THE CHROMATOGRAPHY OF 2,4-DINITROPHENYLHYDRAZONES ON ACETYLATED PAPER

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INTRODUCTION

Aliphatic carbonyl compounds play a major role in the odours of plants, insects and oxidized foods. Since these compounds often occur in amounts below one part per million, special techniques are necessary for their identification[1]. One of the most widely used techniques is the study of their 2,4-dinitrophenylhydrazones, especially by paper chromatography. The relative rates of movement of the 2,4-dinitrophenylhydrazones and the light absorption maxima of the spots, which may be measured directly on the paper after chromatography[2], are important qualitative criteria. Moreover, the pure compounds may be extracted from the paper for further examination such as determination of infrared spectra[3] or melting points.

There is a considerable variety of methods for the paper chromatography of 2,4-dinitrophenylhydrazones in which either treated or untreated paper is used[4]. MEIGH[5], HUELIN[6] and SCHEPARTZ[7], all using untreated paper with the same solvent system, report good separation for the lower homologues but decreasing separation for the higher homologues of the hydrazones of *n*-alkanals and *n*-alkan-2-ones. Better separation throughout the range is obtained by treating the paper with an involatile stationary phase such as phenoxyethanol[8], paraffin oil[9] or vaseline[10]. However, RITTER AND HARTEL[11], discussing these methods, point to the disadvantages associated with lack of reproducibility of the amount and distribution of the stationary phase, possible effects on the stationary phase during spotting, and interference by the non-volatile stationary phase during subsequent measurements. Acetylated paper has been found useful in the chromatography of the 2,4-dinitrophenylhydrazones of carbonyl fission products of sugars[12], polycyclic aromatic hydrocarbons[13], steroids[11], indole derivatives[14], polonium and other ions[15], 3,5-dinitrobenzoates of aliphatic alcohols[16] and the 2,4-dinitrophenylhydrazones of lower aliphatic carbonyl compounds[17]. Although the use of acetylated paper obviates the need for the impregnation with its attendant disadvantages, the acetylation procedures are tedious and not particularly reproducible. Little use has been made of commercial papers, possibly due to their high price.

EXPERIMENTAL

In this study a commercially available acetylated paper (Schleicher & Schüll 2043b/21ac, 20–25 % acetylated; 54 × 57 cm) was used for the paper chromatography of 2,4-dinitrophenylhydrazones of the C_{3-13} *n*-alkan-2-ones, the C_{1-14} *n*-alkanals, the $C_{3-11,16}$ *n*-alk-2-enals and the $C_{5-12,14,16,18}$ *n*-alka-2,4-dienals. The first batch re-

ceived in 1959 had a softer and more open texture than five batches received in 1962.

Using the first batch, excellent chromatograms were obtained with the solvent system described by MEIGH[6]. The 2,4-dinitrophenylhydrazones were dissolved in methanol (except the hydrazones of the C_{11-14} *n*-alkanals which were dissolved in ether) and spots 0.5 cm in diameter and 2.5 cm apart were placed on the baseline 10 cm from one end of the paper. The chromatogram was run parallel to the 57 cm edge of the paper which is apparently at right angles to the fibre direction. The paper was equilibrated overnight in a Shandon 12-in. Universal Strip Chromatank (57 cm high) with methanol saturated with "heptane" (light petroleum, b.p. 100–120°) and then developed by the descending method with "heptane" saturated with methanol. Small circular spots were obtained and the separation can be seen from the data presented in Fig. 1.

With the five later batches of paper good separation was obtained but the faster moving compounds gave "streaked" spots. This streaking was unaffected by the size

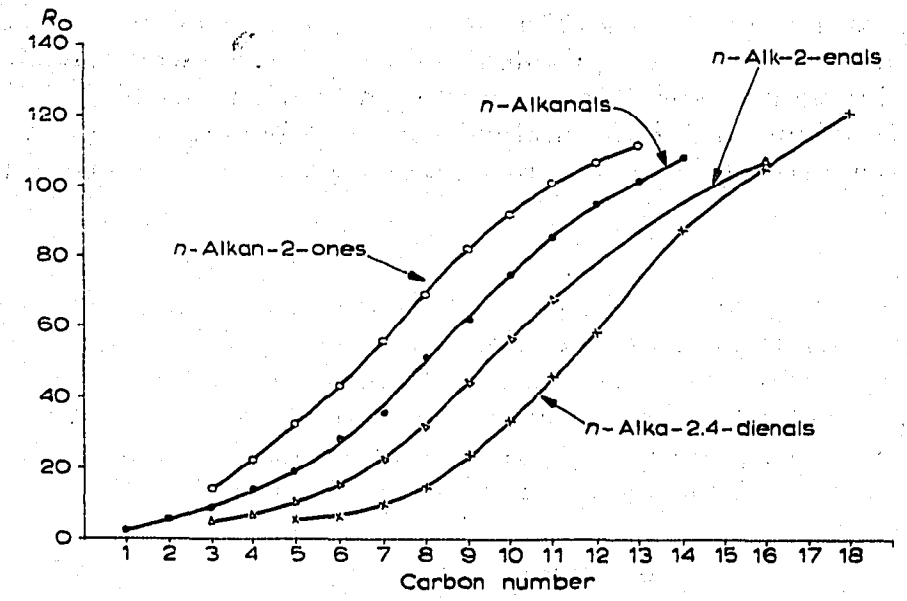

Fig. 1. Mobility of 2,4-dinitrophenylhydrazones. $R_O = \frac{\text{Distance moved by a hydrazone}}{\text{Distance moved by } n\text{-octanal hydrazone}} \times 50$.
O—O *n*-Alkan-2-ones; ●—● *n*-Alkanals; △—△ *n*-Alk-2-enals; ×—× *n*-Alka-2,4-dienals.

of the initial spot or by the distance of the baseline from the end of the paper but was increased by running the compounds in the direction parallel to the 54 cm edge. Several other solvent systems, see for example refs. [13,14], were tried but none were better than the methanol–"heptane". Drying the methanol–"heptane" with calcium sulphate increased the streaking while the addition of 5 % water had no effect.

The acetylated papers were compared by macroscopic and microscopic examination, staining and washing tests, solubility in chlorinated solvents, resistance to air flow and measurement of thickness. While the papers varied considerably, it was not possible to determine the reason for the superiority of the paper received in 1959. This aspect was not pursued as good chromatograms could be obtained by the simple techniques described below.

Two other grades of paper were tested on a limited scale (Schleicher & Schüll

2045b/21ac, 20–25 % acetylated; and 2043a/45ac, 40–45 % acetylated). The former was a harder paper and the latter a more highly acetylated but neither was markedly superior to the 2043b/21ac.

Two modifications with the methanol–"heptane" system each eliminated the streaking. In one, the paper was equilibrated with methanol alone instead of methanol saturated with "heptane". Alternatively, as suggested by RITTER AND HARTEL[11] and SPOTSWOOD[13], the mobile phase was run through about 12 cm of a "retardation strip" of slow paper (Whatman No. 2 or No. 20) before the acetylated paper. Both modifications increased the development time to 4–7 h with two papers in the tank. Experiments were carried out at 15°, 20°, 30° and 40°. The best chromatograms were obtained at 30° with a "retardation strip".

As was noticed by BURTON[18], the spots obtained by acetylated paper chromatography of 2,4-dinitrophenylhydrazones were smaller than those obtained with untreated Whatman paper particularly for the lower homologues. Individual members of the homologous series were separated from each other. The R_F values of the 2,4-dinitrophenylhydrazones of the C_x alkan-2-one, C_{x+1} alkanal, C_{x+3} alk-2-enal and C_{x+5} alka-2,4-dienal were roughly equivalent (*cf.* refs. [2,4]). However, each series could be characterized by light absorption measurements of the spot[2,19]. In this way, a combination of R_F values and λ_{max} enables any spot belonging to the above series to be identified (see also ref. [20] for a comparison with C_{4-10} *n*-alk-1-en-3-one hydrazones).

CONCLUSIONS

The advantages of using acetylated paper for chromatography are the speed and simplicity of preparing the chromatograms, the excellent separation obtained, and the ease with which subsequent measurements may be effected. With the current availability of reagents for the formation of colored derivatives from amines, alcohols, carbonyl compounds, mercaptans and other compounds, it is likely that acetylated paper may be used with advantage in their chromatography.

ACKNOWLEDGEMENTS

We wish to thank Dr. D. P. SCHWARTZ of the Eastern and Dr. E. L. PIPPEN of the Western Utilization Research and Development Divisions, U.S. Department of Agriculture, for gifts of some 2,4-dinitrophenylhydrazones and Mr. P. R. EDWARDS of the Division of Dairy Research, C.S.I.R.O., for his help in the preparation of many chromatograms.

SUMMARY

The paper chromatography of the 2,4-dinitrophenylhydrazones of the C_{3-13} *n*-alkan-2-ones, the C_{1-14} *n*-alkanals the $C_{3-11,16}$ *n*-alk-2-enals and the $C_{5-12,14,16,18}$ *n*-alka-2,4-dienals on six batches of acetylated paper (Schleicher & Schüll 2043b/21ac) is described. With a batch obtained in 1959 excellent chromatograms were obtained by equilibration overnight with methanol saturated with "heptane" followed by descending development with "heptane" saturated with methanol. With the other five batches obtained in 1962, the separation of all compounds was also excellent but the faster moving spots tended to streak. This was prevented by equilibration with

methanol alone or preferably by running the mobile solvent through a "retardation strip" of about 12 cm of a slow paper (Whatman No. 2 or No. 20) before the acetylated paper. The optimum temperature for the latter chromatograms was 30°.

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