

IMPROVED METHODS FOR THIN-LAYER CHROMATOGRAPHIC SEPARATION OF 2,4-DINITROPHENYLHYDRAZONES

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(Received December 7th, 1965)

INTRODUCTION

2,4-Dinitrophenylhydrazones (2,4-DNPH) have often been used for the identification of carbonyl compounds. As these derivatives are intensely colored, they are also well suited for quantitative determination of the carbonyl compounds by colorimetric analysis and for separation by chromatographic procedures. Many methods involving column, paper or thin-layer chromatography have already been published; the thin-layer chromatographic methods seem to be the most promising, as they are in general characterized by simplicity and speed, see for example refs. 1-6*.

The original aim of the present investigation was to develop a rapid and simple procedure for separation and identification of the lower aliphatic carbonyl compounds. However, many aromatic carbonyl compounds have also been included. Thin-layer chromatography is of importance for many practical as well as educational purposes and the methods discussed in this paper have been included in the course in organic identification at the Technical University of Denmark.

EXPERIMENTAL

Preparation of the derivatives

The 2,4-DNPH's were prepared by the "diglyme method"⁷, using pure, commercial carbonyl compounds as starting materials. The melting points were determined and agreed fairly well with the recorded values. In some cases, the nitrogen contents were also determined (by Mr. P. HANSEN, The Microanalytical Laboratory, University of Copenhagen). In most cases, the products prepared were very pure, but a few of the compounds contained isomers which, however, did not influence the investigation.

Adsorbents

The following adsorbents were used:

- (A) Silica Gel H or HF 254 (the latter containing a luminescent indicator), both obtained from Merck.
- (B) Alumina (Fluka, 5 % plaster of Paris).
- (C) Kieselguhr G (Merck).
- (D) Avicel-SF (technical grade microcrystalline cellulose from FMC Corp.).
- (E) Eastman Chromagram sheet (K301R2).

* Note added in proof: See also ref. 9.

Preparation of the plates

The microchromatoplates (40 × 76 mm) were coated by dipping them into a well stirred suspension of 75 g adsorbent in 350 ml of chloroform. In the case of (B), (C) and (D), 2 % methanol was added to the suspension.

Procedure

The plates were developed in a simple 250 ml beaker covered with a cork. When using adsorbent A, the mobile phase was petroleum ether (62–82° from Shell). With adsorbent A, it was also found suitable to employ reversed phase chromatography; this was effected by allowing a solution of 20 % by vol. of dimethylformamide in chloroform, to impregnate the plates for 15 min. The now fully impregnated plates were dried in air for 1 min and were then used immediately.

The alumina plates (B) were developed in the same way as (A) but the mobile phase in this case was a mixture of ethyl acetate and petroleum ether (62–82°) saturated with water.

For the kieselguhr (C) and the microcrystalline cellulose (D) adsorbents both mobile phases mentioned above were used. Similar experiments were carried out in the case of the Chromagram (E); this material was impregnated with dimethylformamide in chloroform as was adsorbent (A).

Detection of the spots

As 2,4-DNPH's are intensely colored, they can often be observed on the plates without further treatment. Smaller amounts are more easily detected by using an U.V.-lamp—especially when HF 254 (containing a luminescent indicator) is employed as the adsorbent.

In the present work, 2,4-DNPH's were detected on the chromatoplates by placing the latter in a beaker containing a small amount of a liquid lower, aliphatic amine. Propylamine, isopropylamine and diethylamine have all been used for this purpose, and cause an increase in color of the 2,4-DNPH's. The color developed disappears in a few seconds after the plates are removed from the beaker; the aliphatic aldehydes—especially methanal—may give rise to a permanent color change on treatment with the amine vapour.

RESULTS AND DISCUSSION

(A) Silica gel plates

Silica gel plates have been employed for separation of the lower aliphatic aldehydes and ketones, but the results obtained were not very satisfactory.

In the literature on the paper chromatographic separation of 2,4-DNPH's good results have been obtained by prior impregnation of the paper with polar compounds, as *e.g.* dimethylformamide⁸. Similar results were observed in the present work, using 10–20 ml of ethyl acetate per 100 ml of petroleum ether as the mobile phase. Using this concentration ethanal, propanal, and butanal were fully separated. The ketones moved a little faster than the aldehydes with the same number of carbon atoms, *e.g.* acetone faster than propanal and 2-butanone faster than butanal. A branched chain causes the compound to move more slowly (2-methylpropanal is slower than butanal).

Double bonds retard the movement, *e.g.* 3-buten-2-one moves more slowly than 2-butanone, and 2-butenal is slower than butanal.

Ethanal moves only a little faster than methanal, but separation can be obtained by repeating the chromatographic procedure once or twice. In this case, it will often be advantageous to allow the mobile phase to evaporate from the upper edge of the plate; the time necessary is 30–45 min. The last-mentioned method also allows the separation of benzaldehyde and ethanal (the former moving faster than the latter).

(B) Alumina

With this adsorbent, different amounts of ethyl acetate in petroleum ether were used for the chromatography of a mixture of four aldehydes (methanal, ethanal, propanal, and butanal); the results appear in Fig. 1. For further work a mobile phase consisting of 15 ml of ethyl acetate in 100 ml of petroleum ether was chosen, as this concentration of ethyl acetate also gave good results when used for the other lower, aliphatic carbonyl compounds. The time of development was 15 min.

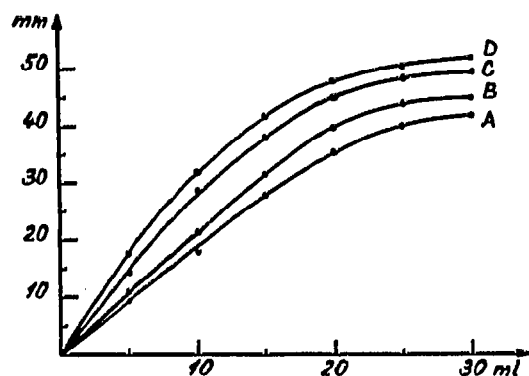


Fig. 1. Relation between the amount of ethyl acetate per 100 ml petroleum ether and the movement on alumina of some lower, aliphatic aldehydes. A = methanal; B = ethanal; C = propanal; D = butanal.

With the procedure used, it is not possible to characterize the movement of the individual compounds by R_F values as the chromatography continues after the front has reached the upper edge of the plate. Therefore, the data reported in this paper represent relative values, with the movement of butanal taken as the standard. In this way, good reproducibility of the results is obtained, as deviations from the normal due to variations in temperature, composition of mobile phase, layer thickness etc. are largely eliminated. The reproducibility is illustrated by the results from 15 plates, chromatographed one after the other, with 2 reference samples of the four aldehyde 2,4-DNPH's one on each edge of the plate. The relative distances and the standard deviations for the 30 runs are given in Table I.

In Table II, relative values are given for all the 2,4-DNPH's dealt with, and color reactions with the amines are recorded as well. The compounds were chromatographed on 3–6 different plates with a standard sample of the four aldehydes mentioned above on each edge.

It can be seen in Table II that in most cases the phenolic compounds do not

TABLE I
REPRODUCIBILITY IN CHROMATOGRAPHING 2,4-DNPH'S

2,4-DNPH	Average value $\left(\pm \sqrt{\frac{\Sigma \lambda^2}{n(n-1)}}\right)$	Standard deviation $\left(\pm \sqrt{\frac{\Sigma \lambda^2}{n-1}}\right)$
Methanal	0.629 \pm 0.004	\pm 0.019
Ethanal	0.729 \pm 0.004	\pm 0.023
Propanal	0.900 \pm 0.003	\pm 0.015
Butanal	1.00 \pm 0	—

TABLE II

R_x VALUES (RELATIVE TO BUTANAL) ON ALUMINA AND REACTIONS FOR 2,4-DINITROPHENYLHYDRAZONES

No.	2,4-DNPH	R_x (relative to butanal)	Colour with amine vapour
1	Methanal	0.63	brown
2	Ethanal	0.73	brown
3	Propanal	0.90	brown
4	Acetone	0.90	brown
5	Butanal	1.00	brown
6	2-Butanone	1.03	brown
7	2-Methylpropanal	1.03	brown
8	Pentanal	1.04	brown
9	2-Pentanone	1.09	brown
10	3-Pentanone	1.09	brown
11	3-Methyl-2-butanone	1.07	brown
12	2-Hexanone	1.12	brown
13	4-Methyl-2-pentanone	1.20	brown
14	Heptanal	1.11	brown
15	2-Heptanone	1.07	brown
16	3-Heptanone	1.19	brown
17	4-Heptanone	1.31	brown
18	5-Methyl-2-hexanone	1.08	brown
19	4,4-Dimethyl-2-pentanone	1.20	brown
20	Acrolein	0.86	red-brown
21	3-Buten-2-one	1.01	red-brown
22	2-Butenal	0.88	red-brown
23	5-Hexen-2-one	1.03	brown
24	Glyoxal	0.06	red
25	Butane-2,3-dione	0.70	red
26	Pentanedial	0.10	brown
27	3-Hydroxy-2-butanone	0.59	red
28	Cyclopentanone	1.00	brown
29	Cyclohexanone	1.03	brown
30	Benzaldehyde	0.87	red
31	2-Chlorobenzaldehyde	1.01	red
32	4-Chlorobenzaldehyde	0.86	red
33	2,4-Dichlorobenzaldehyde	1.04	red
34	4-Bromobenzaldehyde	0.86	red
35	2-Nitrobenzaldehyde	0.51	violet
36	3-Nitrobenzaldehyde	0.47	violet
37	4-Nitrobenzaldehyde	0.23	blue

(continued on p. 265)

TABLE II (continued)

No.	2,4-DNPH	R_F (relative to butanal)	Colour with amine vapour
38	Acetophenone	0.95	red
39	2,5-Dichloroacetophenone	1.12	red
40	3,4-Dichloroacetophenone	0.88	red
41	2-Nitroacetophenone	0.39	purple
42	3-Nitroacetophenone	0.38	red
43	4-Nitroacetophenone	0.38	purple
44	2-Methylbenzaldehyde	0.94	red
45	4-Methylbenzaldehyde	0.90	red
46	Propiophenone	1.05	purple
47	1-Phenyl-2-propanone	0.95	brown
48	Butyrophenone	1.10	red
49	4-Phenyl-2-butanone	0.92	brown
50	Isobutyrophenone	1.12	brown
51	4-Methylpropiophenone	1.08	purple
52	Cinnamaldehyde	0.80	red
53	Crotonophenone	1.11	red-brown
54	1,3-Diphenyl-2-propanone	1.04	red-brown
55	2-Hydroxybenzaldehyde	0.07	red
56	2-Methoxybenzaldehyde	0.83	red
57	3-Hydroxybenzaldehyde	0	red
58	3-Methoxybenzaldehyde	0.71	red
59	4-Hydroxybenzaldehyde	0	red
60	4-Methoxybenzaldehyde	0.61	red
61	2-Hydroxyacetophenone	0	red
62	4-Hydroxyacetophenone	0	red
63	4-Methoxyacetophenone	0.77	red
64	Benzoin	0.17	blue
65	2,4-Dihydroxybenzaldehyde	0	red
66	3,4-Dihydroxybenzaldehyde	0	red
67	2,6-Dihydroxyacetophenone	0	red
68	2,4,6-Trihydroxybenzaldehyde	0	red
69	3,4,5-Trihydroxyacetophenone	0	red
70	4-Dimethylaminobenzaldehyde	0.69	red

move. The only exception is 2-hydroxybenzaldehyde, which moves a little. Aliphatic hydroxy groups also retard the compounds, but to a much lesser degree than do phenolic systems. Nitro groups also retard the movement to some degree; methoxy groups have no great effect in this direction. The *ortho*-compounds in all cases move faster than the *meta*- and *para*-compounds. The *para*-compounds move more slowly than the *meta*-compounds; in some cases the *para*-compounds seem to be so insoluble in the mobile phase that poor chromatograms with pronounced tailing result. Nevertheless good chromatograms can be prepared by taking small samples as the color reaction allows detection of small amounts. The reagent, 2,4-dinitrophenylhydrazine, does not move.

(C), (D) and (E)—other methods

The kieselguhr (C) and the microcrystalline cellulose (D) were used in a manner similar to that described for silica gel and alumina, but the materials did not effect a separation sufficient for the present purposes.

The Chromagram (E) yielded separations comparable to those on silica gel

plates. When impregnated with dimethylformamide, the results obtained were nearly as good as those of the impregnated silica gel plates.

SUMMARY

A simple method for the separation of 2,4-dinitrophenylhydrazones of aldehydes and ketones has been developed. The separation is achieved on thin layers of alumina using 15 ml of ethyl acetate per 100 ml of petroleum ether (62–82°) saturated with water. Detection of the spots is carried out by a color reaction with either propylamine, isopropylamine or diethylamine. R_F values relative to butanal are given for 70 carbonyl compounds and the colors with the amines are presented.

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