p-Phenylazophenacyl Esters

Rates of Movement Relative to p-Phenylazophenacyl Bromide on Silicic Acid and Identification by Paper Partition Chromatography

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▶ The relative rates of travel (R_r values) of p-phenylazophenacyl derivatives compared to p-phenylazophenacyl bromide on silicic acid columns and a technique for the chromatography of p-phenylazophenacyl derivatives on paper are presented and their applications to analysis and identification of organic acids are discussed. An improved method for the preparation of p-phenylazophenacyl bromide and the melting points of 30 additional p-phenylazophenacyl derivatives are also included.

UTILIZATION of the *p*-phenylazophenacyl derivatives in the investigation of the very small amounts of organic acids, present either in the free state or as esters in the volatile flavor materials in grapes and wines, necessitated the improvement of chromatographic techniques for the separation and identification of very small amounts of these derivatives. This paper presents the melting points of the additional *p*-phenylazophenacyl derivatives which have been prepared and the results of investigations concerning the identification of comparatively small amounts of these derivatives both on silicic acid columns and by paper partition chromatography techniques.

MATERIALS

p-Phenylazophenacyl Bromide. The *p*-phenylazoacetophenone was prepared as previously described (*2*). The *p*-phenylazoacetophenone was then brominated, following to a considerable extent the procedure for bromination of acetophenone given in "Organic Syntheses" (*1*), except that dry chloroform was used as the solvent and the bromine was added in chloroform solution. Removal of the solvent and hydrogen bromide gave a solid residue which contained essentially no tar. Extraction of the residue with several portions of hot Skellysolve B resulted in the isolation of crude product, melting point, 96°–102°, in 85 to 90% yield, in the bromination step. Purification of this material by recrystallization from Skellysolve B or by chromatography on silicic acid gave excellent recovery of pure *p*-phenylazophenacyl bromide as orange colored needles, corrected melting point, 103–4° C.

Adsorbent. Silicic acid (Mallinckrodt, analytical reagent, No. 2847, 100-mesh) was activated as described (*2*).

Developing Solvents. Thiophene-free benzene and 2-phenoxyethanol (Eastman Kodak Co., No. 4861) were used without further purification. The Skellysolve B and the *n*-heptane were redistilled before use.

***p*-Phenylazophenacyl Esters.** The derivatives were prepared and purified as previously described (*2*).

APPARATUS AND PROCEDURE FOR DETERMINATION OF RELATIVE RATES OF MOVEMENT ON SILICIC ACID

The apparatus and procedure for packing the column have been described (*2*). A 0.3- to 0.5-mg. sample of each of one or more *p*-phenylazophenacyl derivatives and *p*-phenylazophenacyl bromide was placed on the column and the chromatogram was developed as described. Development was continued until the reagent band had progressed almost to the bottom of the column (30 to 35 cm. in length). At several points during the development process the distances which the reagent band and any derivative bands had traveled down the column were measured to the closest millimeter, from the top of the silicic acid column to the point of greatest color density in the band. The ratios of the distances traveled by a given *p*-phenylazophenacyl derivative relative to *p*-phenylazophenacyl bromide were calculated and the average value of the ratio was designated as the R_r value.

APPARATUS AND PROCEDURE FOR PAPER PARTITION CHROMATOGRAPHY

Sheets of Whatman No. 7 filter paper were dipped in a solution of 10% 2-phenoxyethanol in acetone, blotted free of excess solution, and dried in air for 0.5 hour to an hour. The *p*-phenylazophenacyl derivatives, dissolved in benzene, were applied to the paper to give spots 0.5 cm. or less in diameter. The sheets of paper were then suspended from a trough in a Chromatocab saturated with *n*-heptane and 2-phenoxyethanol vapors, and development of the chromatogram by the descending technique was started using *n*-heptane saturated with 2-phenoxyethanol as the developing solvent. The derivative spots were placed on the 18 × 22 inch sheets of paper, so that they moved down the long direction of the paper during development.

RESULTS AND DISCUSSION

Table I shows the corrected melting

Table I. Rates of Movement (R_r) of p-Phenylazophenacyl Esters Relative to p-Phenylazophenacyl Bromide on Silicic Acid

	M.P., ° C.	R_r
Acetate	125–127[a]	0.047
Propionate	104–105[a]	0.057
Butyrate	98–99[a]	0.087
Isobutyrate	101.5–102[a]	0.11
Valerate	73–74[b]	0.11
Isovalerate	93.8–94.3[c]	0.14
2-Methylbutyrate	82.5–83.5	0.13
Caproate	83–84[a]	0.16
Isocaproate	83.2–84.0	0.16
Enanthate	72.8–73.2	0.19
Isoenanthate	69.0–70.0	0.20
Caprylate	83.0–83.5[a]	0.24
2-Ethylhexanoate	61.5–62.8	0.31
Pelargonate	80.7–81.3	0.29
Caprate	84.5–85.5[a]	0.34
Undecylate	88.0–88.5[c]	0.40
Laurate	87.0–87.5[a]	0.45
Tridecylate	89.0–89.5	0.50
Myristate	92–93[a]	0.55
Palmitate	95–96[a]	0.63
Stearate	99–100[a]	0.73
3-Hexenoate	71.5–72.0	0.11
2,4-Hexadienoate	139.5–140.2	0.072
10-Undecenoate	74.5–76.0	0.24
12-Hydroxystearate	111.5–112.5	0.10
dl-Lactate	157–158	0.0
Succinate	220.5–222.0	[d]
Tartrate	235–240	[d]
Malate	217–218	[d]
Benzoate	171–172	0.15
Phenylacetate	99–100	0.061
Hydrocinnamate	112.5–113.0	0.080
Cinnamate	178–179	0.093
Phenylglyoxylate	128–131	0.11
o-Toluate	105.5–107.0	0.23
Salicylate	167.0–167.5	0.21
p-Hydroxybenzoate	234–235	[d]
Syringate	197.6–199.6	0.0
Benzilate	182–184	0.028
p-Chlorobenzoate	151–153	0.28
p-Nitrobenzoate	197–199	0.058

[a] Listed in (*2*).
[b] Previously reported (*2*) as 69.5–70.0.
[c] Observed m.p. was considerably higher than value reported by Sugiyama, Harada, Mita, and Ueno (*7*): isovalerate, m.p. 77.5–78.0°; undecylate, m.p. 73–4°.
[d] Insoluble in developing solvent.

points for the additional *p*-phenylazophenacyl esters prepared in this investigation and the R_r values on silicic acid for all the *p*-phenylazophenacyl esters prepared in the authors' laboratory. In calculating the R_r values, any ratio of distances which was obtained from data where the reagent had moved less than approximately one third of the length of the column was usually discarded. In these cases the derivative band often had moved such a short distance down the column that appreciable errors in measurement were unavoidable. The width of the band at the top of the column before development was started also tended to cause some error in the measurements in such cases, as the distance traveled was measured from the top of the silicic acid column. By the same line of reasoning, the R_r values of those derivatives which were strongly adsorbed are subject to somewhat greater error than the values for the derivatives that moved through the column at more appreciable rates. The maximum errors usually observed however were of the order of a few per cent. The R_r values listed in Table I were all determined using the same preparation of adsorbent and the same solvent mixture, in order to avoid errors due to variations in these materials.

Experiments with a number of the derivatives in which the amount of derivative was varied severalfold while the amount of *p*-phenylazophenacyl bromide was maintained constant at 0.3 mg. indicated a small but steady increase in R_r value as the amount of derivative was increased. This effect of the amount of material on the R_r value is clearly shown by the fact that when 5 to 10-mg. quantities of the materials are used, the stearate derivative and the reagent are inseparable (*2*). On the other hand, with the smaller amounts of materials used in the present investigation the two are readily separable, and the stearate derivative has an R_r value of 0.73. Indeed, consideration of the data shown in Figure 1, where the R_r values for the derivatives of the straight-chain saturated fatty acids are plotted against the number of carbon atoms in the fatty acid, would indicate that good separations might be expected in the case of the derivatives of fatty acids somewhat larger even than stearic acid with the reagent band still passing on down the column first. In utilizing these R_r values in the identification of unknown acids, the amount of the derivative placed on the column is thus important and if the amount of material placed on the column is large, the R_r value is likely to be too great.

The data in Table I again demonstrate the expected small decrease in strength of adsorption (increase in R_r value) expected with increased chain branching in the acid portion of the molecule.

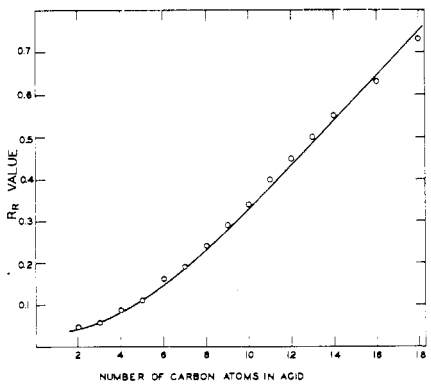

Figure 1. R_r values of *p*-phenylazophenacyl derivatives of normal saturated fatty acids

These differences are not sufficient to allow separation of such structural isomers even under the conditions of this investigation. Also in evidence in the data in Table I is the large increase in strength of adsorption (decrease in R_r value) expected (*3*) when the acid portion of the molecule contains a group, such as a hydroxyl group, capable of strong hydrogen-bonding effects with the adsorbent, or a group, such as an aromatic ring or an aliphatic carbon-carbon double bond, capable of strong electron donor-acceptor interactions with the adsorbent.

The paper partition chromatographic system using 2-phenoxyethanol–impregnated paper as the stationary phase and *n*-heptane saturated with 2-phenoxyethanol as the mobile phase, as described for the separation of steroids (*6*) and for the separation of homologs of low molecular weight 2,4-dinitrophenylhydrazones (*4*), is well adapted to the chromatography of *p*-phenylazophenacyl derivatives of acids. Table II presents the R_f values for the saturated fatty acid derivatives and also mobilities for the lower molecular weight straight-chain saturated fatty acid derivatives as well as certain unsaturated aliphatic and aromatic acid derivatives. In all the values listed in Table II, the spots were well defined and the distances traveled were determined by measuring to the lead edge of the spot.

The R_f values were determined by using a development time of approximately 3 hours, by which time the solvent front had moved to within 2 or 3 inches of the bottom of the paper. Samples of 50 to 100 γ gave small well-defined spots for the lower molecular weight derivatives, with the spots tending to become somewhat more elongated as the molecular weight increased. The R_f values as such are subject to changes in experimental conditions and are not sufficiently different in magnitude to permit direct use for identification purposes. It is necessary to use known substances for comparison on the paper. For such comparisons it is usually desirable to develop the chromatograms for much longer than the 3-hour maximum possible when R_f values are determined. The mobilities of the C_2 to C_7 straight-chain saturated fatty acid derivatives obtained by development of the chromatogram for an 8-hour period are listed in Table II. Any saturated fatty acid derivatives of larger molecular weight would be washed off the paper in this time. The mobilities of the relatively slow-moving aromatic acid derivatives and unsaturated aliphatic acid derivatives were also determined under the same conditions, and the values are listed in Table II for each derivative that developed as a relatively well formed spot. The benzoate, benzilate, cinnamate, salicylate, *p*-chlorobenzoate, and 12-hydroxystearate derivatives all gave streaks on development for 8 hours and apparently are not subject to investigation by this technique. The lactate, syringate, tartrate, malate, and *p*-hydroxybenzoate derivatives were not sufficiently soluble in benzene to allow them even to be placed on the paper by the method described.

This paper also presents an improved method for the preparation of the reagent, *p*-phenylazophenacyl bromide. Bromination of *p*-phenylazoacetophenone in this laboratory by the methods previously described (*2*, *5*, *7*) always resulted in the formation of large amounts of tars and a low-melting crude product which could not be purified

Table II. Chromatography of *p*-Phenylazophenacyl Esters on Paper[a]

	R_f	Mobility in 8 Hours, Cm. from Origin
Acetate	0.11	20.5
Propionate	0.16	24.5
Butyrate	0.20	29.5
Isobutyrate	0.24	...
Valerate	0.26	35.0
Isovalerate	0.27	...
2-Methylbutyrate	0.28	...
Caproate	0.27	40.0
Isocaproate	0.25	...
Enanthate	0.30	45.0
Isoenanthate	0.30	...
Caprylate	0.32	...
Pelargonate	0.35	...
Caprate	0.40	...
Undecylate	0.42	...
Laurate	0.45	...
Tridecylate	0.48	...
Myristate	0.49	...
Palmitate	Streak	...
Stearate	Streak	...
3-Hexenoate	...	37.0
2,4-Hexadienoate	...	21.0
o-Toluate	...	24.0
Hydrocinnamate	...	17.0
Phenylacetate	...	14.0
Phenylglyoxylate	...	13.0
p-Nitrobenzoate	...	0.0

[a] Whatman No. 7 paper. Results similar in nature were obtained using Schleicher and Schuell No. 598 paper.

satisfactorily by recrystallization. Purification of this product by chromatography on silicic acid usually resulted in the separation of a considerable amount of unreacted ketone and a low yield of the pure reagent. In the present work bromination of the ketone in the presence of a small amount of anhydrous aluminum chloride catalyst resulted in the formation of essentially no tars and gave crude *p*-phenylazophenacyl bromide in excellent yields, usually 85 to 90%, which was easily purified by recrystallization or by chromatographic techniques.

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Analysis of Hydrocarbon Blends by Gas-Liquid Partition Chromatography

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▶ Nine hydrocarbon blends of known composition were analyzed on a commercially available vapor-phase chromatographic instrument, and in each instance the analyses more closely approximated weight per cent than mole per cent. The result for each component was calculated from the ratio of its peak area to the total area of all peaks observed. These analyses were contradictory to some data in which results were obtained in mole per cent, but agree well with recent interpretations of peak areas used for quantitative analysis.

GAS-liquid partition chromatography (GLPC) offers an easy method of determining the composition of hydrocarbon mixtures. Some workers had assumed that the results of such analyses were derived as mole per cent (*4, 5*). Recent investigations contradict that assumption (*2, 3*). The following investigation was undertaken in an effort to determine whether the areas under the peaks were related to mole per cent or weight per cent.

Since the initiation of this work, an excellent article has been written on the interpretation of areas under the peaks when used for quantitative analysis (*1*). In water–ethyl alcohol–ether solutions and other systems, Browning and Watts found that the areas under the peaks are proportional to the weight per cent of the components, if the thermal conductivities of the components are similar. They suggested caution in the general use of areas for mole per cent calculations. Because of the importance of these results, analyses of hydrocarbon blends are presented here which seem to add to the general significance of the peak area-weight per cent relationship.

Seven hydrocarbon blends were prepared from components which are frequently encountered in the analytical work of the petroleum industry. In preparing each blend only components were selected which would appear as completely resolved peaks on the chromatogram. It was necessary that the area under these peaks be sufficiently great to be measured accurately by the method used. Representative hydrocarbon types—cyclic, aromatic, and paraffinic—were frequently mixed in a single blend.

Two Phillips hydrocarbon mixtures were selected with the same factors in mind.

The concentrations of components were assumed to be proportional to the area under the peaks in the determinations presented. The area under each peak was obtained by multiplying the peak height by the width of the peak at half the height. For most components the width could not be obtained to better than two significant figures, thus limiting the precision of the method The average deviation of duplicate analyses, which were run on a three-component blend, did not exceed 1.0%.

The percentage composition obtained from the areas was then compared to the known composition of the blends which were expressed as both weight per cent and mole per cent. The absolute magnitude of the difference between the experimentally obtained percentage and the known per cent composition for each component was termed the "individual deviation." For every blend the summation of these individual deviations has been called the "total deviation." The total deviation illustrates the discrepancy between the experimental analysis and the known per cent composition. These data on the prepared blends are shown in Table I. Results obtained from the analyses of Phillips hydrocarbon mixtures 33 and 41 are given in Table II.

APPARATUS

A Model 154, Perkin-Elmer vapor fractometer was used in these investigations. This instrument uses a thermistor-type thermal conductivity cell. The construction of this detector cell is such that the reference side is of the convection-diffusion design and the sensing side is of the direct flow design.

Helium was the carrier gas and the flow rates were approximately 40 cc. per minute. Liquid sample sizes were of the order of magnitude of 20 μl., but no attempt was made to control sample size accurately.

The stationary phase consisted of two columns in series, which gave a total column length of 4 meters. The first column contained didecyl phthalate and the second, ethyl hexyl sebacate as the liquid phases, both deposited on Celite, 40 to 80 mesh. Analyses were run at 50°, 100°, or 120° C., depending on the volatility of the blends.

The degree of purity of hydrocarbons used for blending was such that the total resolvable impurities in any one blend did not exceed 0.6% by weight.

PROCEDURE

Serum bottles of 20-cc. capacity with aluminum-sealed rubber stoppers were used for blending.

The bottle was evacuated by applying vacuum to a hypodermic needle inserted into the rubber diaphragm. The evacu-