THIN-LAYER CHROMATOGRAPHY OF HOMOLOGUES AND VINYLOGUES OF HIGHER FATTY ALDEHYDES, DIMETHYL ACETALS, AND 2,4-DINITROPHENYLHYDRAZONES

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INTRODUCTION

Plasmalogens are present in a variety of animal tissues. The aldehydes bound in them have been analyzed by gas–liquid chromatography (GLC) as their dimethyl acetals by a number of investigators[1–5]. Methanolysis of mixtures of acyl esters and plasmalogens with methanolic HCl or BF_3–methanol yields a mixture of methyl esters of fatty acids and aldehyde dimethyl acetals (DMAs). Some investigators have found it necessary to effect a separation of the DMAs from the methyl esters before analyzing them by GLC. FARQUHAR[3] made this separation by chemical means, and ENG *et al.*[4] by thin-layer chromatography.

Caution should be exercised in the interpretation of results obtained by GLC of DMAs from methanolyzed lipids. The presence of free fatty aldehydes existing in such mixtures either due to incomplete conversion of fatty aldehydes to the DMAs[2] or by reversion of DMAs to the aldehydes[5] should be verified by independent methods. Further, the behavior of DMAs on stationary liquid phases used in GLC needs further investigation in the light of observations by several investigators[2,5,6], who have reported that DMAs are not found or partially decomposed in columns commonly used in the GLC analysis of methyl esters of fatty acids. TLC should prove to be a valuable adjunct to GLC in these cases.

Separations by TLC of homologues and vinylogues of free aldehydes and DMAs have not been reported in the literature. Usually aldehyde separations have been made in the form of their 2,4-dinitrophenylhydrazones (DNPHs). Even with this approach, separations of DNPHs of longer chain saturated and unsaturated aldehydes have not been described. In 1952, ONOE[7] separated the DNPHs of *n*-aliphatic aldehydes up to C_{10} on silica gel plates, using benzene saturated with water as developing solvent. KAUFMANN AND KIRSCHNEK[8] reported the separation of DNPHs of saturated even numbered (C_8–C_{18}) and unsaturated C_{18} aldehydes (oleyl, linoleyl and linolenyl) by reversed-phase paper chromatography. Use was made of mercuric acetate adducts in separating unsaturated aldehydes and critical pairs. URBACH[9] separated the DNPHs of *n*-aliphatic aldehydes (C_1–C_{14}) by multiple development on Kieselguhr G plates impregnated with 2-phenoxyethanol. LIBBEY AND DAY[10] separated the DNPHs of the same aldehydes on Silica Gel G plates impregnated with mineral oil.

This papers describes a simple procedure for separation of mixtures of aldehydes, DMAs and methyl esters into classes by TLC. The separation of individual fatty alde-

hydes, their DMAs and DNPHs according to chain length and degree of unsaturation is described also.

EXPERIMENTAL

Materials

The various aldehydes, DMAs and DNPHs were prepared as described previously[11,12]. Solvents were reagent grade and redistilled.

Preparation of plates

Using a thin-layer applicator (Desaga, Heidelberg), glass plates (20 × 20 cm) were coated as usual with a well stirred suspension of silica Gel G or Kieselguhr G (E. Merck, Darmstadt; 30 g in 60 ml water) to give a layer approximately 250 μ in thickness. The plates were air-dried at room temperature for 15–30 min and activated at 110° for 1 h.

Siliconized plates for reversed-phase chromatography were made by dipping the activated Kieselguhr G plates in a 10 % (v/v) solution of silicone (Dow Corning 200 fluid) in petroleum ether (30–60°). The solvent was removed by exposing the plates to the atmosphere for about 5 min.

Silver nitrate impregnated plates were made by dipping activated Silica Gel G plates in a saturated solution of silver nitrate in 95 % aqueous methanol. After removal of the solvent by evaporation at room temperature, the plates were activated at 110° for 30 min and allowed to cool (10 min) before applying the solutions.

Development of chromatograms

The compounds were dissolved in petroleum ether, except the DNPHs, which were dissolved in ethyl ether. One μl of 0.1 % solution of each of the several compounds were spotted on the plates 2 cm from the base. Each spot, therefore, contained 1 μg of the individual compound. The spotted plates were dried in a stream of nitrogen and developed by the ascending technique in a tank lined with filter paper soaked with the solvent. Spots of aldehydes and their DMAs were made visible on siliconized plates by spraying the plates with a saturated solution of $K_2Cr_2O_7$ in 70 % (by volume) sulfuric acid[13] and charring at 160°. The spots on $AgNO_3$ impregnated plates were made visible by spraying with 50 % aqueous H_2SO_4 and charring. The DNPH spots were clearly visible as yellow spots against the white background.

RESULTS

Separation into classes

Fig. 1 shows a representative two-dimensional chromatogram demonstrating the separation of a mixture containing methyl esters of fatty acids, DMAs and aldehydes into classes using Silica Gel G plates. A solution of the mixture was spotted on the lower left hand corner of a plate 2 cm away from each of the nearest edges. The plate was first developed in toluene until the solvent was 12 cm above the spot (20 min). This resulted in the separation of the DMAs from the methyl esters and the aldehydes. In order to separate the methyl esters and the aldehydes, the plate was dried in a stream of nitrogen for 5 min and developed in the second dimension (90

degree rotation counterclockwise) with petroleum ether–ethyl ether–acetic acid, (90:10:1, v/v); the new solvent front was allowed to move for 18 cm (45 min). Mixtures containing 1 μg of each class could be detected.

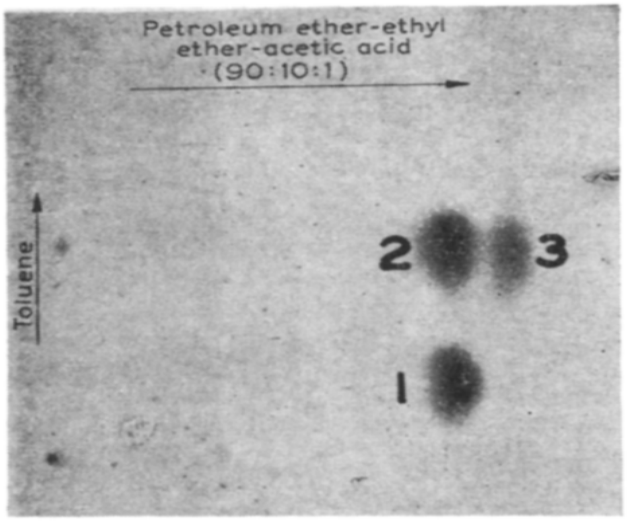

Fig. 1. Photograph of a two-dimensional adsorption thin-layer chromatogram of methyl esters, aldehydes and dimethyl acetals on Silica Gel G. The first dimension was developed for 20 min with toluene (12 cm) and the second dimension, with petroleum ether (b.p. 30–60°)–ethyl ether–acetic acid (90:10:1) for 45 min (18 cm). 1 = Dimethyl acetals; 2 = aldehydes; 3 = methyl esters.

Separation of aldehydes

Fig. 2A shows the separation of the saturated and unsaturated aldehydes achieved by reversed-phase partition chromatography. The plate was developed with 85 % aqueous acetone saturated to 90 % with silicone. It was then removed from the tank, dried in a stream of nitrogen for 5 min, and redeveloped once more. The saturated aldehydes were separated from one another, as were also the unsaturated aldehydes. The critical pairs, palmitaldehyde and oleylaldehyde, and myristaldehyde and linoleylaldehyde, had identical R_F values and could not be separated by this technique. However, they were separated by adsorption chromatography using $AgNO_3$–impregnated Silica Gel G plates.

Fig. 2B shows a separation of the aldehydes according to unsaturation achieved by $AgNO_3$–Silica Gel G plates. The plates were developed with petroleum ether–ethyl ether (76:24, v/v). The saturated aldehydes as a class moved to the solvent front. The R_F values of the unsaturated aldehydes varied with the degree of unsaturation; the lower the degree of unsaturation, the higher the R_F value. The saturated aldehydes were recovered from the plate by extracting the relevant portion with ether, and separated from one another by reversed-phase partition chromatography as shown in Fig. 2A. Thus, by a combination of $AgNO_3$-impregnated adsorption chromatography and reversed-phase partition chromatography, a complete separation of all the aldehydes was made.

Separation of dimethyl acetals

The separation of the DMAs of the saturated and unsaturated aldehydes made by reversed-phase partition chromatography is shown in Fig. 3A. The developing solvent was 85 % aqueous acetone saturated to 90 % with silicone. The plate was

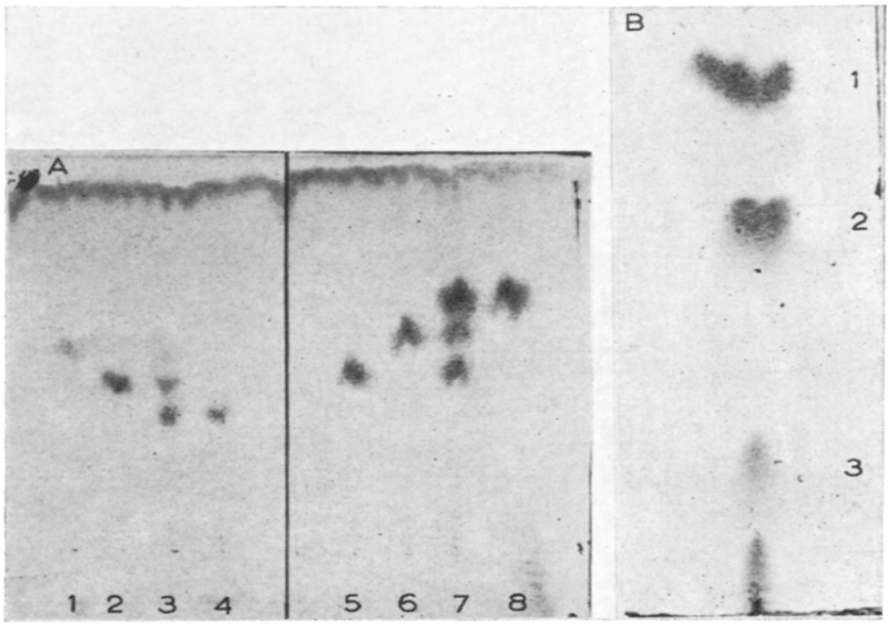

Fig. 2. (A) Photograph of a reversed-phase thin-layer chromatogram of fatty aldehydes. Support: Kieselguhr G. Impregnation: 10% silicone in petroleum ether. Solvent: 85% aqueous acetone saturated to 90% with silicone. Conditions: the mobile solvent was allowed to ascend 16 cm from the starting line two times (2 h). 1 = Myristaldehyde; 2 = palmitaldehyde; 3 = mixture of 1, 2, and 4; 4 = stearaldehyde; 5 = oleyl aldehyde; 6 = linoleyl aldehyde; 7 = mixture of 5, 6 and 8; 8 = linolenyl aldehyde. (B) Separation of fatty aldehydes according to degree of unsaturation by $AgNO_3$–Silica Gel G TLC. Solvent: petroleum ether–ethyl ether (76:24, v/v). Conditions: The solvent was allowed to ascend once for 16 cm from the starting line (30 min). 1 = Mixture of saturated aldehydes; 2 = oleyl aldehyde; 3 = linoleyl aldehyde; 4 = linolenyl aldehyde.

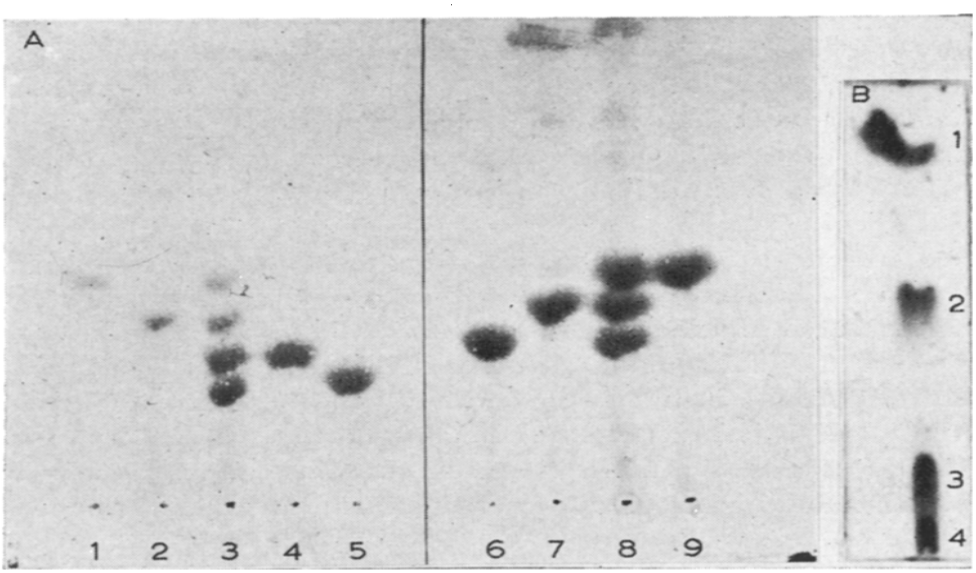

Fig. 3. (A) Photograph of a reversed-phase thin-layer chromatogram of dimethyl acetals. Support and solvent used were the same as in Fig. 2 A. Conditions: The solvent was allowed to ascend 16 cm from the starting line three times (3 h). 1 = Lauraldehyde dimethyl acetal; 2 = myristaldehyde dimethyl acetal; 3 = mixture of 1, 2, 4 and 5; 4 = palmitaldehyde dimethyl acetal; 5 = stearaldehyde dimethyl acetal; 6 = oleyl aldehyde dimethyl acetal; 7 = linoleyl aldehyde dimethyl acetal; 8 = mixture of 6, 7 and 9; 9 = linolenyl aldehyde dimethyl acetal. (B) Separation of the dimethyl acetals according to degree of unsaturation by $AgNO_3$–Silica Gel G TLC. Solvent: petroleum ether–ethyl ether (88:12, v/v). Conditions were the same as those in Fig. 2 B. 1 = Saturated fatty aldehyde dimethyl acetals; 2 = oleyl aldehyde dimethyl acetal; 3 = linoleyl aldehyde dimethyl acetal; 4 = linolenyl aldehyde dimethyl acetal.

developed thrice in the same direction with the same solvent, after drying each time for 5 min in a stream of nitrogen. As in the case of the free aldehydes, the separation of critical pairs of DMAs by reversed-phase alone is difficult.

Fig. 3B shows the separation of the DMAs on $AgNO_3$–Silica Gel G plates on the basis of unsaturation. Like the aldehydes, the saturated DMAs moved to the solvent front and the unsaturated DMAs separated in the same order as the corresponding aldehydes. The saturated DMAs were recovered from the plate and rechromatographed by the reversed-phase method to give separations as shown in Fig. 3A.

Separation of DNPHs

Fig. 4A shows the separation of the DNPHs of the saturated fatty aldehydes (C_{10}–C_{18}) and the unsaturated aldehydes, oleyl, elaidyl, linoleyl and linolenyl. Critical pairs also occur among such derivatives. In addition, only a slight difference in R_F values between the *cis* and *trans* pairs, oleyl and elaidyl-DNPHs, was observed in this system.

Fig. 4B shows the separation of the DNPHs on the basis of unsaturation on $AgNO_3$–Silica Gel G layers. The solvent was petroleum ether–ethyl ether (85:15, v/v). A well-defined separation of the geometrical isomers, oleyl and elaidyl-DNPHs, was obtained.

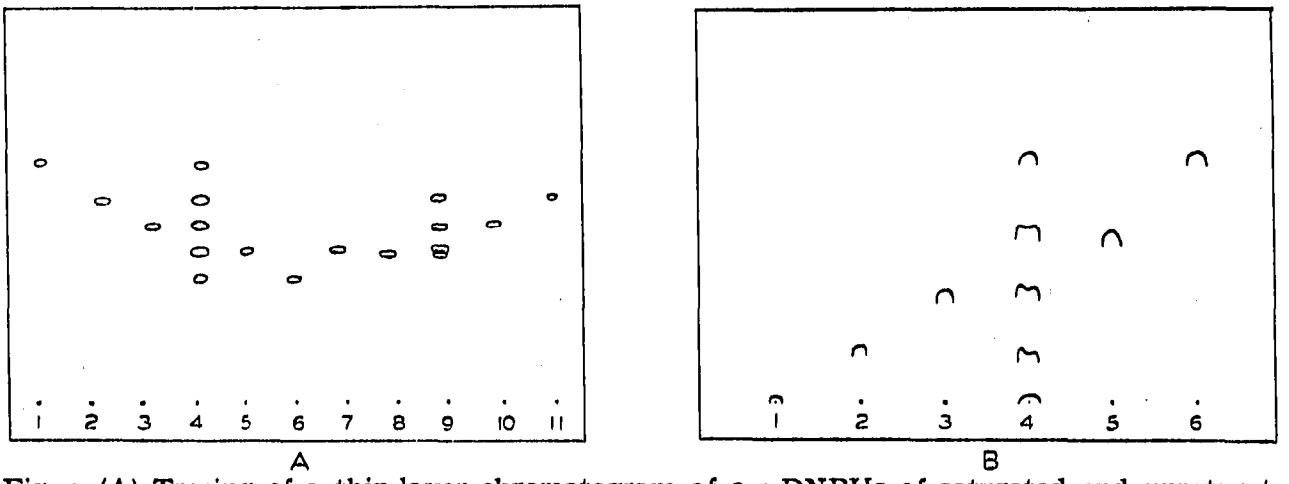

Fig. 4. (A) Tracing of a thin-layer chromatogram of 2,4-DNPHs of saturated and unsaturated fatty aldehydes. Support and solvent were the same as those in Fig. 2A. The solvent was allowed to ascend once 16 cm from the starting line (40 min). DNPHs of 1 = capryl aldehyde; 2 = lauryl aldehyde; 3 = myristyl aldehyde; 4 = mixture of 1, 2, 3, 5 and 6; 5 = palmityl aldehyde; 6 = stearyl aldehyde; 7 = oleyl aldehyde; 8 = elaidyl aldehyde; 9 = mixture of 7, 8, 10 and 11; 10 = linoleyl aldehyde; 11 = linolenyl aldehyde. (B) Tracing of a thin-layer chromatogram of 2,4-DNPHs of fatty aldehydes according to degree of unsaturation of $AgNO_3$–Silica Gel G plates. Solvent: petroleum ether–ethyl ether (85:15, v/v). Conditions: The solvent was allowed to ascend 16 cm (40 min). DNPHs of 1 = linolenyl aldehyde; 2 = linoleyl aldehyde; 3 = oleyl aldehyde; 4 = mixtures of 1, 2, 3, 5 and 6; 5 = elaidyl aldehyde; 6 = mixture of saturated aldehydes.

DISCUSSION

Separations of free fatty aldehydes and their dimethyl acetals by TLC have not been previously reported in the literature. By a combination of reversed-phase partition chromatography and $AgNO_3$-impregnated Silica Gel G adsorption chromato-

graphy, complete separations of the saturated and unsaturated aldehydes and of geometrical isomers of DNPHs of monounsaturated aldehydes were accomplished. The dimethyl acetals were also separated by a combination of these techniques.

The free aldehydes apparently were not oxidized to the corresponding carboxylic acids during chromatography, since spraying them with the 2,4-dinitrophenylhydrazone reagent[14] gave yellow spots characteristic of the aldehydes. Further, spraying with the sulfuric acid reagents and charring yielded only single spots. Spots corresponding to acids were not obtained. Aldehydes lower than C_{14} chain length could not be detected in less than 1 μg amounts, possibly due either to failure to char or to high volatility. DMAs could be detected down to C_{12} chain length in μg amounts. However, the DNPHs of even lower chain aldehydes could be detected, even in smaller amounts.

In contrast to the DNPH derivatives which were separated from one another by reversed-phase technique with only one development, the free aldehydes required two developments and the dimethyl acetals required three developments. The number of developments increased with the reduction in polarity of these classes.

In the reversed-phase system used in this study the higher the number of carbon atoms in the free aldehyde, the DMA and the DNPH, the slower was the migration. URBACH[9] separated the DNPHs of C_1–C_{14} *n*-alkanals on Kieselguhr G plates impregnated with 2-phenoxyethanol. The separation was achieved by developing the plates four times with light petroleum. In that system, the higher members of the series moved the fastest. LIBBEY and DAY[10] separated the DNPHs of the same aldehydes on Silica Gel G plates impregnated with mineral oil. The plates were given a single development with dioxane–water (65:35, v/v). Under their conditions, they observed that the migration rate varied inversely with the number of carbon atoms.

Finally, we have found TLC very useful for separation of methanolyzed tissue lipids into methyl esters of fatty acids and DMAs. The presence of free aldehydes in these lipid mixtures is also easily detected by this technique. Detection of aldehyde impurities in DMAs by GLC is not always feasible. We have found that in EGS columns, for example, both the aldehydes and the dimethyl acetals have the same retention times.

GLC alone may not resolve completely complex mixtures of dimethyl acetals. As in the case of methyl esters of fatty acids, dimethyl acetals of saturated normal and branched-chain aldehydes may overlap with those of unsaturated aldehydes of lower chain lengths. In this respect TLC is useful as an adjunct to GLC. Combination of TLC with GLC should lead to a complete resolution and identification of aldehydes present in naturally occurring plasmalogens.

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SUMMARY

A method is described for the separation of fatty aldehydes, aldehyde dimethyl acetals and methyl esters of fatty acids into classes by two-dimensional thin-layer chromatography.

Separations of individual members of long-chain even-numbered saturated fatty aldehydes, their dimethyl acetals and 2,4-dinitrophenylhydrazones and the C_{18} unsaturated aldehydes (oleyl, linoleyl, and linolenyl) and their corresponding derivatives were achieved by reversed-phase partition thin-layer chromatography. The critical pairs having identical R_F values in the reversed-phase system were separated by adsorption chromatography using silver nitrate-impregnated Silica Gel G plates. With this technique the *cis–trans* isomers also could be separated as shown by 2,4-dinitrophenylhydrazine derivatives.

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