THE SEPARATION OF 2,4-DINITROPHENYLHYDRAZONES BY THIN LAYER CHROMATOGRAPHY

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Since the work of STRAIN[1] in 1935, at least 70 papers on the chromatography of 2,4-dinitrophenylhydrazones (DNPHs) have been published. Paper chromatography on untreated[2–4], treated[5–12] and acetylated[13–15] papers, and adsorption[16–22] and partition[23,24,25] column chromatography have been used. In 1952, ONOE[26] reported the separation of DNPHs of *n*-aliphatic aldehydes up to C_{10} by a "chromatostrip" technique. More recently, the thin-layer of chromatography (TLC) of DNPHs of aromatic carbonyl compounds has been reported by DHONT AND DE ROOY[27] and DNPHs of simple aliphatic carbonyl compounds have been separated by ROSMUS AND DEYL[28], and NANO AND SANCIN[29]. DNPHs of hydroxycarbonyl compounds have been separated by ANET[30]. URBACH[31] reported the separation of mixtures of DNPHs of the homologous series of *n*-alkan-2-ones, alk-1-en-3-ones, alkanals, alk-2-enals, alka-2,4-dienals and alka-2,6-dienals on kieselguhr impregnated with phenoxyethanol, and aluminium oxide impregnated with silver nitrate. BADINGS[32] discussed the effects of atmospheric humidity on the separation of some of these compounds by adsorption chromatography on basic zinc carbonate, and by partition chromatography on basic zinc carbonate impregnated with Carbowax 400. LIBBEY AND DAY[33] separated DNPHs of *n*-alkanals and *n*-alkan-2-ones on silica gel impregnated with mineral oil, while SCHWARTZ AND PARKS[34] achieved similar separations by adsorption on magnesia-celite.

It is the purpose of this paper to describe the TLC technique for the separation of a wide range of DNPHs with particular application to those formed from the products of pyrolysis of cotton cellulose[35,36].

EXPERIMENTAL

Materials

Silica gel G and aluminium oxide G (Merck) were used as adsorbents. Mesoxaldehyde was prepared by the method of WOLFROM AND ARSENAULT[37] and hydroxypyruvaldehyde by the method of EVANS AND WARING[38].

Preparation of DNPHs

Most DNPHs were prepared from the parent carbonyl compound by the method of NEUBERG, GRAUER AND PISHA[39]. Derivative 41 (Table I) was prepared from the di-sodium salt of ketomalonic acid. To prevent osazone formation, derivatives 27,

32, 33, 35 and 36 were prepared in the absence of acid by the method of REICH AND SAMUELS[40]. Derivative 34 was prepared by stirring a solution of the carbonyl compound (1 mole) and 2,4-dinitrophenylhydrazine (0.9 mole) in dimethylsulphoxide for 10 h at room temperature; this method could also be used for the preparation of other DNPHs. Derivatives 15, 16 and 17 were prepared by stirring a solution of the carbonyl compound (1 mole) and 2,4-dinitrophenylhydrazine (0.9 mole) in ethanol[41] at room temperature for 5 h. DNPHs of hydroxycarbonyl compounds were acetylated with acetic anhydride–pyridine (1:2 v/v). In agreement with REICH AND SAMUELS[40], it was found that diacetoxydihydroxyacetone DNPH could not be obtained in a crystalline form.*

All DNPHs were recrystallised to constant melting point. Any slight impurities were removed by preparative TLC. The infrared spectra (as Nujol mull or potassium bromide disc), visible spectra (alcoholic sodium hydroxide), and ultraviolet spectra (chloroform) of most DNPHs were measured.

Preparation of plates

Glass plates, 20 × 20 cm, 20 × 10 cm and 20 × 5 cm were spread with a layer 0.25 mm thick of silica gel G (30 g with 60 ml water) or aluminium oxide G (30 g with 60 ml water) by means of a Desaga spreader; they were dried for 30 min at room temperature and then activated by heating at 110° for 2 h, or dried without heating.

Application of sample and development

The samples were applied as a line of small spots (0.5–1.0 mm diameter) 1 cm from the edge of the plate by means of drawn-out melting point tubes. Compounds readily soluble in benzene were applied as dilute (0.01–0.1 %) solutions in this solvent, otherwise they were first dissolved in a small quantity of ethyl acetate or tetrahydrofuran and benzene was added. For preparative TLC the sample was applied as a line of closely spaced spots, or sometimes as a streak by means of a fine paint brush.

The atmosphere inside a Shandon chromatographic tank (fitted with filter paper on two sides to ensure saturation of the air) was first equilibrated for 1 h with 100 ml of the solvent to be used for development. Development was started and after the solvent had ascended to a height of 10–15 cm the plate was removed from the tank and dried in a stream of warm air.

Modified procedure for use with derivatives sparingly soluble in volatile solvents

When the highly insoluble mesoxaldehyde tris-DNPH was spotted onto plates from solutions in volatile solvents, it was often "precipitated" on the adsorbent, and remained at the origin or streaked badly when the plate was developed. This difficulty was overcome by application from a dilute solution in nitrobenzene. The nitrobenzene was removed by repeated elution with light petroleum (b.p. 80–100°) in which mesoxaldehyde tris-DNPH and most other DNPHs were immobile. The top 5 cm of adsorbent containing the accumulated nitrobenzene was removed before development. Several easily soluble DNPHs were used to confirm that the mobilities of DNPHs applied by this method did not differ from those of DNPHs applied by the normal method from volatile solvents on the same plate.

* A crystalline form (m.p. 77°) has since been obtained by prolonged cold storage.

Spray reagent

The colours obtained after spraying with ethanolamine were different for different classes of DNPHs (Table I).

TABLE I

DNPHs EXAMINED BY TLC AND COLOURS GIVEN WHEN SPRAYED WITH ETHANOLAMINE

	DNPH of	*Colour with ethanolamine**		*DNPH of*	*Colour with ethanolamine**
1	Formaldehyde	B	22	Diacetyl (bis)	P
2	Acetaldehyde	B	23	Mesoxaldehyde (1,2-bis)	P
3	Propionaldehyde	B	24	Mesoxaldehyde (tris)	P
4	*n*-Butyraldehyde	B	25	Hydroxypyruvaldehyde (bis)	P
5	*n*-Valeraldehyde	B	26	Erythrose (bis)	P
6	Acrolein	RB	27	Glucose (mono)	B
7	Crotonaldehyde	RB	28	Glucose (bis)	P
8	Acetone	B	29	3-Deoxy-D-*erythro*-hexosone (bis)	P
9	Methyl ethyl ketone	B	30	5-(Hydroxymethyl)-furfural	M
10	Methyl *n*-propyl ketone	B	31	Hydroxymethyl 2-furyl ketone	M
11	Diethyl ketone	B	32	Glycolaldehyde	B
12	Furfural	M	33	Acetol	B
13	5-Methylfurfural	M	34	Acetoin	B
14	Furil (bis)	V	35	Glyceraldehyde	B
15	Glyoxal (mono)	OR	36	Dihydroxyacetone	B
16	Pyruvaldehyde (mono)	DR	37	Pyruvic acid	B
17	Diacetyl (mono)	DR	38	Glyoxylic acid	RB
18	Glutaraldehyde (bis)	B	39	Laevulinic acid	B
19	Bis-(5-methylenefurfural)-ether	M	40	α-Oxoglutaric acid	B
20	Glyoxal (bis)	P	41	Ketomalonic acid	B
21	Pyruvaldehyde (bis)	P			

* Colour code: B = brown; DR = dark red; M = mauve; OR = orange red; P = purple to blue; RB = reddish brown; V = violet.

Reactions on plates

(*i*) *Acetylation.* DNPHs of hydroxycarbonyl compounds were acetylated directly on the thin-layer plate by spotting the acetylating agent (acetic anhydride–pyridine, 2:3 v/v) directly over the DNPHs and heating the plate at 80° for 15 min ("single treatment"), when acetylation occurred and excess reagent vaporised. Sometimes, after "single treatment", the sample spot was again treated with acetylating agent and the plate was heated for a further 20 min ("double treatment"). The mobilities of the acetylated derivatives could then be compared with those of standard compounds.

(*ii*) *Treatment with acid.* The reactions that occur when DNPHs of hydroxycarbonyl compounds and mono-DNPHs of dicarbonyl compounds were treated with concentrated hydrochloric acid directly on the thin-layer plate were studied. The technique was similar to that described for acetylation.

Care was taken to ensure that the diameter of the resultant spot did not exceed about 2 mm during both acetylation and treatment with acid.

Methods for examining complex mixtures of DNPHs

Useful information on the number and nature of components of complex mixtures of DNPHs derived from the products of pyrolysis of cotton cellulose was obtained by normal, multiple, two-dimensional, and continuous horizontal development, and by spraying the developed plates with ethanolamine. Sometimes mixtures were chromatographed on plates on which the adsorbents had been shaped as a sector. The components of any mixtures were identified in the following way. The mixture was preparatively applied to 20–40 (20 × 20 cm) plates. Double development gave a partial separation. Each plate was divided into 6–8 zones, each containing several components. Corresponding zones from every plate were collected and the DNPHs were extracted with solvent. After concentration of the solutions and examination by normal TLC further separation was done by the use of one or two plates for each collective zone, yielding sufficient amounts of each component for comparative TLC, reactions on plates, and ultraviolet and visible spectra. Sometimes sufficient material was separated for measurement of the melting point and infrared spectrum. Ketoacid DNPHs were examined separately. They were separated from other DNPHs by extraction from solution in benzene or ethyl acetate with 1 *N* sodium bicarbonate. After acidification the ketoacid DNPHs were extracted from the aqueous phase, and examined by normal TLC.

RESULTS AND DISCUSSION

Standard compounds

Of the many solvents tried those found to be suitable for the separation of all the DNPHs examined were:

(1) 80–100° light petroleum–diethyl ether (70:30) (P/E).

(2) Benzene–tetrahydrofuran (98:2; 96:4; 95:5; 93:7; 80:20; 70:30) B/THF).

(3) Benzene–tetrahydrofuran–glacial acetic acid (60:36:4) (B/THF/A).

It is important to have a small starting spot. Application of samples from solution in benzene gave much smaller spots than application from solvents such as acetone, ether, and tetrahydrofuran. Drawn-out melting point tubes also gave smaller spots than a commercial pipette. During development the amount of diffusion of the spots depended on the type of DNPH and the developing solvent. Compounds 1–17 exhibited the greatest diffusion in both the P/E and B/THF solvents, the spot diameters ranging from 2.5 to 4.0 mm. The spot diameters of compounds 18–41 in the B/THF solvents were about 2–3 mm. Single solvents are generally preferable to mixed solvents, but although the DNPHs of hydroxycarbonyl compounds could be separated on silica gel with diethyl ether as developing solvent, the B/THF solvents were preferred because there was less diffusion.

Several authors[4,7,9,18,28,43–49] have reported the separation of *cis*- and *trans*-isomers of DNPHs during chromatography, whereas others[17,20] have reported the "double zoning" phenomenon which occurs on adsorption columns. When examined by TLC the DNPHs of furfural, 5-methylfurfural, 5-(hydroxymethyl)-furfural, glycoaldehyde, and acetol sometimes gave two spots derived from the *cis*- and *trans*-isomers. The only bis-DNPH to give two spots was that from furil. For every DNPH the predominant spot had the lower mobility, and after development the distance between the isomers of any one DNPH was usually less than 1.5 cm.

DNPHs of simple aliphatic aldehydes and ketones, three furan carbonyl compounds, and mono-DNPHs of dicarbonyl compounds were best separated by the P/E solvent. Fig. 1 shows the mobilities of these DNPHs (compounds 1–17 of Table I) on both activated silica gel and aluminium oxide plates, and the reversals of order

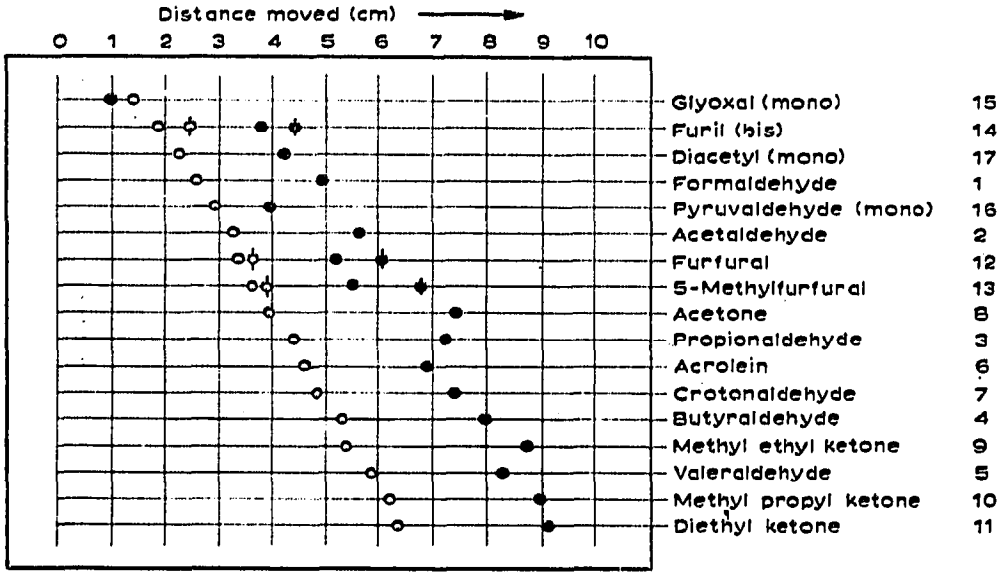

Fig. 1. Separation of 2,4-dinitrophenylhydrazones on activated silica gel (○) and aluminium oxide (●). Distance moved by solvent [80–100° light petroleum–diethyl ether (70:30)] = 15 cm. Positions of fast moving isomers shown by —○— and —●—.

that occur on changing the adsorbent. In the P/E solvent, with the exception of glyoxal mono-DNPH, whose strong adsorption is due to the remaining aldehyde group, all the DNPHs moved faster on aluminium oxide than on silica gel; the same was generally true in the B/THF solvents. Most of compounds 18–41 were immobile in the P/E solvent; those that were not did not move more than 0.7 cm when the solvent front travelled 13 cm.

Fig. 2 shows the separation of some mono-, bis-, and tris-DNPHs on silica gel by B/THF (93:7). The separation of the bis-DNPHs of glyoxal, pyruvaldehyde and diacetyl could be improved by using a 98:2 mixture of B/THF.

Fig. 3 shows the separation of DNPHs of hydroxycarbonyl compounds, together with some other DNPHs, on silica gel by B/THF (80:20). The separation of the slower moving compounds was improved by increasing the amounts of tetrahydrofuran in the solvent, but some reversals of order occurred (Fig. 4). The strong adsorption of these DNPHs is due largely to the hydroxyl groups. It is interesting, however, that as the amount of tetrahydrofuran is increased, the slightly soluble bis-DNPHs with 1, 2, 3, and 4 hydroxyl groups become less strongly adsorbed than the easily soluble mono-DNPHs with 1 and 2 hydroxyl groups. The positions of the minor isomers of glycolaldehyde, acetol, and 5-(hydroxymethyl)-furfural have been omitted from Fig. 4 for clarity. Fig. 5 shows the separation of the DNPHs of six furan carbonyl compounds on silica gel by B/THF (95:5).

The separation of geometrical isomers of DNPHs of α-ketoacids by paper chromatography has been reported[42–48]; BUSH AND HOCKADAY[4] separated the *cis*-

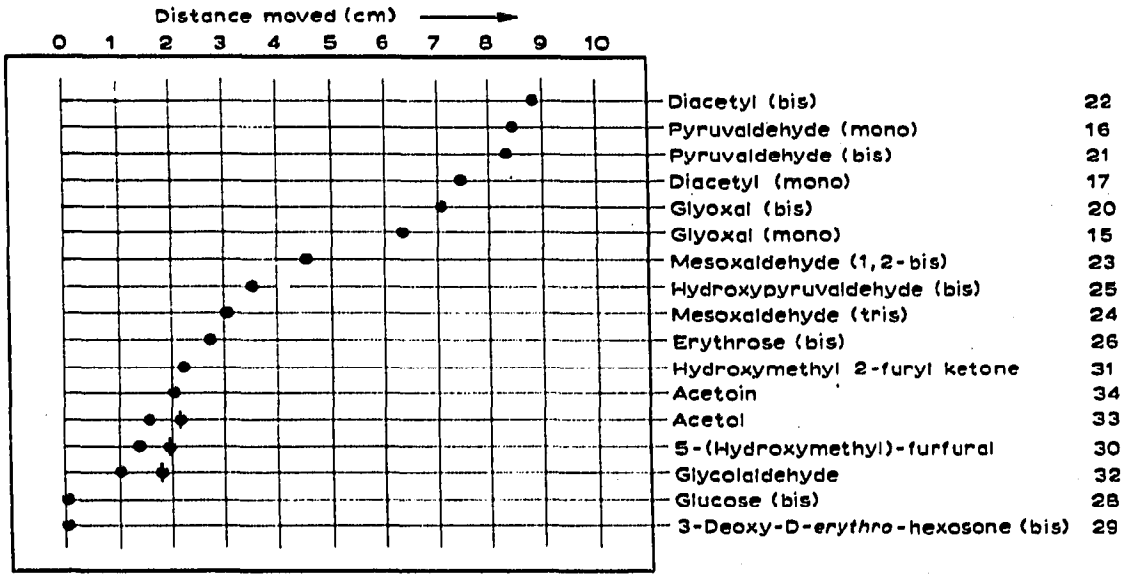

Fig. 2. Separation of 2,4-dinitrophenylhydrazones on silica gel. Distance moved by solvent [benzene–tetrahydrofuran (93:7)] = 15 cm.

and *trans*-isomers of DNPHs of some α-ketoacids by paper chromatography under alkaline conditions, and showed that under acidic conditions only one spot was usually seen. They suggested that the differences in behaviour might be due to differences in intra-molecular hydrogen bonding in acidic and alkaline solvents. The five α-ketoacid DNPHs studied in this work were immobile in the P/E solvent and in B/THF solvents containing less than 20% tetrahydrofuran. However, they were separated as single spots on silica gel by B/THF/A (60:36:4) (Fig. 6).

Although most of derivatives 18–41 could be separated on aluminium oxide, the resolution on silica gel was better. The effect of functional groups on the adsorption affinity for silica gel and aluminium oxide is well shown by a comparison of

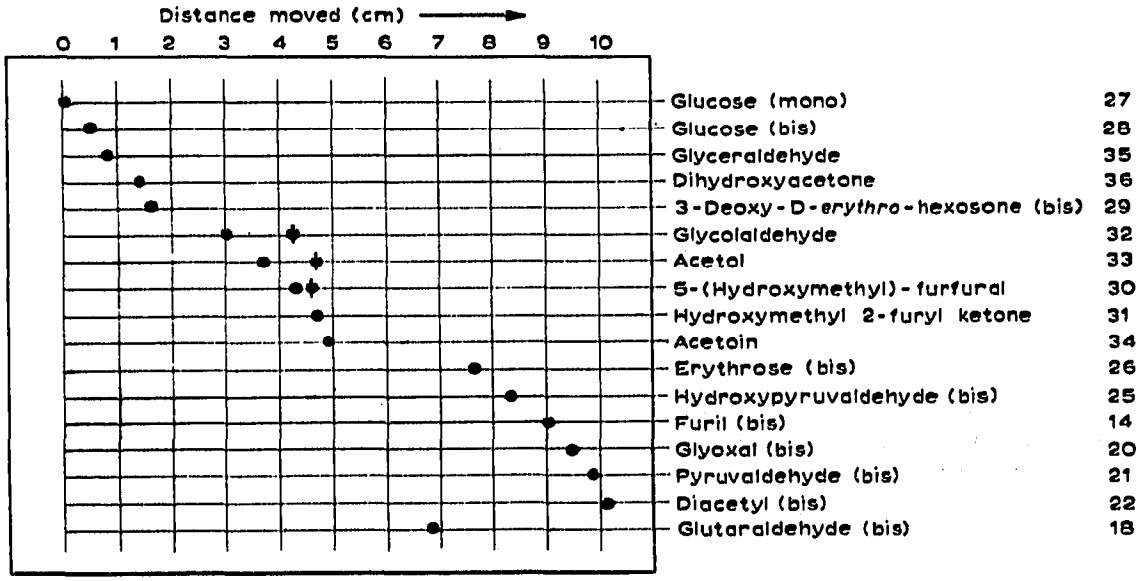

Fig. 3. Separation of 2,4-dinitrophenylhydrazones of hydroxycarbonyl compounds on silica gel. Distance moved by solvent [benzene–tetrahydrofuran (80:20)] = 15 cm.

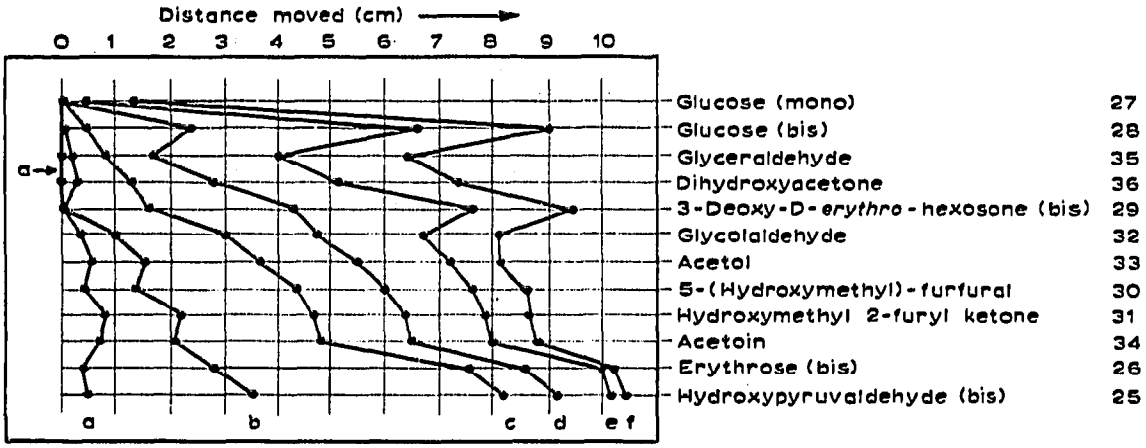

Fig. 4. Comparison of mobilities of 2,4-dinitrophenylhydrazones of hydroxycarbonyl compounds on silica gel, when distance moved by solvent = 15 cm. Solvents are benzene–tetrahydrofuran in the ratios: (a) 98:2; (b) 93:7; (c) 80:20; (d) 70:30; (e) 50:50; (f) 30:70.

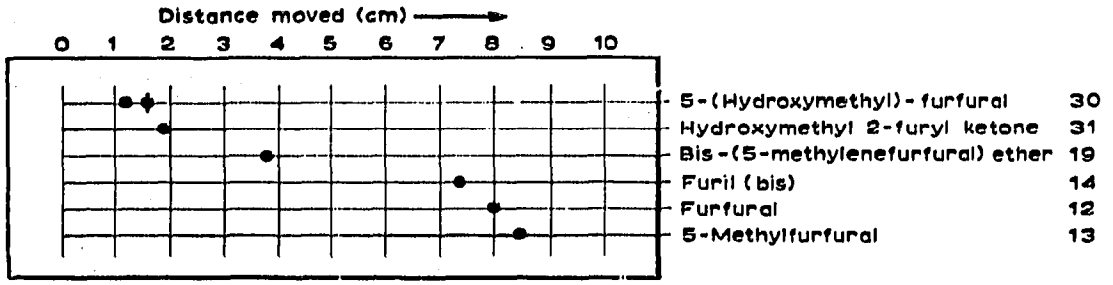

Fig. 5. Separation of 2,4-dinitrophenylhydrazones of furan carbonyl compounds on silica gel. Distance moved by solvent [benzene–tetrahydrofuran (95:5)] = 15 cm.

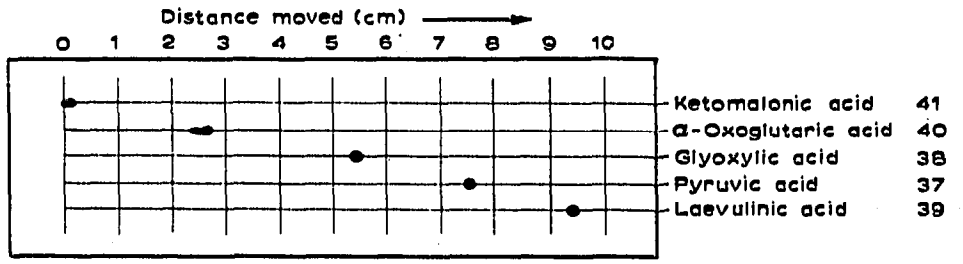

Fig. 6. Separation of 2,4-dinitrophenylhydrazones of α-ketoacids on silica gel. Distance moved by solvent [benzene–tetrahydrofuran–acetic acid (60:36:4)] = 15 cm.

the mobilities of a series of DNPHs of two-carbon-atom molecules, $(NO_2)_2 \cdot C_6H_3 \cdot NH \cdot N \cdot CH \cdot R$ in B/THF (98:2 and 93:7) where, in order of decreasing adsorption affinity, R is COOH, CH_2OH, CHO, or CH_3.

Since DNPHs are coloured no spray reagent is needed to detect them. However, transient colours, characteristic of different classes of DNPHs, are observed when chromatograms are sprayed with ethanolic sodium or potassium hydroxide. SCHWARTZ[25] has reported that the colours associated with DNPHs in alkaline media were observed when DNPHs were chromatographed on columns of Celite treated with ethanolamine. Ethanolamine was found to be a useful spray reagent for DNPHs separated by TLC. The colours listed in Table I were strong and distinctive and lasted for ½ to 1 hour.

Confirmatory techniques – reactions on plates

The preparation of derivatives directly on thin-layer adsorbents and comparison of their mobilities with those of authentic specimens has been used to confirm the identity of compounds[49–53]. Quantitative acetylation of DNPHs of hydroxycarbonyl compounds (derivatives 25–36) was not usually achieved with "single treatment" with acetic anhydride–pyridine, but was usually effected with "double treatment". Those compounds whose isomers normally separated as described earlier only gave one spot when acetylated. The instability of the minor isomer of glycolaldehyde DNPH was shown by the following experiment. Glycolaldehyde DNPH was applied to one corner of a plate (Fig. 7) which was developed in B/THF (80:20) until a good

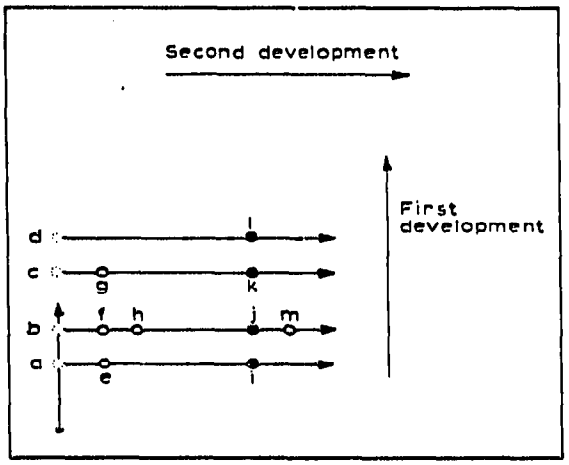

Fig. 7. Separation of compounds formed by treating glycolaldehyde 2,4-dinitrophenylhydrazone with acetic anhydride–pyridine directly on a silica gel plate.

separation of the isomers, a and b was achieved. Glycolaldehyde DNPH was spotted at position c and acetoxyglycolaldehyde DNPH at position d. The spots at a, b and c were then acetylated once and the plate developed in B/THF (96:4) at right angles to the first development. a and c gave i and k which were the same acetylated DNPH l, and small amounts of unacetylated DNPHs e and g. The minor isomer b gave mainly j, which has the same mobility as acetoxyglycolaldehyde DNPH l, and f, and h which are the isomers a and b while m is presumably the acetylated derivative of isomer b; f, h and m were present in trace amounts and could only be detected

easily by spraying the plate with ethanolamine. When sprayed with ethanolamine, acetylated DNPHs gave the same colours as those given by the corresponding unacetylated DNPHs.

Acetylation of DNPHs containing more than two hydroxyl groups was more difficult. Five spots, all giving purple colours with ethanolamine, were separated by B/THF (90:10) when glucose bis-DNPH was acetylated on a silica gel plate. The fastest and slowest moving spots were the fully acetylated and unacetylated DNPHs respectively. The other three spots arose from partial acetylation; on further acetylation and development at right angles to the first development, their mobilities were changed to that of the fully acetylated DNPH. A similar result was observed when 3-deoxy-D-*erythro*-hexosone bis-DNPH was acetylated on silica gel, four spots being separated. Glucose mono-DNPH was the only compound that could not be acetylated to an appreciable extent.

Acetylation can often be used to detect hydroxyl groups in a complex mixture of DNPHs. If a single spot of a mixture is developed in the normal way and the separated components are then treated with the acetylating agent *in situ*, subsequent development at right angles in the same solvent will reveal acetylated derivatives. They will lie other than on the diagonal line that would normally result from two-dimensional development in the same solvent. For identification, the mobilities of the acetylated spots can be compared with those of standard acetates spotted alongside the straight line of spots resulting from the first development. However, it is best to isolate the hydroxycarbonyl DNPH by preparative TLC and carry out the reactions with single compounds. Although the acetates of hydroxypyruvaldehyde, 5-(hydroxymethyl)-furfural, and acetol DNPHs are not well separated in B/THF (96:4) (Fig. 8),

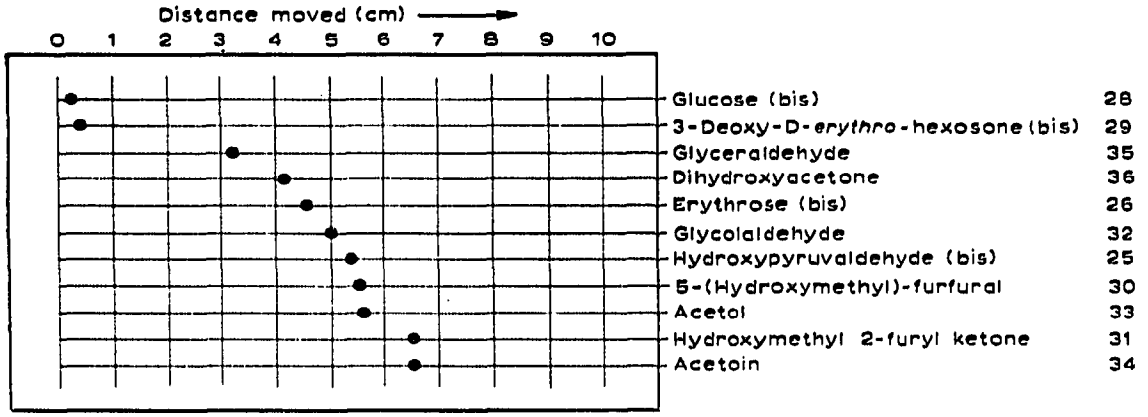

Fig. 8. Separation of acetylated 2,4-dinitrophenylhydrazones on silica gel. Distance moved by solvent [benzene–tetrahydrofuran (96:4)] = 12 cm.

they could be distinguished by the colours that they gave with ethanolamine; a similar effect was observed with the acetates of hydroxymethyl 2-furyl ketone and acetoin DNPHs. The unacetylated derivatives were, however, well separated by B/THF (80:20).

During exploratory work it was seen that, when some mono-DNPHs of α-hydroxycarbonyl compounds were acetylated with acetyl chloride, osazone formation occurred, presumably due to the presence of hydrogen chloride. The reactions of concentrated hydrochloric acid with the mono-DNPHs of glycolaldehyde, acetol,

TABLE II

PRODUCTS FROM TREATING DNPHs OF HYDROXYCARBONYL COMPOUNDS WITH CONCENTRATED HYDROCHLORIC ACID ON SILICA GEL

	Mono-DNPH of	*DNPHs formed by acid treatment*
32	Glycolaldehyde	Glyoxal (mono and bis)
33	Acetol	Pyruvaldehyde (mono and bis)
35	Glyceraldehyde	Pyruvaldehyde (mono and bis) and hydroxypyruvaldehyde (bis)
36	Dihydroxyacetone	Pyruvaldehyde (mono and bis) and hydroxypyruvaldehyde (bis)
34	Acetoin	Diacetyl (mono and bis)
27	Glucose	Glucose (bis) with difficulty

glyceraldehyde, dihydroxyacetone, acetoin, and glucose adsorbed on silica gel were therefore investigated. The technique was similar to that described for acetylation; the plates were developed in B/THF (98:2) and the products are described in Table II.

In some experiments small amounts of unreacted hydroxycarbonyl DNPH remained. Usually after "double treatment", mono-DNPHs of dicarbonyl compounds were absent, and the amount of hydroxypyruvaldehyde bis-DNPH formed from compounds 35 and 36 increased. When hydroxypyruvaldehyde bis-DNPH was formed, another spot intermediate in mobility between pyruvaldehyde bis-DNPH and hydroxypyruvaldehyde bis-DNPH was seen. This was at first thought to be hydroxypyruvaldehyde mono-DNPH since it gave a red colour with ethanolamine similar to that given by other mono-DNPHs of dicarbonyl compounds. However, it could not be acetylated and could not be converted to the bis-DNPH on treatment with acid. In addition to the products mentioned there usually appeared another, which moved faster and gave a brown colour with ethanolamine. This was thought to be derived from 2,4-dinitrophenylhydrazine. The mono-DNPHs of glyoxal, pyruvaldehyde, and diacetyl yielded bis-DNPHs by similar treatment with acid.

Complex mixtures of DNPHs

When complex mixtures of DNPHs were examined by normal TLC it was often necessary to overload the adsorbent, in order to detect the minor components. Resolution was, however, often impaired by diffusion of those DNPHs present in large amounts, but could be improved by chromatographing the mixture on a "sector shaped" adsorbent as shown in Fig. 9. Continuous horizontal TLC was sometimes used to separate spots that were not completely separated by normal TLC. During normal TLC DNPHs in admixture often moved faster, but occasionally slower than the single DNPH, even when the concentrations of the DNPHs were approximately the same singly and in admixture. Displacement analysis might account for the former phenomenon, but both effects could be explained by changes in adsorption isotherms[54] on mixing the DNPHs. Comparison of mobilities of DNPHs in such mixtures with those of known DNPHs is not a reliable method of identification. For these reasons, preparative TLC and zone isolation were used to separate components before identification by comparative TLC, reactions on plates, and spectroscopic methods. Fig. 10 shows a typical preliminary separation of a complex mixture of DNPHs. Details of the analysis of DNPHs derived from the products of pyrolysis of cotton cellulose by these methods have been described elsewhere[36].

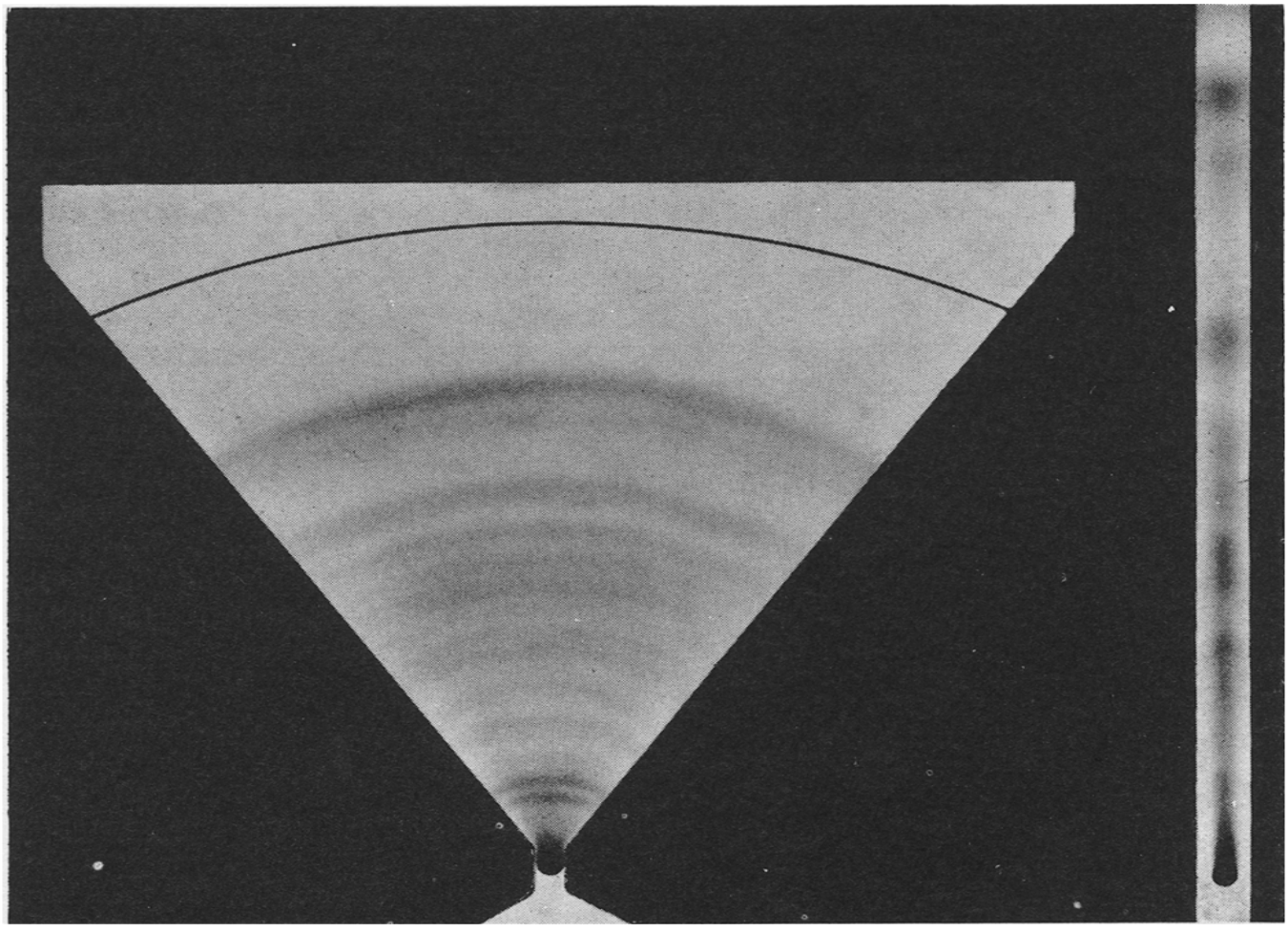

Fig. 9. Separation of an overloaded complex mixture of 2,4-dinitrophenylhydrazones by "sector shaped" and normal development. Solvent = benzene–tetrahydrofuran (90:10).

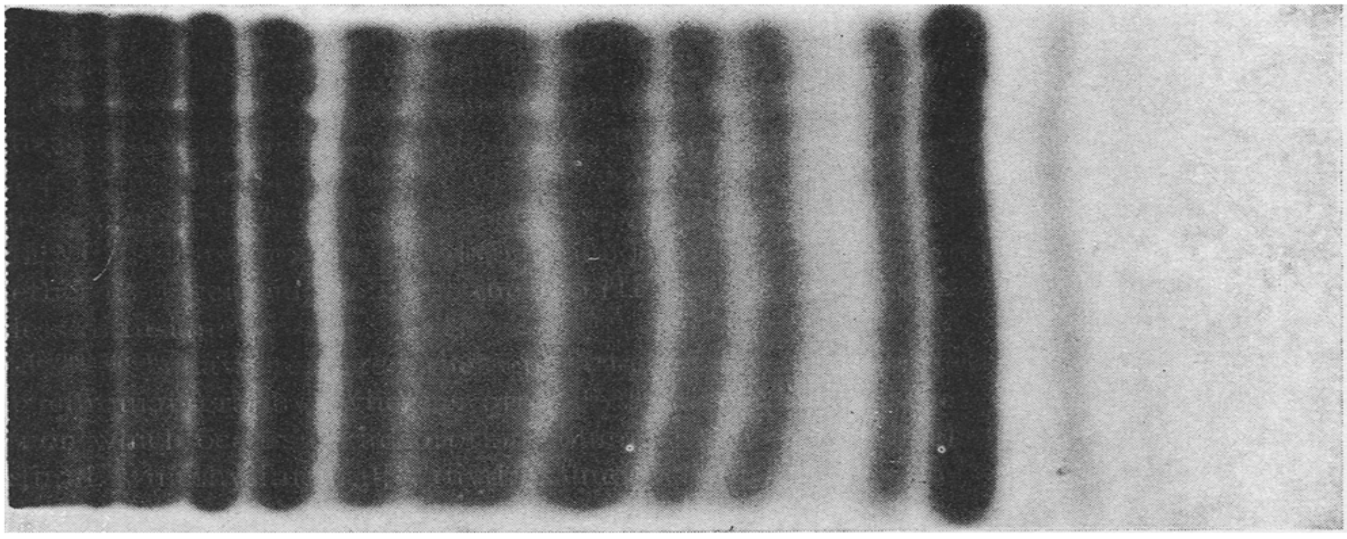

Fig. 10. Preliminary separation of a complex mixture of 2,4-dinitrophenylhydrazones derived from the products of pyrolysis of cotton cellulose, on silica gel. First development in 80–100° light petroleum–diethyl ether (70:30) for 12 cm; second development in benzene–tetrahydrofuran (90:10) to top of plate.

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SUMMARY

The separation of 41 mono-, bis-, and tris-2,4-dinitrophenylhydrazones derived from simple saturated aldehydes and ketones, unsaturated aldehydes, di-, tri-, and hydroxycarbonyl compounds, and α-ketoacids by thin-layer chromatography is described. The *cis*- and *trans*-isomers of some of these compounds have been separated. The characteristic colours produced by different classes of 2,4-dinitrophenylhydrazones sprayed with ethanolamine are more lasting and more distinctive than those with alcoholic sodium or potassium hydroxide. A method for the chromatography of highly insoluble 2,4-dinitrophenylhydrazones is described. 2,4-Dinitrophenylhydrazones have been treated with acetylating agents, and with concentrated hydrochloric acid, directly on the thin-layer plates, and the chromatographic behaviour of the products has been studied. Methods for separating and identifying the components of complex mixtures of 2,4-dinitrophenylhydrazones are discussed.

REFERENCES

1 H. H. STRAIN, *J. Am. Chem. Soc.*, 57 (1935) 758.
2 R. G. RICE, G. J. KELLER AND J. G. KIRCHNER, *Anal. Chem.*, 23 (1951) 194.
3 W. J. SCHMITT, E. J. MORICONI AND W. F. O'CONNOR, *Anal. Chem.*, 28 (1956) 249.
4 I. E. BUSH AND T. D. R. HOCKADAY, *J. Chromatog.*, 8 (1962) 433.
5 J. G. KIRCHNER AND G. J. KELLER, *J. Am. Chem. Soc.*, 72 (1950) 1867.
6 R. B. SELIGMAN AND M. D. EDWARDS, *Chem. Ind. (London)*, (1955) 1406.
7 D. F. MEIGH, *Chem. Ind.*, (1956) 986.
8 W. S. LYNN JR., L. A. STEELE AND Z. STAPLE, *Anal. Chem. (London)*, 28 (1956) 132.
9 D. A. BUYSKE, L. H. OWEN, P. WILDER, JR., AND M. E. HOBBS, *Anal. Chem.*, 28 (1956) 910.
10 R. ELLIS, A. M. GADDIS AND G. T. CURRIE, *Anal. Chem.*, 30 (1958) 475.
11 E. SUNDT AND M. WINTER, *Anal. Chem.*, 30 (1958) 1620.
12 A. M. ASATOOR, *J. Chromatog.*, 7 (1962) 415.
13 H. S. BURTON, *Chem. Ind. (London)*, (1954) 576.
14 T. ZAIKOV, *Zh. Analit. Khim.*, 15 (1960) 639.
15 D. A. FORSS AND E. H. RAMSHAW, *J. Chromatog.*, 10 (1963) 268.
16 J. D. ROBERTS AND C. GREEN, *Anal. Chem.*, 18 (1946) 335.
17 J. W. WHITE, JR., *Anal. Chem.*, 20 (1948) 726.
18 B. E. GORDON, F. WOPAT, JR., H. D. BURNHAM AND L. C. JONES, JR., *Anal. Chem.*, 23 (1951) 1754.
19 E. W. MALMBERG, *J. Am. Chem. Soc.*, 76 (1954) 980.
20 M. L. WOLFROM AND G. P. ARSENAULT, *Anal. Chem.*, 32 (1960) 693.
21 D. A. FORSS, A. E. DUNSTONE AND W. STARK, *Australian J. Chem.*, 13 (1960) 584.
22 D. P. SCHWARTZ, O. W. PARKS AND M. KEENEY, *Anal. Chem.*, 34 (1962) 669.
23 K. J. MONTY, *Anal. Chem.*, 30 (1958) 1350.
24 E. A. CORBIN, D. P. SCHWARTZ AND M. KEENEY, *J. Chromatog.*, 3 (1960) 322.
25 D. P. SCHWARTZ, *J. Chromatog.*, 9 (1962) 180.
26 K. ONOE, *J. Chem. Soc. Japan, Pure Chem. Sect.*, 73 (1952) 337.
27 J. H. DHONT AND C. DE ROOY, *Analyst*, 86 (1961) 74.

28 J. ROSMUS AND Z. DEYL, *J. Chromatog.*, 6 (1961) 187.
29 G. M. NANO AND P. SANCIN, *Experientia*, 19 (1963) 323.
30 E. F. L. J. ANET, *J. Chromatog.*, 9 (1962) 291.
31 G. URBACH, *J. Chromatog.*, 12 (1963) 196.
32 H. T. BADINGS, *J. Chromatog.*, 14 (1964) 265.
33 L. M. LIBBEY AND E. H. DAY, *J. Chromatog.*, 14 (1964) 273.
34 D. P. SCHWARTZ AND O. W. PARKS, *Microchem., J.*, 7 (1963) 403.
35 F. H. HOLMES AND C. J. G. SHAW, *J. Appl. Chem. (London)*, 11 (1961) 210.
36 G. A. BYRNE, D. GARDINER AND F. H. HOLMES, *J. Appl. Chem. (London)* to be submitted for publication.
37 M. L. WOLFROM AND G. P. ARSENAULT, *J. Org. Chem.*, 25 (1960) 205.
38 W. L. EVANS AND C. E. WARING, *J. Am. Chem. Soc.*, 48 (1926) 2678.
39 C. NEUBERG, A. GRAUER AND B. V. PISHA, *Anal. Chim. Acta*, 7 (1952) 238.
40 H. REICH AND B. K. SAMUELS, *J. Org. Chem.*, 21 (1956) 68.
41 H. REICH AND L. HEFLE, JR., *J. Org. Chem.*, 21 (1956) 708.
42 D. CAVALLINI, N. FRONTALI AND G. TOSCHI, *Nature*, 163 (1949) 568.
43 S. M. ALTMANN, E. M. CROOK AND S. P. DATTA, *Biochem. J.*, 49 (1951) 634.
44 D. SELIGSON AND B. SHAPIRO, *Anal. Chem.*, 24 (1952) 754.
45 H. B. STEWART, *Biochem. J.*, 55 (1953) 26P.
46 B. AXELROD, R. S. BUNDURSHI, C. M. GREINER AND R. JANG, *J. Biol. Chem.*, 202 (1953) 619.
47 F. A. ISHERWOOD AND D. H. CRUICKSHANK, *Nature*, 173 (1954) 121.
48 A. MEISTER AND P. A. ABENDSCHEIN, *Anal. Chem.*, 28 (1956) 171.
49 J. M. MILLER AND J. G. KIRCHNER, *Anal. Chem.*, 25 (1953) 1107.
50 H. P. KAUFMANN AND T. H. KHOE, *Fette, Seifen, Anstrichmittel*, 64 (1962) 81.
51 H. P. KAUFMANN, Z. MAKUS AND T. H. KHOE, *Fette, Seifen, Anstrichmittel*, 64 (1962) 1.
52 E. V. TRUTER, *Thin Film Chromatography*, Cleaver Hulme Press Ltd., London, 1963.
53 R. D. BENNETT AND E. HEFTMANN, *J. Chromatog.*, 9 (1962) 353.
54 E. J. SHELLARD, *Lab. Pract.*, 13 (1964) 290.