FLUORESCENT DETECTION AND DETERMINATION OF ORGANIC COMPOUNDS

III. CARBONYL DERIVATIVE SEPARATION BY CHROMATOGRAPHY

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INTRODUCTION

Since carbonyl compounds are widespread in both natural products and air impurities, much research has been devoted to the aim of developing a rapid method of separation and identification of aromatic and aliphatic carbonyl compounds, both saturated and unsaturated, on the semimicro and micro scale.

The most often applied method for derivatization of carbonyl compounds is the formation of 2,4-dinitrophenyl (DNP) hydrazones. The chromatography of small amounts of these materials has been attempted on both untreated[1-3] and treated papers[4-7]. The latter method, that of reversed phase chromatography, has generally been more successful. R_F values have been reported for homologous members of aliphatic aldehyde 2,4-DNP-hydrazones from C_1 to C_6 on propylene glycol treated paper[7], and for the C_7 to C_{11} derivatives on vaseline-treated paper. Excellent separations were obtained using paraffin as a paper impregnant for DNP-hydrazones of lower aliphatic carbonyls[8] with the results expressed relative to formaldehyde DNP-hydrazone movement. Paper impregnated with sodium bisulfite was used to separate carbonyl compounds due to differential rates of reaction[9] followed by location with DNP-hydrazine spray.

Other carbonyl derivatives separated on paper include Girard P and T[10], benzene sulfohydroxamates[11] and cyanoaceto hydrazones[12].

The application of vaseline-treated paper to separate carbonyl derivatives has previously been reported from this laboratory[13]. The rapidity of the reaction of 2-diphenylacetyl-1,3-indandione-1-hydrazone[14] with carbonyls, the ease of purification, the high melting points of the derivatives (azines) and the ease and sensitivity of detection have led to this further study on the separation and detection of these compounds[15].

EXPERIMENTAL

A "Thomas-Kolb" jar for 10 in. × 10 in. paper was used for ascending and a round tank equipped with frame and trough was used for descending chromatography. The

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tanks were lined with Whatman No. 1 paper saturated with the developing solvent. Methanol–water (12:1) was used as a developing solvent for Whatman No. 1 paper impregnated with vaseline ("Blue Seal" Chesebrough-Ponds, Inc., New York, N.Y.).

Reagent grade solvents and single distilled water were used. The carbonyl compounds were reagent grade obtained from commercial sources and used without purification. The azines were prepared as previously reported[13] from 2-diphenyl-acetyl-1,3-indandione-1-hydrazone and were recrystallized from chloroform–methanol until a constant melting point compound was obtained (Kofler Hot Stage, corrected).

PROCEDURE

The papers were impregnated by rapidly drawing them through a glass tray containing 7% w/v of vaseline in petroleum ether (30–60°, fraction). Each solution was immediately used for two papers. Stock solutions of the azine and the hydrazone were prepared in chloroform at 1 mg/ml. Samples for chromatography of 2 μl were applied from 1 mg/ml solutions. The average time for ascending chromatography was 6 h for an 8 in. front, and for descending, 6 h for a 10 in. front. The spots were located by their fluorescence using a 3660 Å ultraviolet hand lamp (Mineralite Ultraviolet Products, Inc., San Gabriel, Calif.).

RESULTS AND DISCUSSION

Various combinations of polar and non polar solvents for development of chromatograms on untreated and vaseline-treated paper were used. In early experiments ethyl ether was used as a vaseline solvent but petroleum ether as a solvent resulted in more even treatment of the paper. Developing systems on untreated paper gave separation from the reagent, but were not successful in the resolution of homologous members. The R_F values of various azines using both treated and untreated papers are shown in Table I.

R_F values for methyl ketone azines from C_3 to C_{11} are shown in Table II. The

TABLE I

R_F VALUES OF CARBONYL AZINES ON TREATED AND
UNTREATED PAPER

Azine	R_F[a]	
	Vaseline-treated[b]	Untreated[c]
Acetaldehyde	0.74	0.53
Citraldehyde	0.58	0.57
Vanillin[d]	0.75	0.02
Veratraldehyde[d]	0.80	0.06
Piperonal[d]	0.67	0.14
Salicylaldehyde	0.71	0.21
Benzophenone	0.57	0.58

[a] 25° ± 2°.
[b] Descending, methanol–water (12:1), 6 h for 10 in. front.
[c] Descending, hexane saturated with water, 2 h for 10 in. front.
[d] Previously unreported derivatives.

TABLE II

R_F VALUES FOR METHYL KETONE AZINES ON TREATED PAPER:
ASCENDING DEVELOPMENT

Azine of methyl ketone	R_F[a]	R_F mixture
C_3	0.80	—
C_4	0.83	0.81
C_5	0.79	0.76
C_6	0.75	0.71
C_7	0.68	0.66
C_8	0.59	0.59
C_9	0.55	0.52
C_{10}	0.48	0.46
C_{11}	0.40	0.39

[a] Methanol–water (12:1), 6 h for an 8 in. front at 25°. Average of five determinations.

average deviations are $\pm$ 0.04 for C_3 to C_7 and $\pm$ 0.02 R_F units for C_8 to C_{11} for five separate vaseline-treated papers. The deviations between duplicates on the same sheet are less, indicating variations in the impregnation process between separate papers. Treatment with 14% w/v vaseline slowed development time, the front moving only 5 in. in 24 h for ascending chromatography. R_F values were lower and no better separation was obtained for the aliphatic series. A mixture of 1 ml of 1 mg/ml each of the C_3 to C_{11} azines was combined and evaporated *in vacuo* to 1 ml and an aliquot was chromatographed. The results correspond to those of the separate azines (Table II) but with only one spot at the C_3–C_5 position. The C_4 azine was mixed with an equal amount of each of the C_5 to C_9 azines and successfully separated as compared to the pure azines in an adjacent lane on the same treated paper (Table III).

By a sequential decrease in factors of 10 of the concentration of the samples chromatographed, the lower limit range of detection was found to be between 0.02–0.2 μg. 2-Hexanone and 2-decanone azines were used as representative compounds and the R_F values corresponded to $\pm$ 0.02 units to those in Table II.

A modification of the rapid derivatization technique of these authors[13] was attempted first at 10 μg/ml and then at 1 μg/ml for reaction detection. A drop of the hydrazone (at 1 mg/ml) and a drop of hydrochloric acid were added to the carbonyl in chloroform and the mixture was heated. This was then rapidly evaporated *in vacuo*

TABLE III

R_F VALUES OF AZINE PAIR MIXTURES:
ASCENDING DEVELOPMENT

Azine of methyl ketone	R_F[a]		R_F pure azine adjacent lane	
$C_4 + C_5$	0.83	0.79	C_4	0.83
$C_4 + C_6$	0.83	0.75	C_5	0.77
$C_4 + C_7$	0.82	0.67	C_6	0.75
$C_4 + C_8$	0.83	0.62	C_7	0.66
$C_4 + C_9$	0.83	0.56	C_8	0.61
			C_9	0.55

[a] Methanol–water (12:1), 25°.

to approximately 0.1 ml, and 1 μl was applied and chromatographed. At the 10 μg/ml level using benzaldehyde, benzophenone and 2-heptanone, only benzaldehyde gave the same R_F value as its previously prepared azine derivative (0.69 ascending, using methanol–water, 12:1). The two other carbonyls had R_F values of 0.79 and 0.80. A faint yellow spot was observed at 0.83 for the hydrazone treated in the same manner. This must be interpreted as being a reaction between a carbonyl present in the solvent and the reagent. The reaction indicated with benzaldehyde probably is due to the greater reactivity of the unhindered aromatic azine as compared to the carbonyl present in chloroform. This confirms an earlier report that the aromatic compounds are more reactive than the aliphatic[1]. At a carbonyl concentration of 1 μg/ml, R_F values were found only at 0.80 to 0.83 corresponding to an acetone impurity in the chloroform.

Chromatography of some carbonyl group and hydrazone reaction solutions was attempted in order to observe any interference in the detection of carbonyl compounds. Although no definite conclusions were reached, spots obtained from these solutions are different from those of carbonyl derivatives with regard to their resolution and their pale yellow fluorescence in ultraviolet light. However, these materials may be contaminated with aldehydes or ketones or they have reacted with the hydrazone to form other fluorescent products than azines.

TABLE IV

COMPARISON OF AZINE AND 2,4-DINITROPHENYLHYDRAZONE MELTING POINTS OF METHYL KETONES

Compound	M.p. of azine	Δ m.p. with increase in -CH_2-	M.p. of 2,4-DNPH	Δ m.p. with increase in -CH_2-
2-Propanone	226–227	—	126	—
2-Butanone	197.5–198	—29	116–117	— 9
2-Pentanone	166–167	—21	143–144	+27
2-Hexanone	135–136	—30	110	—34
2-Heptanone	147–148.5	+12	89	—21
2-Octanone	128.5–130.5	—18	58	—30
2-Nonanone	126.5–127.5	— 3	—	—
2-Decanone*	121–122	— 5	—	—
2-Undecanone	105–106	—16	—	—

* New compound

A comparison of the 2,4-DNP-hydrazone melting points[15] to those of the azine methyl ketones (Table IV) shows that the higher aliphatic azines (C_7 to C_{11}) have distinct melting points while the hydrazones are low melting or oils.

CONCLUSION

The 2-diphenylacetyl-1,3-indandione-1-azines must rank as one of the better derivatives for carbonyl determination. Their distinct R_F values, melting points and high order of formation may eventually lead to these derivatives replacing some of the presently more familiar non-fluorescent derivatives.

SUMMARY

R_F values of various aliphatic and aromatic fluorescent 2-diphenylacetyl-1,3-indandione-1-azines of carbonyl compounds were given for vaseline-treated and untreated paper. The melting points of the C_3–C_{11} methyl ketone azines were contrasted to the corresponding 2,4-dinitrophenylhydrazones and found in the C_7–C_{11} compounds to be more distinct.

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