provided a profitable approach to the problem.

In the present study it has succeeded in obtaining clean separations of saturated higher fatty acids by the reversed-phase chromatography of their acetol ester derivatives, which has been efficacious for the above improvement.

The acetol esters of fatty acids were first prepared by Locquin⁸⁾ and later by Kling⁹⁾ by the action of chloroacetone on the fatty acids. Polgar¹⁰⁾ and Paquot and Hiep¹¹⁾ prepared some functional derivatives of these compounds. In our present experiments the preparation of the acetol esters has been accomplished in high yield and much shorter reaction time by using bromoacetone instead of chloroacetone as shown in the following equation:



As the suitable derivatives to be employed for paper chromatography, the 2,4-dinitrophenylhydrazones and thiosemicarbazones of the acetol esters have been prepared; the latter have not hitherto been reported in the literature.

Preparation

Fatty Acids. — Arachidic acid was prepared from stearyl alcohol by malonic ester synthesis. The other even-numbered fatty acids from acetic to stearic were all commercial preparations which were completely repurified and found of high purity by the determination of their physical and chemical constants.

Acetol Esters of Fatty Acids. — A solution of fatty acid in alcohol was neutralized with a *N* solution of potassium hydroxide in alcohol until the solution became just neutral or slightly acidic. A small excess of bromoacetone was then added to the neutralized solution, and the mixture was refluxed at 80–90° for about 30 minutes, after which a large quantity of water was added. The mixture was extracted with ether, and the ether extract washed first with a dilute sodium carbonate solution and then with water repeatedly. After drying the ether extract with anhydrous sodium sulfate, the solvent was removed, and the residue recrystallized from alcohol. In the cases of the esters of lower fatty acids than lauric, they were purified by distillation *in vacuo*. The acetol esters thus purified were obtained as colorless needles or plates in the esters of higher members and as colorless liquids in those of lower members. The yields were nearly theoretical.

The physical properties of these compounds are listed in Table 1.

2,4-Dinitrophenylhydrazones of Acetol Esters. — A warm solution of a slight excess of 2,4-dinitrophenylhydrazine in 2 *N* methanolic hydrochloric acid was added to acetol ester dissolved in alcohol with warming. After standing

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Table 1. Acetol Esters of Fatty Acids

Acid	M. p., °C.	B. p., °C. ^a	$n_D^{t,b}$
Acetic	67–70 ^{13.5}	1.4194 ^{13.5}
Butyric	87–88 ^{14.0}	1.4230 ^{14.0}
Caproic	108–110 ^{13.5}	1.4304 ^{15.0}
Caprylic	134–136 ^{13.5}	1.4360 ^{15.0}
Capric	17.5	161–162 ^{14.0}
Lauric	30.5
Myristic	42.0
Palmitic	50.0
Stearic	56.5
Arachidic	61.0

^a Superior figure indicates the barometric pressure in millimeters of mercury at which the boiling point was determined.

^b Superior number represents the temperature (°C.) recorded at *t*.

for about 30 minutes, a small quantity of water was added to the reaction mixture, and the crude 2,4-dinitrophenylhydrazone was collected by filtration and freed from hydrochloric acid by washing with water. Recrystallization from alcohol gave yellow needles in nearly quantitative yield.

For paper-chromatographic purposes, the pure 2,4-dinitrophenylhydrazones were applied as their 1–2% solutions in benzene, but the sample

solutions sufficient for paper chromatography could also be prepared directly from fatty acids by the simplified procedure described below.

Thiosemicarbazones of Acetol Esters.

A mixture of thiosemicarbazide and a slight excess of acetol ester in absolute methanol was refluxed at 70° for 30–40 minutes. After cooling a small quantity of water was added to the reaction mixture, and the product was filtered with suction. The solid thiosemicarbazone was recrystallized from 80–95% alcohol to give an approximately 90% yield of colorless plates.

Data for the melting points and analyses of these 2,4-dinitrophenylhydrazones and thiosemicarbazones are given in Table 2. Table 3 shows the solubilities of the compounds in various solvents.

Simplified Method of Preparation of 2,4-Dinitrophenylhydrazones for Paper Chromatography.

— A solution of fatty acids in alcohol was neutralized with a *N* solution of potassium hydroxide in alcohol in presence of phenolphthalein until a pink color was produced, and then slightly acidified by adding one drop of 0.05 *N* hydrochloric acid in methanol. To the solution was added a slightly less amount of bromoacetone than the theoretical. After refluxing at 80–90° for about 30 minutes, the mixture was allowed to cool to about 50°, and a warm

Table 2. 2,4-Dinitrophenylhydrazones and Thiosemicarbazones of Acetol Esters of Fatty Acids

Acid	2,4-Dinitrophenylhydrazone			Thiosemicarbazone				
	M. p., °C.	N, %		M. p., °C.	N, %		S, %	
		Calcd.	Found		Calcd.	Found	Calcd.	Found
Acetic	112.0 ^a	18.91	18.86	146.5	22.21	22.12	16.94	16.65
Butyric	65.5	17.28	17.21	153.0	19.57	19.63	14.76	14.61
Caproic	52.5	15.90	16.01	112.0	17.13	17.17	13.07	12.90
Caprylic	61.0	14.73	14.56	120.5	15.37	15.21	11.73	11.53
Capric	70.0 ^a	13.72	13.74	114.0	13.94	13.97	10.64	10.57
Lauric	78.0 ^a	12.84	12.80	113.5	12.75	12.70	9.73	9.68
Myristic	83.5 ^a	12.06	12.15	114.0	11.75	11.69	8.97	8.91
Palmitic	87.5 ^a	11.37	11.24	114.5	10.90	10.83	8.32	8.28
Stearic	90.0 ^a	10.76	10.63	115.0	10.16	10.12	7.75	7.70
Arachidic	91.5	10.21	10.12	115.0	9.51	9.45	7.26	7.22

^a Previously prepared.^{10,11)}

Table 3. Solubilities of 2,4-Dinitrophenylhydrazones and Thiosemicarbazones of Fatty Acid Acetol Esters^a

Acid	2,4-Dinitrophenylhydrazone					Thiosemicarbazone				
	C ₂	C ₆	C ₁₀	C ₁₄	C ₁₈	C ₂	C ₆	C ₁₀	C ₁₄	C ₁₈
Solvent										
Methanol	+	+	+	+	+	+++	+++	+	+	+
Ethanol	+	+	+	+	+	+	+	+	+	+
Pyridine	+++	+++	+++	+++	+++	+++	+++	+++	+++	+++
Acetone	+++	+++	+++	+	+	+++	+++	+++	+++	+
Ethyl acetate	+++	+++	+++	+++	+	+	+++	+++	+++	+++
Ether	+	+++	+++	+	+	+	+	+	+	+
Benzene	+++	+++	+++	+++	+++	+	+	+	+	+++
Carbon tetrachloride	+	+++	+++	+	+	±	±	+	+	+
Petroleum ether	±	+	+	+	+	±	±	±	+	+

^a At room temperature. Solubilities are indicated by the scale +++ very soluble to ± very slightly soluble.

solution of a slight excess of 2,4-dinitrophenylhydrazine in 2 *N* methanolic hydrochloric acid was then poured into this mixture. The reaction mixture thus obtained was allowed to stand for about 30 minutes after stirring, and benzene was added with shaking. The benzene layer separated upward by adding a large quantity of water was directly used as the sample solution for paper chromatography.

Paper Chromatography of 2,4-Dinitrophenylhydrazones

Filter Paper.— Sheets (35×40 cm.) or strips (2.5×40 cm.) of Toyo No. 2 filter paper were used. A starting line was penciled 5 cm. above one of the narrower edges of the paper and kept at a distance of approximately 4 cm. above the surface of the solvent.

Solvent Systems.— Reversed-phase systems were employed. As the stationary solvent decalin was used. The developing solvents used were methanol–decalin (8 : 1 by volume) (MD), methanol–ethyl acetate–decalin (40 : 3 : 7) (MSD), methanol–acetic acid–decalin (50 : 2 : 7) (MAD), and 90% (*V/V*) ethanol–acetic acid–decalin (30 : 5 : 3) (EAD).

Procedure.— From 0.5 to 2 μ l. of the sample solutions in benzene, containing 5–20 μ g. of each 2,4-dinitrophenylhydrazone of fatty acid

acetol ester, were placed at original points along the starting line on the filter paper. After complete removal of benzene by drying the paper in air, the paper was uniformly sprayed with decalin, the amount being approximately 7 μ l. per 1 cm². of the paper, and immediately developed upward with the developing solvent in a glass cylinder maintained at 30°. The solvent was allowed to ascend about 25 cm. over a 5-hour period in the cases of the systems MD, MSD, and MAD, and about 16 cm. over a 7-hour period in the case of EAD. After development, the paper was removed and air-dried. The yellow distinct spots of 2,4-dinitrophenylhydrazones of acetol esters were well located on the chromatogram without spraying of any reagent, and they showed no considerable fading for a long time. Even 1 μ g. of the compounds were detectable. By spraying with a *N* solution of potassium hydroxide in alcohol, the yellow spots changed to intense reddish-brown spots which, however, were rapidly discolored into the original yellow.

***R_F* Values and Separability.**— The *R_F* values of the 2,4-dinitrophenylhydrazones of fatty acid acetol esters measured with their pure samples are recorded in Table 4.

Among the solvent systems employed, the systems MD and MSD displayed the highest

Table 4. R_F Values of 2,4-Dinitrophenylhydrazones and Thiosemicarbazones of Fatty Acid Acetol Esters^a

Acid	2,4-Dinitrophenylhydrazone				Thiosemicarbazone	
	MD ^b	MSD ^b	MAD ^b	EAD ^b	MD ^b	MAT ^b
Acetic	0.73	0.73	0.79	0.90	0.91	0.82
Butyric	0.71	0.75	0.77	0.84	0.86	0.81
Caproic	0.67	0.68	0.67	0.70	0.82	0.76
Caprylic	0.60	0.61	0.61	0.59	0.78	0.67
Capric	0.51	0.52	0.53	0.50	0.74	0.58
Lauric	0.43	0.43	0.45	0.40	0.72	0.49
Myristic	0.33	0.34	0.37	0.31	0.67	0.39
Palmitic	0.25	0.26	0.29	0.22	0.58	0.26
Stearic	0.17	0.20	0.21	0.16	0.48	0.01
Arachidic	0.12	0.15	0.12	0.12	0.45	0.00

^a Ascending chromatography at 30°C.

^b Solvent systems: MD, methanol-decalin; MSD, methanol-ethyl acetate-decalin; MAD, methanol-acetic acid-decalin; EAD, ethanol-acetic acid-decalin; MAT, methanol-acetic acid-tetralin.

separability. A mixture of the derivatives of all the even-numbered saturated fatty acids from

caproic to arachidic can be successfully separated from each other with these solvent systems as

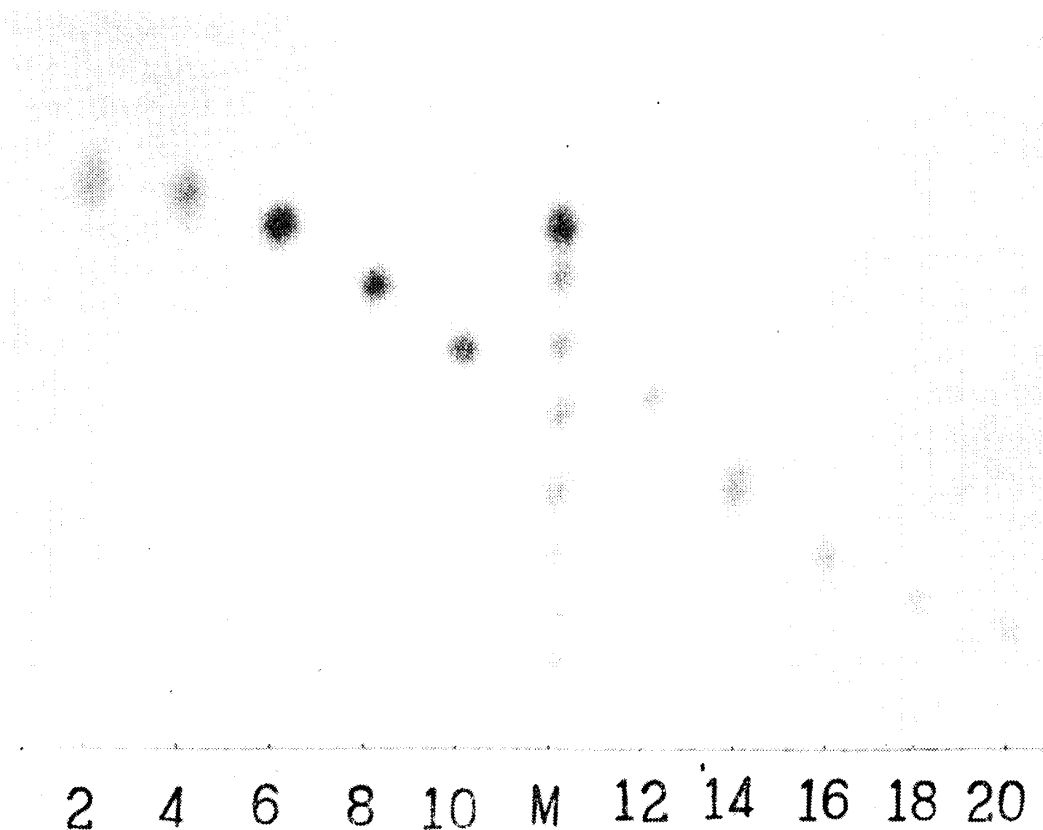


Fig. 1. Paper chromatogram of the 2,4-dinitrophenylhydrazones derived from the acetol esters of saturated fatty acids. Ascending chromatography with the solvent system MD. Number represents the number of carbon atoms in the original acid. M, a mixture of the derivatives of all the even-numbered saturated acids from C₆ to C₂₀.

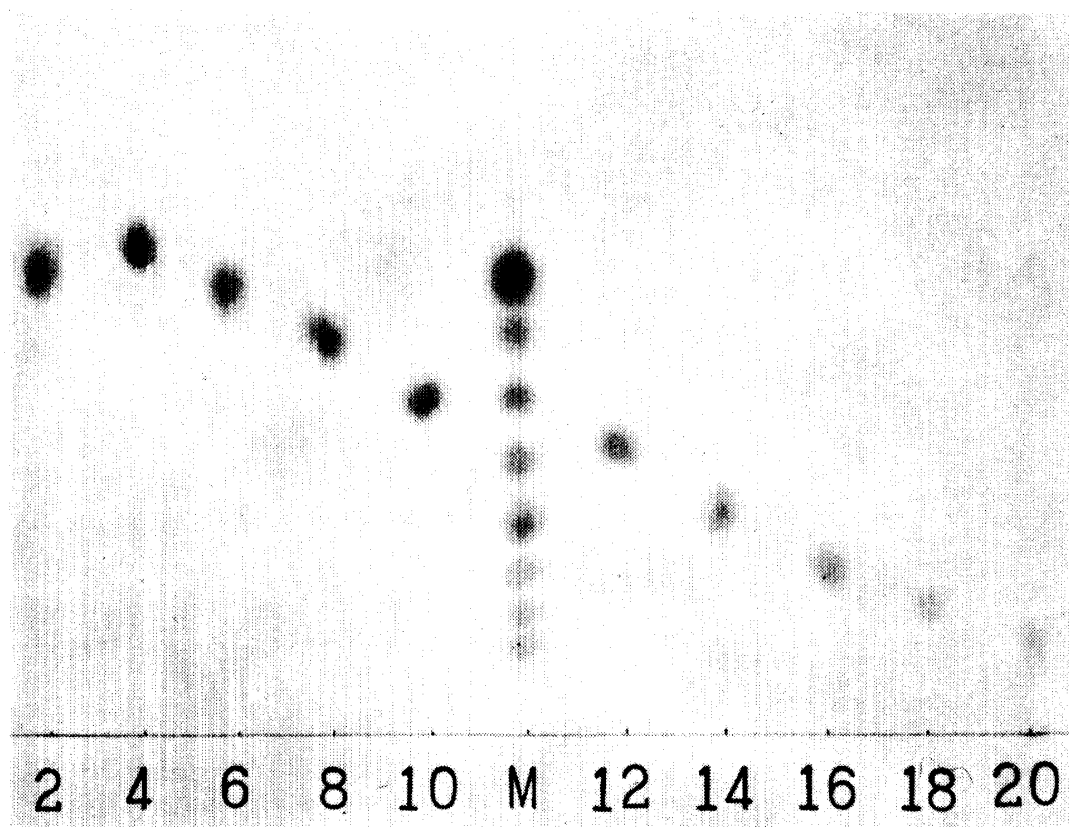


Fig. 2. Paper chromatogram of the 2,4-dinitrophenylhydrazones derived from the acetol esters of saturated fatty acids. Ascending chromatography with the solvent system MSD. Number represents the number of carbon atoms in the original acid. M, a mixture of the derivatives of all the even-numbered saturated acids from C_6 to C_{20} .

shown in Figs. 1 and 2.

Paper Chromatography of Thiosemicarbazones

Filter Paper.— The filter papers used were the same as those described above.

Solvent Systems.— Decalin or tetralin was used as the stationary solvent in this reversed-phase chromatography. The developing solvent used against decalin was methanol—decalin (8:1 by volume) (MD), and that against tetralin was 90% (V/V) methanol—acetic acid—tetralin (10:2:1) (MAT).

Procedure.— A solution of the thiosemicarbazones of fatty acid acetol esters in ethyl acetate was applied as the sample solution. The procedure of development was similar to that employed in the above paper-chromatographic separation of 2,4-dinitrophenylhydrazones with the exception of the technique of color de-

velopment. The developed and air-dried paper was first soaked in warm 5% mercuric acetate solution for 5 minutes, then washed in a stream of water for about 2 hours, dried in air, and sprayed with a 0.05% solution of diphenylcarbazone in alcohol. The thiosemicarbazones of acetol esters appeared by spraying as purple spots on a white background.

R_f Values and Separability.— Table 4 also gives the R_f values of the thiosemicarbazones of the acetol esters of saturated fatty acids. The separability of these thiosemicarbazones on the chromatogram was somewhat inferior to that of 2,4-dinitrophenylhydrazones.

Discussion

All of the 2,4-dinitrophenylhydrazones of saturated acid acetol esters are colored crystalline compounds melting above room temperature and have relatively large differences in melting

points between adjacent members. These compounds are therefore also useful for characterizing saturated fatty acids as their crystalline derivatives.

The irregularity in the melting points of the thiosemicarbazones of saturated acid acetol esters is found as their interesting behavior; wide fluctuations similar to those observed in fatty acid amides occur in the melting points of these derivatives of even-numbered lower acids.

As the stationary solvent for paper chromatography of 2,4-dinitrophenylhydrazones, decalin contrasted strikingly with tetralin which was ineffectual because it detained all the spots of these derivatives near the original points. On the contrary, in the case of paper chromatography of thiosemicarbazones, there was no remarkable difference in characteristic as the stationary solvent between decalin and tetralin. It seems that the above specialities of these derivatives in relation to the stationary solvents used are due to differences in their solubilities in the stationary solvents, which may be affected by the affinities in chemical structure between the solutes and the solvents.

The paper-chromatographic separation of the 2,4-dinitrophenylhydrazones of fatty acid acetol esters is especially excellent in its high sensitivity and separability as well as in almost quantitative yields of the derivatives on their preparation. The technique should be effectively applicable to the separation and identification of natural mixed fatty acids, and it may also be possible to accomplish the simultaneous separation of all the saturated and unsaturated acids.

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Summary

A series of acetol esters of the even-numbered saturated fatty acids from C₂ to C₂₀ have been prepared by the action of bromoacetone on the corresponding fatty acids and subsequently converted into their 2,4-dinitrophenylhydrazones and thiosemicarbazones which have been used as the fatty acid derivatives suited for reversed-phase paper chromatography.

The best separation has been achieved in the paper chromatography of the 2,4-dinitrophenylhydrazones of acetol esters, using decalin as the stationary solvent and methanol-decalin or methanol-ethyl acetate-decalin as the moving solvent. In this case, all the saturated fatty acids from C₆ to C₂₀ have been clearly separated from each other on a chromatogram with high sensitivity.

This method is useful in the microanalysis of mixed fatty acids.

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