

THIN-LAYER CHROMATOGRAPHY OF ALIPHATIC 2,4-DINITROPHENYLHYDRAZONES

GERDA URBACH

*Division of Dairy Research, C.S.I.R.O.,
Melbourne (Australia)*

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Carbonyl compounds play an important role in desirable and undesirable flavours. No comprehensive study of aliphatic 2,4-dinitrophenylhydrazones by thin-layer chromatography (TLC) has been published. This paper describes methods for separating members of homologous series and for separating different homologous series from one another by TLC.

I. SEPARATION OF MEMBERS OF AN HOMOLOGOUS SERIES

In 1952 ONOE¹ separated the 2,4-dinitrophenylhydrazones of *n*-aliphatic aldehydes up to C₁₀, propenal, but-2-enal and citral on silica gel plates using benzene saturated with water as developing solvent. Later DHONT AND DE ROOY² used a 3:1 mixture of benzene and light petroleum (b.p. 60–80°) to separate the 2,4-dinitrophenylhydrazones of *n*-aliphatic aldehydes, and benzene containing 5% ethyl acetate to separate the 2,4-dinitrophenylhydrazones of aromatic aldehydes, both on silica gel plates. ROSMUS AND DEYL³ separated the 2,4-dinitrophenylhydrazones of some aldehydes and ketones on alumina using either benzene-hexane (1:1) or ether as solvent. The present author tried these systems on the homologous series of *n*-alk-1-en-3-ones but could not separate homologues above C₆.

KAUFMANN AND MAKUS⁴, working in the lipid field, used adsorption TLC to separate substances into classes, but found that partition TLC with impregnated plates was more suitable for separation of members of homologous series. FORSS AND STARK⁵, in their discussion of the separation of 2,4-dinitrophenylhydrazones by four^{6–9} paper chromatographic systems consider the phenoxyethanol-light petroleum (b.p. 100–120°) system⁶ the most satisfactory. This system was therefore tried on kieselguhr G plates with the result that separations of all the members of the normal homologous series of alkanals C_{1–14}, alkan-2-ones C_{3–13}, alk-1-en-3-ones (vinyl ketones) C_{4–10}, alk-2-enals C_{3–11,16}, alka-2,4-dienals C_{5–12,14,16,18} and alk-3-en-2-ones C_{6,7,10} were obtained as shown in Fig. 1. A slight difference in *R_F* value between the *trans*-2,*trans*-6- and *trans*-2,*cis*-6-isomers of nonadienal was also obtained, as illustrated. However, this pair is readily separated by the system described in Section II (B).

Experimental

Dry kieselguhr G plates* (E. Merck A. G., Darmstadt) were rocked in a shallow dish

* The Desaga (Heidelberg, Germany) equipment was used throughout.

filled with a 10% solution of 2-phenoxyethanol in acetone. The impregnated plates were removed, allowed to dry and ether solutions of the 2,4-dinitrophenylhydrazones were applied 4 cm from one edge of the plate. The plate was developed with light petroleum (b.p. 100–120°, not aromatic-free) in a tank lined with filter paper soaked with the light petroleum. The plate was removed from the tank and, after the light

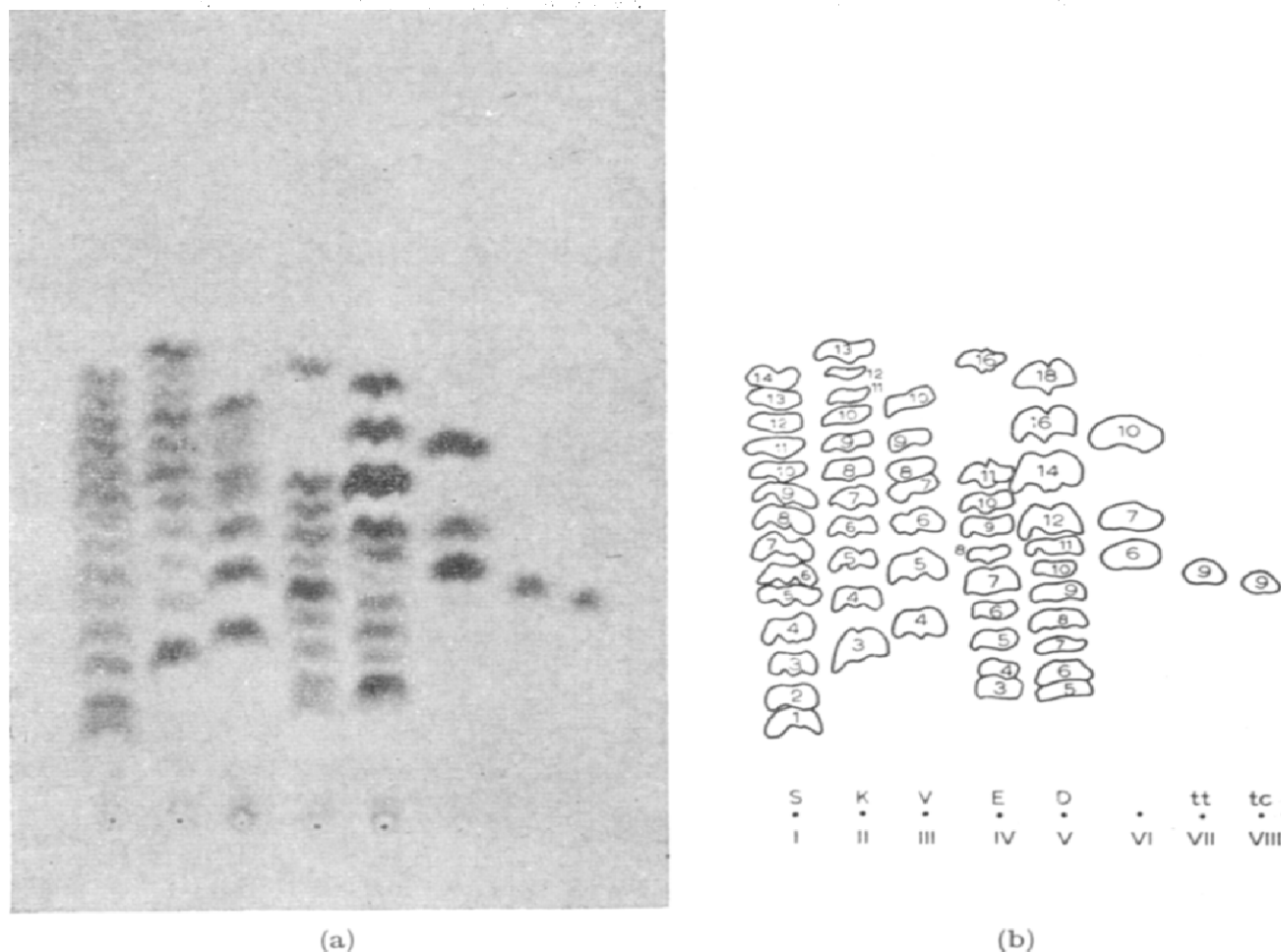


Fig. 1. Separation of members of normal homologous series of 2,4-dinitrophenylhydrazones of (i) alkanals (S) C_{1-14} ; (ii) alkan-2-ones (K) C_{3-13} ; (iii) alk-1-en-3-ones (V) C_{4-10} ; (iv) alk-2-enals (E) $C_{3-11,16}$; (v) alka-2,4-dienals (D) $C_{5-12,14,16,18}$; (vi) alk-3-en-2-ones $C_{6,7,10}$; (vii) nona-*trans*-2, *trans*-6-dienal (viii) nona-*trans*-2, *cis*-6-dienal. Carrier: kieselguhr G. Impregnation: 10% phenoxyethanol in acetone. Solvent: light petroleum (b.p. 100–120°). Conditions: the mobile solvent was allowed to ascend 9 cm from the starting line three times and 11 cm from the starting line the fourth time.

petroleum had evaporated, was photographed and replaced in the tank. In this way, the solvent was allowed to ascend the plate four times. Figs. 1–4 show the effect of multiple ascent. Obviously, fewer ascents are needed for simpler mixtures. In multiple development, compounds with a high R_F value tend to become crowded together whereas compounds with low R_F values are further separated. This effect has been discussed by LENK¹⁰ who also pointed out that with multiple development, as opposed to longer running distance, the spots become flattened out and hence resolution is improved.

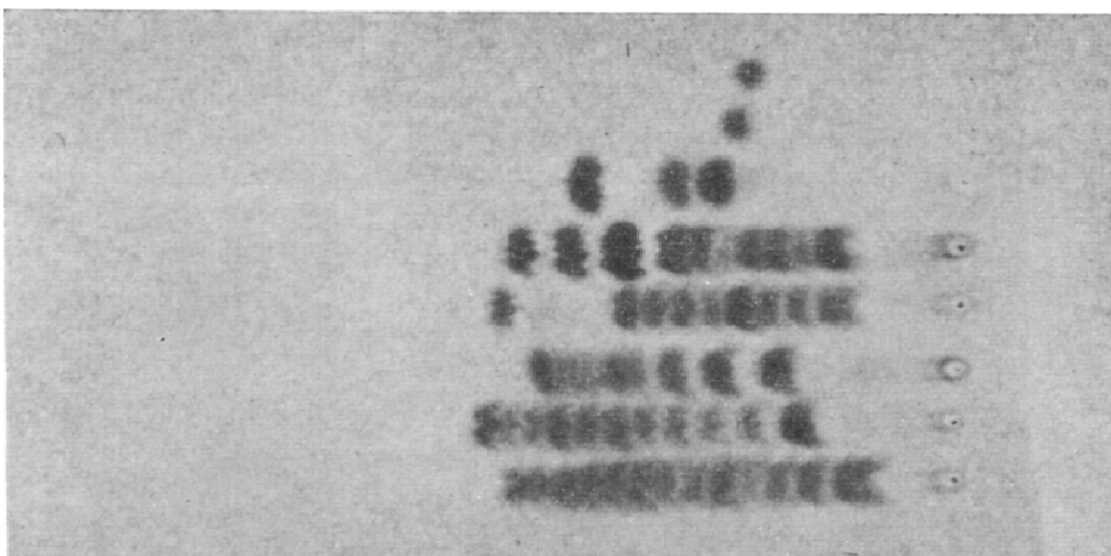


Fig. 4. The plate as in Fig. 1 after three ascents.

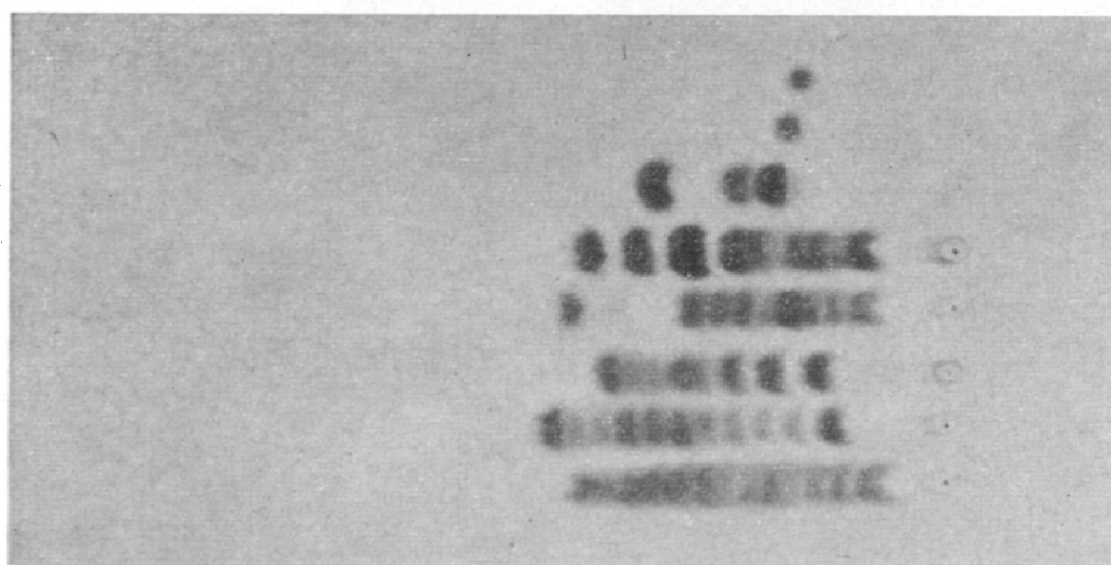


Fig. 3. The plate as in Fig. 1 after two ascents.

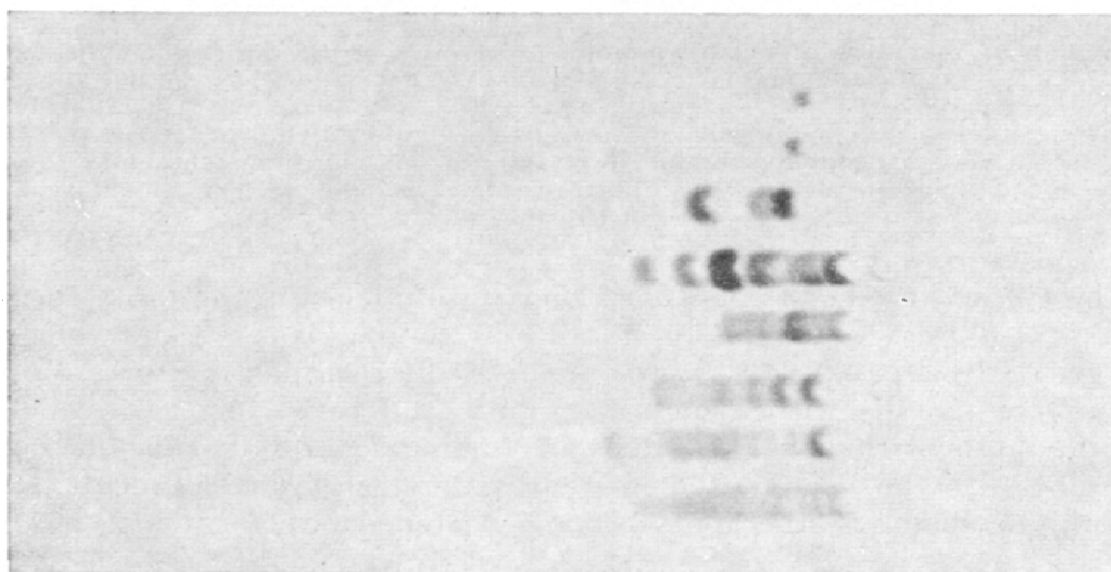


Fig. 2. The plate as in Fig. 1 after one ascent.

At the bottom of the plate the phenoxyethanol has been eluted by the light petroleum as shown by the pale area in the photographs, particularly Fig. 1. In order that the eluted area should not interfere with the separation, the starting line was made 4 cm above the edge of the plate instead of the usual 1 cm.

The plates should be photographed within 24 h as the spots tend to diffuse in the phenoxyethanol, and alka-2,4-dienals fade on standing. Orthochromatic film was used for Figs. 1-4 whilst Figs. 5-8 were photographed in U.V. light with a Polaroid camera.

The system described above separates individual members of any homologous series but it is apparent from Fig. 1 that there are several groups of 2,4-dinitrophenylhydrazones from different homologous series which have the same R_F values in this system. The separations of such groups are described in the following sections.

II. SEPARATION INTO CLASSES

(A) Separation into aldehyde, saturated methyl ketone and vinyl ketone 2,4-dinitrophenylhydrazones

FORSS, DUNSTONE AND STARK¹¹ used acid-washed alumina columns to separate 2,4-dinitrophenylhydrazones according to classes. This suggested that some separation into classes should be possible on thin layers of alumina. It was found that 4% diethyl ether in light petroleum readily separated aldehydes, saturated methyl ketones and vinyl ketones but only gave slight separation of aldehydes of different degrees of unsaturation. A separate system, described in Section II (B) was therefore developed for the separation of aldehydes into classes.

Experimental

Plates were prepared from a slurry consisting of 30 g of aluminium oxide G (E. Merck A. G., Darmstadt) to 50 ml of distilled water. The plates were allowed to set for 10 min, dried in an oven at 115° for 15 min and stored open to the atmosphere. The duration and temperature of heating of the plates were not found to be critical provided the plates were allowed to equilibrate with the atmosphere. Once the plates were so equilibrated, they could be stored indefinitely, open to the atmosphere.

Compounds were applied in CCl_4 solution. The solvent used was 4% diethyl ether in light petroleum (b.p. 30-40°). The solvent was allowed to ascend to the top of the plate. The tank need not be sealed completely air-tight, in fact, a leak in the seal increases separation. The boiling range of the light petroleum is not critical. Similar separations were obtained with 4% ether in light petroleum (b.p. 100-120°). The spots are intensified and turned bright red with 2% NaOH in ethanol-water (9:1, v/v)¹².

Fig. 5 shows typical separations of groups of *n*-alkanals, *n*-alkan-2-ones and *n*-alk-1-en-3-ones which are inseparable with phenoxyethanol (Section I).

It can be seen that from C_4 onwards all the *n*-alkanals have the same R_F value whereas the R_F 's of the *n*-alkan-2-ones increase slightly from C_4 to C_{12} and the R_F 's of the *n*-alk-1-en-3-ones increase slightly from C_5 to C_{10} . In both series of ketone derivatives the first member of the series has an appreciably lower R_F value than the second member. Thus the methyl vinyl ketone 2,4-dinitrophenylhydrazone has the

same R_F value as the methyl ethyl ketone derivative and acetone 2,4-dinitrophenylhydrazone has the same R_F value as the alkanal derivatives from butanal onwards and it does not separate very well from the propanal derivative. The pairs not separated on this adsorbent separated well on the adsorbent described in the next section.

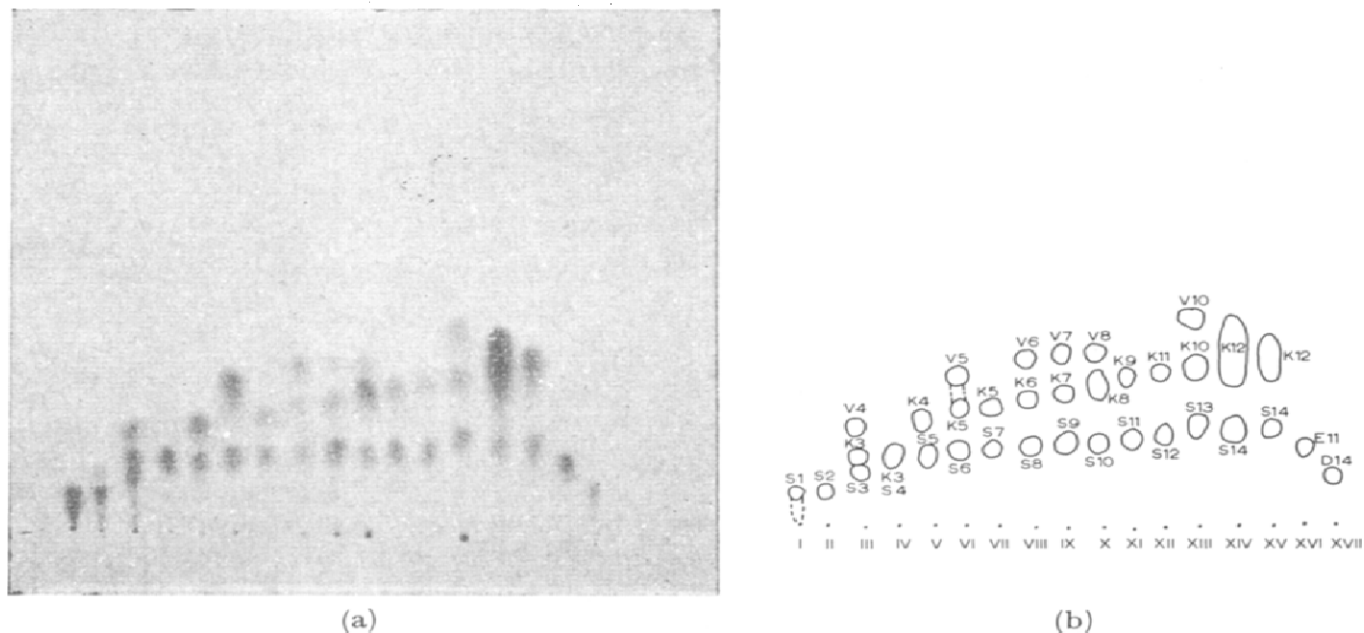


Fig. 5. Separation of mixtures of normal 2,4-dinitrophenylhydrazones of alkanals (S), alkan-2-ones (K) and alk-1-en-3-ones (V) which have the same R_F value in the partition system illustrated in Fig. 1. (a) Photograph. (b) Tracing. (xiv) dodecan-2-one has been spotted too heavily; (xvi) undec-2-enal; (xvii) tetradeca-2,4-dienal. Adsorbent: aluminium oxide G. Solvent: 4% diethyl ether in light petroleum (b.p. 30–40°), one ascent to edge of plate.

In general there was poor separation between aldehydes of different degrees of saturation. However, Fig. 5 shows that some separation has been obtained between *n*-tetradecanal, *n*-undec-2-enal and *n*-tetradeca-2,4-dienal.

(B) *Separation of aldehydes into alkanals, alk-2-enals, alka-2,4-dienals and nona-2,6-dienals*

Dr. J. H. RUSSEL* drew the attention of the author to a paper by BARRETT, DALLAS AND PADLEY¹³ describing the use of silica gel impregnated with silver nitrate to separate isomeric glycerides and glycerides having the same number of carbon atoms but different degrees of unsaturation. Alumina containing 25% AgNO₃ was therefore tried, with success, for the separation of aldehydes into classes. This adsorbent also separated the inseparable pairs of compounds listed in the previous section.

Experimental

Plates were prepared from a slurry made from 30 g aluminium oxide G suspended in a solution of 7.5 g of AgNO₃ in 50 ml of water. The plates were allowed to set for 10 min and then heated for 20 min, the oven temperature rising gradually from 115° to 135°. The plates were stored overnight open to the atmosphere, protected from bright light.

* Division of Organic Chemistry, C.S.I.R.O., Melbourne.

Compounds were spotted from CCl_4 solution. The plates were developed with freshly prepared 16% ether in light petroleum (b.p. 30–40°) in tanks lined with filter paper soaked with solvent. Fresh solvent was used for each run as it was found that used solvent gave unsatisfactory separation.

Decreasing the moisture content of Al_2O_3 - AgNO_3 plates decreases the R_F value and increases the stability of AgNO_3 complexes. Thus it would be expected that plates with a low activity would give R_F values in an order similar to that found on an Al_2O_3 plate containing no AgNO_3 . This was found to be so. On relatively wet plates ketones travel ahead of aldehydes and there is very little separation between the classes of aldehydes, particularly alkanals and alk-2-enals. With decreasing water content the effect of complexing becomes more evident. Apparently alkan-2-one 2,4-dinitrophenylhydrazones form complexes more readily than the alkanal derivatives and therefore the R_F values of alkan-2-one derivatives are decreased to less than those of alkanal derivatives. In both cases complex formation must be assumed to occur at the $\text{C}=\text{N}$ bond. In this case, the R_F value of the vinyl ketone derivative is also decreased relative to that of the methyl ketone derivative, the additional double bond of the vinyl compound causing the greater decreases in R_F value in the presence of AgNO_3 .

Several factors influence the stability of the AgNO_3 complex.

1. The stability increases with increase in the number of double bonds¹⁴—the R_F values decrease in the order alkanal, alkenal, alkadienal.

2. Compounds with conjugated double bonds form less stable complexes than compounds with unconjugated double bonds¹⁴—the nona-2,6-dienals have a lower R_F value than the alka-2,4-dienals.

3. *cis*-Double bonds are complexed more readily than *trans*-double bonds¹⁵—nona-*trans*-2,*trans*-6-dienal has a higher R_F value than nona-*trans*-2,*cis*-6-dienal.

If one bears these factors in mind it will be apparent that if compounds forming relatively stable complexes, such as the *trans,trans* and *trans,cis* forms of nona-2,6-dienal are to be separated, a plate of lower activity is preferable, whereas a wider separation of the relatively less stable complex forming alkanals and alk-2-enals will be obtained with a drier plate. However, all five classes of compounds can be separated on one plate as shown in Fig. 6. There is a wide range of moisture contents which will give a satisfactory separation of alkanals, alk-2-enals and alka-2,4-dienals. As the position of ketones varies from R_F values less than alk-2-enals to R_F values greater than alkanals with small changes in water content it is therefore desirable before an unknown mixture is examined, to remove ketones by using an Al_2O_3 plate.

If a group of plates as prepared is found to be too wet, as indicated by poor separation between alkanals and alk-2-enals, a satisfactory plate is often produced by heating for 1 min at about 115°. A plate which has been somewhat overheated will usually give a satisfactory separation with two ascents.

Fig. 6 shows typical separations of groups of aldehyde 2,4-dinitrophenylhydrazones which do not separate on phenoxyethanol-impregnated kieselguhr and only slightly on alumina. The R_F values in each group decrease with increase in the number of double bonds. Except for the very early members of each homologous series R_F values on any one plate are characteristic of a particular series. Thus, providing one member of each series has been added as marker (preferably internal) and ketone derivatives are known to be absent (from a previous chromatogram run on alumina),

aldehyde 2,4-dinitrophenylhydrazones can be classified according to degree of unsaturation, and position and configuration of double bonds. The figure also shows the separation of nona-*trans*-2,*trans*-6-dienal from nona-*trans*-2,*cis*-6-dienal 2,4-dinitrophenylhydrazone, the *trans*-isomer travelling ahead of the *cis*-isomer. The nona-2,6-dienal 2,4-dinitrophenylhydrazones are well separated from the alka-2,4-dienal

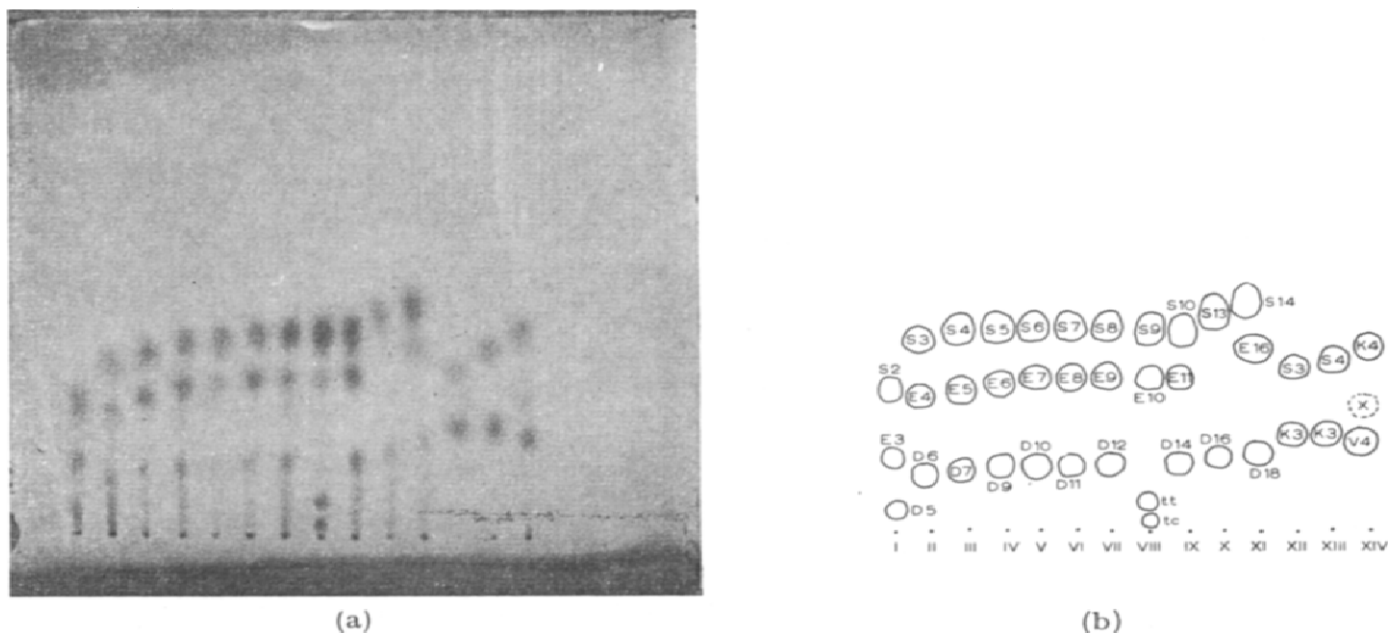


Fig. 6. Separation of normal 2,4-dinitrophenylhydrazones of alkanals (S); alk-2-enals (E); alka-2,4-dienals (D); (viii) nona-*trans*-2,*trans*-6-dienal (tt) and nona-*trans*-2,*cis*-6-dienal (tc); (xii) propanal (S₃) and acetone (K₃); (xiii) butanal (S₄) and acetone (K₃); (xiv) methyl ethyl ketone (K₄) and methyl vinyl ketone (V₄). (X is an impurity.). Adsorbent: aluminium oxide G containing 20% AgNO₃ (w/w). Solvent: 16% diethyl ether in light petroleum (b.p. 30–40°) freshly prepared, allowed to ascend twice to the edge of the plate. (a) Photograph. (b) Schematic drawing.

derivatives. The R_F value of acetone 2,4-dinitrophenylhydrazone is always considerably lower than that of the other members of the series. Because of this the Al₂O₃-AgNO₃ system can be used to achieve a wide separation between acetone 2,4-dinitrophenylhydrazone and the propanal or butanal derivatives. Fig. 6 illustrates these separations and also that of methyl vinyl ketone and methyl ethyl ketone 2,4-dinitrophenylhydrazones. This pair does separate on the phenoxyethanol system but not on alumina plates. On the Al₂O₃-AgNO₃ adsorbent a good separation is achieved with one ascent.

A further aid in the identification of classes is the rate at which they blacken. *n*-Alkanal 2,4-dinitrophenylhydrazones became black within a day while 2,4-dinitrophenylhydrazones of unsaturated compounds take several days to blacken. 2,4-Dinitrophenylhydrazones of *n*-alkan-2-ones do not become black at all but eventually fade completely.

III. TWO-DIMENSIONAL APPLICATION

The systems described in the previous sections can be readily combined in a two-dimensional application to separate a mixture containing several members of different homologous series. Two such separations are illustrated in Figs. 7 and 8.

Fig. 7 shows the separation of a mixture containing *n*-alkanal C_{6,9,13} (S₆, S₉, S₁₃), *n*-alkan-2-one C_{5,7,10} (K₅, K₇, K₁₀), *n*-alk-1-en-3-one C_{5,7,10} (V₅, V₇, V₁₀) 2,4-dinitrophenylhydrazones using aluminium oxide G as adsorbent. The plate was developed twice to the edge of the plate in the first direction with 4% diethyl ether in light petroleum (b.p. 30–40°) as solvent. Before development in the second direction the plate was dipped rapidly but steadily and evenly into a 10% solution of 2-phenoxyethanol in acetone. Great care must be exercised in this impregnation to

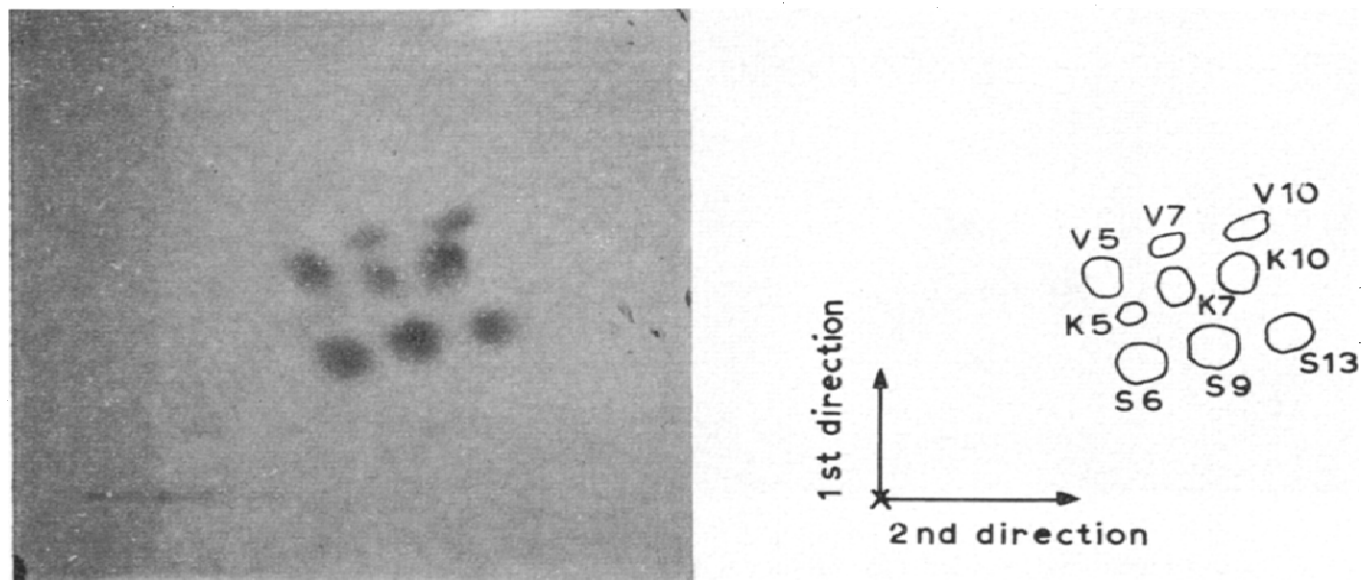


Fig. 7. Two-dimensional separation of normal 2,4-dinitrophenylhydrazones of hexanal (S₆); nonanal (S₉); tridecanal (S₁₃); pentan-2-one (K₅); heptan-2-one (K₇); decan-2-one (K₁₀); pent-1-en-3-one (V₅); hept-1-en-3-one (V₇); dec-1-en-3-one (V₁₀). Adsorbent: aluminium oxide G. Solvent: *first direction* 4% diethyl ether in light petroleum (b.p. 30–40°) allowed to run twice to the edge of the plate; *second direction* plate dipped in 10% phenoxyethanol in acetone and developed once with light petroleum (b.p. 100–120°) to the edge of the plate.

avoid washing off the spots. The plate should be dipped in the impregnating solution to just above the line of spots and then rapidly withdrawn from the solution. After impregnation of the plate, the acetone was allowed to evaporate off and the plate was developed with light petroleum (b.p. 100–120° not aromatic-free) in a direction at right angles to the first direction. In this case the solvent was allowed to ascend to the edge of the plate. For an unknown mixture, markers can be spotted along the edge of the plate before development in the second direction. The fact that spots appear to streak after development in the first direction does not necessarily mean that the chromatogram is unsatisfactory. In the chromatogram illustrated in Fig. 7 the mixture formed a streak after development in the first direction. However, the apparent streaking was due to overlapping spots which have been separated by the development in the second direction.

Fig. 8 shows a separation of a mixture containing *n*-alkanal C_{4,7,10} (S₄, S₇, S₁₀), *n*-alk-2-enal C_{5,8,11} (E₅, E₈, E₁₁) and *n*-alka-2,4-dienal C_{7,11,14} (D₇, D₁₁, D₁₄) 2,4-dinitrophenylhydrazones. The adsorbent is the aluminium oxide G–AgNO₃ mixture described in Section II B. The plate was heated at 115–135° for 20 min and left overnight as described above. The chromatogram was developed once to the edge of

the plate with 16 % diethyl ether in light petroleum (b.p. 30-40°). The plate was then impregnated with phenoxyethanol as described in the preceding paragraph and developed in the second direction with light petroleum (b.p. 100-120°, not aromatic-free). A satisfactory separation was obtained in this case after the solvent had ascended 14 cm from the starting line.

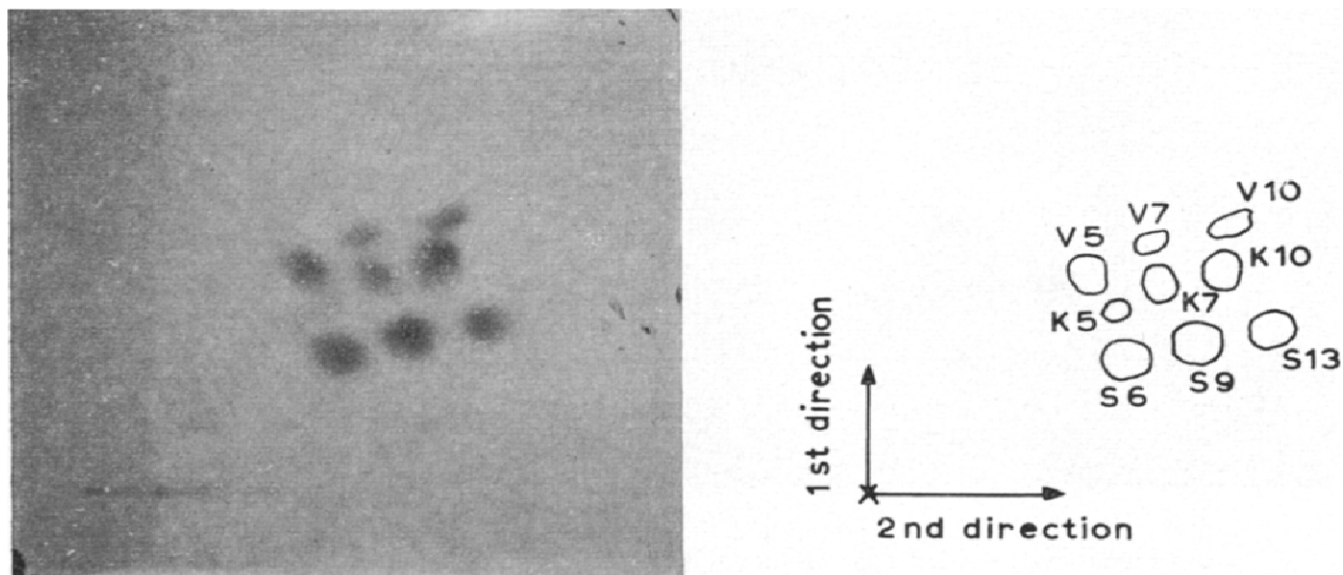


Fig. 8. Two-dimensional separation of normal 2,4-dinitrophenylhydrazones of butanal (S₄); heptanal (S₇); decanal (S₁₀); pent-2-enal (E₅); oct-2-enal (E₈); undec-2-enal (E₁₁); hepta-2,4-dienal (D₇); undeca-2,4-dienal (D₁₁); tetradeca-2,4-dienal (D₁₄). (X is an impurity). Adsorbent: aluminium oxide G containing 20 % AgNO₃ (w/w). Solvent: *first direction* 16 % diethyl ether in light petroleum (b.p. 30-40°) allowed to travel to the edge of the plate once; *second direction* plate dipped in 10 % phenoxyethanol in acetone and developed once with light petroleum (b.p. 100-120°) 14 cm from start to front.

The rate of blackening of spots cannot be used for further identification of classes when the plates have been impregnated with phenoxyethanol because under these conditions the spots tend to fade rather than blacken.

The two chromatograms shown in Figs. 6 and 7 illustrate the separation of mixtures containing either *n*-alkanals, *n*-alkan-2-ones and *n*-alk-1-en-3-ones or only aldehydes of different degrees of unsaturation. To identify a mixture containing members of all these classes the following procedure should be adopted.

1. The unknown mixture is examined two-dimensionally on an alumina plate. This will identify the ketones except acetone which may form one spot with lower aldehydes. This chromatogram will also give an indication of the relative concentration of the various components as well as the carbon number range of the aldehydes present. There may also be some separation between *n*-alkanals and unsaturated aldehydes, the unsaturated aldehydes having a slightly lower *R_F* value in the first direction. The phenoxyethanol does not interfere with the intense red colour for monocarbonyl 2,4-dinitrophenylhydrazones produced by spraying with alcoholic NaOH.

2. One or more spots are developed on an aluminium oxide G plate with 4 % ether in light petroleum (b.p. 30-40°). A clear separation between aldehydes and

ketones is required at this stage and the plate may therefore have to be developed twice. The areas corresponding to aldehydes are scraped off the plate with a spatula and the aldehydes are extracted from the adsorbent with diethyl ether. The extract is evaporated to a small volume and rechromatographed two-dimensionally on an aluminium oxide G-AgNO₃ plate. This will identify the aldehydes according to degree of unsaturation, configuration and position of double bond and carbon number. If acetone is present in the original mixture it will travel with or just ahead of the higher alka-2,4-dienals and propenal in the first direction. However, it travels ahead of propenal and behind the higher alka-2,4-dienals in the second direction.

3. A mixture is prepared containing all the compounds tentatively identified above and mixed in approximately equal proportions with the unknown mixture. Steps 1 and 2 are then repeated with this mixture: if the same number of spots as previously is obtained on both two-dimensional chromatograms, the compounds in the unknown mixture have been identified with the degree of certainty which any chromatographic procedure affords. The possibility of the presence of members of other homologous series of 2,4-dinitrophenylhydrazones has not been excluded.

DISCUSSION

The most comprehensive system for the separation of 2,4-dinitrophenylhydrazones so far published is that of GADDIS AND ELLIS¹⁶ who used adsorption chromatography on paper to separate 2,4-dinitrophenylhydrazones into homologous series of normal alkan-2-ones, alkanals, alk-2-enals and alka-2,4-dienals, and various partition systems with impregnated paper to separate individual members of homologous series. They state that their systems will not separate the pairs acetone-propenal, acetone-butanal and ethanal-propenal. These pairs are separated on the Al₂O₃-AgNO₃ system. The present author also found that vinyl ketones run with the methyl ketones in the GADDIS AND ELLIS adsorption system whereas these series readily separate on alumina plates.

SMITH AND OHLSON¹⁷ have discussed the factors which influence retention time in the gas chromatographic separation of unsaturated hydrocarbons using AgNO₃ in ethylene glycol as the stationary phase. The same considerations apparently apply in TLC of 2,4-dinitrophenylhydrazones of saturated and unsaturated aldehydes and ketones. R_F values of aldehyde 2,4-dinitrophenylhydrazones decrease with increasing number of double bonds, consistent with increased stability of the silver complex¹⁴. Nona-2,4-dienal 2,4-dinitrophenylhydrazone has a higher R_F value than the nona-2,6-dienal derivatives as would be expected from the reported lower stability of the silver complexes of conjugated olefins as compared with unconjugated olefins¹⁴. The separation between the *trans,trans*- and the *trans,cis*-isomers of nona-2,6-dienal 2,4-dinitrophenylhydrazone is also in accord with the easier formation of complexes with *cis*- rather than *trans*-double bonds¹⁵. This fact has recently been used in the very elegant separation of methyl elaidate (the *trans*-isomer) and methyl oleate (the *cis*-isomer) on a column of silica gel impregnated with AgNO₃, the *trans*-isomer having a lower retention volume than the *cis*-isomer¹⁸.

It is to be expected that compounds, other than AgNO₃, which are also known to form complexes with olefinic linkages should also prove effective in the separation of compounds according to number, position and configuration of double bonds,

e.g. I_2^{10} , $AlCl_3$, $FeCl_3$, $ZnCl_2$, $CuCl$, $Hg(OAc)_2^{14}$ or any compounds known to act as Lewis acids, and transition metals such as Pt, Pd, Rh²⁰ etc. Indeed $Hg(OAc)_2$ has been used for this purpose. MANGOLD²¹ describes the use of $Hg(OAc)_2$ for the separation of lipids into classes according to degree of unsaturation and configuration of double bond and PREY, BERGER AND BERBALK²² use $Hg(OAc)_2$ adducts for the separation of olefins. Several column chromatographic procedures are described in the literature for the separation of 2,4-dinitrophenylhydrazones into classes. Although none of these authors offer any explanation for the effectiveness of their methods, complex formation could easily play a part in some of them. VAN DUIN²³ used $ZnCO_3$ columns to separate 2,4-dinitrophenylhydrazones into alkan-2-one, alkanal, alk-2-enal, and alka-2,4-dienal derivatives by displacement with a mixture of light petroleum-benzene (9:1) to which varying quantities of pyridine were added. BADINGS²⁴ adapted this method to TLC for the separation of oct-2-enal and deca-2,4-dienal 2,4-dinitrophenylhydrazones. SCHWARTZ, PARKS AND KEENEY²⁵ separated aliphatic monocarbonyls into classes on magnesia-Celite columns. On their columns the formaldehyde and acetaldehyde derivatives travelled with the higher 2-enals, acrolein with the higher 2,4-dienals, and penta-2,4-dienal had a distinctly greater retention volume than the higher 2,4-dienals. This behaviour is paralleled on Al_2O_3 - $AgNO_3$ plates (Fig. 6) where acetaldehyde 2,4-dinitrophenylhydrazone has the same R_F value as the higher 2-enal derivatives, acrolein 2,4-dinitrophenylhydrazone travels with the 2,4-dienal derivatives and penta-2,4-dienal has a considerably lower R_F value than the higher 2,4-dienal derivatives.

It is also possible that complex formation between metal ions of the paper and unsaturated 2,4-dinitrophenylhydrazones plays a part in the class separation of GADDIS AND ELLIS¹⁶ and the acetylated paper system of FORSS AND RAMSHAW⁹. This is further supported by the marked effect of temperature on these separations. At lower temperatures complex formation is favoured¹⁴ and hence streaky spots are produced on acetylated paper⁹, whereas at higher temperatures, which are less favourable to complex formation, the spots are more compact.

SUMMARY

Thin-layer chromatographic procedures are described for the separation of 2,4-dinitrophenylhydrazones into:

1. Individual members of homologous series by a partition system between 2-phenoxyethanol supported on kieselguhr G and light petroleum (b.p. 100-120°),
2. *n*-Aldehyde, *n*-alkan-2-one and *n*-alk-1-en-3-one derivatives on aluminium oxide G with 4 % diethyl ether in light petroleum (b.p. 30-40°) as the solvent,
3. *n*-Alkanal, *n*-alk-2-enal, *n*-alka-2,4-dienal, *n*-nona-*trans*-2,*trans*-6-dienal and *n*-nona-*trans*-2,*cis*-6-dienal derivatives on plates of aluminium oxide G containing 25 % $AgNO_3$ (w/w) with 16 % diethyl ether in light petroleum (b.p. 30-40°) as the developing solvent.

These procedures have been combined in two-dimensional techniques to give a separation of mixtures of the 2,4-dinitrophenylhydrazones of the normal homologous series of alkan-2-ones, alk-1-en-3-ones, alkanals, alk-2-enals, alka-2,4-dienals, and alka-2,6-dienals.

REFERENCES

- ¹ K. ONOE, *J. Chem. Soc. Japan, Pure Chem. Sect.*, 73 (1952) 337; from *C. A.*, 47 (1953) 3757d.
- ² J. H. DHONT AND C. DE ROOY, *Analyst*, 86 (1961) 74.
- ³ J. ROSMUS AND Z. DEYL, *J. Chromatog.*, 6 (1961) 187.
- ⁴ H. P. KAUFMANN AND Z. MAKUS, *Fette, Seifen, Anstrichmittel*, 62 (1960) 1014.
- ⁵ D. A. FORSS AND W. STARK, *Anal. Chem.*, in preparation.
- ⁶ W. S. LYNN, JR., LOIS A. STEELE AND E. STAPLE, *Anal. Chem.*, 28 (1956) 132.
- ⁷ R. ELLIS, A. M. GADDIS AND G. T. CURRIE, *Anal. Chem.*, 30 (1958) 475.
- ⁸ F. KLEIN AND K. DE JONG, *Rec. Trav. Chim.*, 75 (1956) 1285.
- ⁹ D. A. FORSS AND E. H. RAMSHAW, *J. Chromatog.*, 10 (1963) 268.
- ¹⁰ H. P. LENK, *Z. Anal. Chem.*, 184 (1961) 107.
- ¹¹ D. A. FORSS, E. A. DUNSTONE AND W. STARK, *Australian J. Chem.*, 13 (1960) 584.
- ¹² E. F. L. J. ANET, *J. Chromatog.*, 9 (1962) 295.
- ¹³ C. B. BARRETT, M. S. J. DALLAS AND F. B. PADLEY, *Chem. Ind. (London)*, (1962) 1050.
- ¹⁴ S. WINSTEIN AND H. J. LUCAS, *J. Am. Chem. Soc.*, 60 (1938) 836.
- ¹⁵ P. L. NICHOLS, JR., *J. Am. Chem. Soc.*, 74 (1952) 1091.
- ¹⁶ A. M. GADDIS AND R. ELLIS, *Anal. Chem.*, 31 (1959) 870.
- ¹⁷ B. SMITH AND R. OHLSON, *Acta. Chem. Scand.*, 16 (1962) 351.
- ¹⁸ B. DE VRIES, *Chem. Ind. (London)*, (1962) 1049.
- ¹⁹ J. G. TRAYNHAM AND J. R. OLECHOWSKI, *J. Am. Chem. Soc.*, 81 (1959) 571.
- ²⁰ G. N. SCHRAUZER AND S. EICHLER, *Chem. Ber.*, 95 (1962) 260.
- ²¹ H. K. MANGOLD, *J. Am. Oil Chemists' Soc.*, 38 (1961) 708.
- ²² V. PREY, A. BERGER AND H. BERBALK, *Z. Anal. Chem.*, 185 (1962) 113.
- ²³ H. VAN DUIN, *Neth. Milk Dairy J.*, 12 (1958) 74.
- ²⁴ H. T. BADINGS, *J. Am. Oil Chemists' Soc.*, 12 (1959) 648.
- ²⁵ D. P. SCHWARTZ, O. P. PARKS AND M. KEENEY, *Anal. Chem.*, 34 (1962) 669.

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