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TWO-DIMENSIONAL THIN-LAYER CHROMATOGRAPHY OF Dns-AMINO ACIDS ON REVERSED-PHASE SILICA GEL

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SUMMARY

Ready-for-use reversed-phase high-performance thin-layer chromatographic plates were used for the separation of Dns-amino acids. The development was performed by using methanol–2% acetic acid (75:25) or methanol–0.01 *M* Na_2HPO_4 (75:25). Reversed-phase plates were used also for adsorption chromatography using a non-aqueous solvent system such as benzene–chloroform–acetic acid (50:48:2) or *n*-heptane–ethyl acetate–acetic acid (65:33:2). Good separations were achieved by using both principles in the two-dimensional arrangement.

INTRODUCTION

One of the main advantages of thin-layer chromatography (TLC) over column techniques is the possibility of using a two-dimensional arrangement. By using two different types of interactions in two directions it is possible to effect the separation of very complex mixtures of compounds and concommittantly such interactions help in the identification of individual spots[1]. The success of two-dimensional chromatography depends mainly on the choice of suitable solvent systems. In most separations either pH differences in both phases or differences in the hydrogen acceptor and hydrogen donor properties of the mobile phase components have been exploited.

In contrast, three papers have been published in which a change of the mobile phase caused alterations of the sorbent properties and therefore also caused a change in the separation mechanism in both directions. Wagner *et al.*[2] used an aqueous mobile phase in the first direction followed by a non-aqueous mobile phase in the second direction for the separation of phenols on polyamide. In the aqueous system the separation mechanism resembled a reversed-phase system, whereas in the non-aqueous system the polyamide behaved as a hydrophilic sorbent. Analogous results were obtained by Woods and Wang[3] in polyamide TLC of Dns-amino acids. Vidrine and Nicholas[4] applied a mixture of silica gel and silanized silica gel as the stationary phase for the two-dimensional chromatography of lipophilic substances. In an aqueous mobile phase the reversed-phase effect on silanized silica gel was the effect that governed the separation, whereas in non-aqueous systems the main mechanism was adsorption on non-silanized silica gel.

Much attention has been paid in biochemistry during the last 15 years to the separation of Dns-amino acids by diverse chromatographic procedures (for reviews see, *e.g.*, refs. 5–7). During the present investigation we tried to apply new sorbents with chemically bonded alkyl residues (C_2, C_8, C_{18})[8] on silica gel for the separation of this type of compound. In all previous papers aqueous mobile phases were used with these sorbents and therefore the separation mechanism was that of reversed-phase partition. In this paper we report that the application of non-aqueous phases offers equally good results, but the mechanism of separation is different from that which occurs in aqueous solvent systems. Therefore, we have tried to use this effect for the two-dimensional separation of Dns-amino acids.

EXPERIMENTAL

Preparation of Dns-amino acids

Amino acids were derivatized according to Seiler and Wiechmann[7], with the exception that extraction of the Dns-amino acids from the reaction mixture was effected with diethyl ether.

Thin layers

HPTLC Fertigplatten RP-2 F_{254}, RP-8 F_{254} and RP-18 F_{254} for the nano-DC (Merck, Darmstadt, G.F.R.) were used.

Mobile phases

The following mobile phases were used: S1 = methanol–2% acetic acid (75:25); S2 = methanol–0.01 *M* Na_2HPO_4 (75:25); S3 = benzene–chloroform–acetic acid (50:48:2); S4 = *n*-heptane–ethyl acetate–acetic acid (65:33:2 or 60:38:2).

Chromatographic techniques

A solution of Dns-amino acids in methanol was applied in amounts of 0.1–0.5 μg on 10 × 10 cm plates. Development was carried out in glass chambers saturated with the vapour of the mobile phase at 23 °C until the solvent front had moved 7 cm from the origin. Detection was performed under UV light (minUVIS, Desaga, Heidelberg, G.F.R.) at 366 nm.

RESULTS AND DISCUSSION

High-performance liquid chromatography was used for the separation of Dns-amino acids by Bayer *et al.*[9] and Wilkinson[10], who applied both straight and reversed phases using LiChrosorb RP-8, μBondapak C_{18} and Spherisorb 5-ODS. Their separation conditions could not be transferred to thin-layer separations because of the high proportion of water in the mobile phase. Also, in reversed-phase chromatography gradient elution was applied.

In the first stage of our work we sought optimal conditions for the analysis of Dns-amino acids on a new type of HPTLC ready-for-use plates with chemically bonded RP-2, RP-8 and RP-18. It was found that a suitable solvent system for development is methanol plus about 25% of 2% acetic acid or 0.01 *M* Na_2HPO_4 solution. The running times in these systems on the RP-8 layer were 20 and 70 min,

respectively. An increased content of water in the mobile phase increased considerably the time of development. The replacement of methanol with acetonitrile did not give better results. Of the types of bonded phases tested, comparable results were obtained with RP-8 and RP-18; RP-2 did not give satisfactory results. The effect of the sorbent is illustrated in Fig. 1.

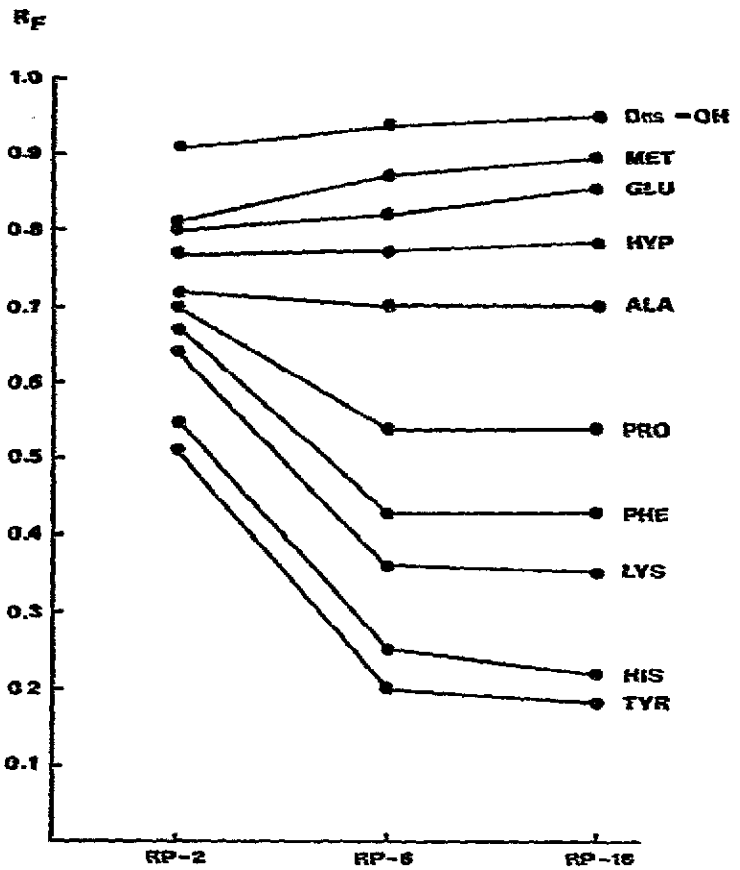


Fig. 1. Effect of type of reversed-phase layer on the R_F values of Dns-amino acids. Mobile phase: methanol–2% acetic acid (75:25).

Because a complete separation of all commom amino acids in a single run was not expected, we sought a suitable mobile phase for the second run in two-dimensional chromatography. It was found that good separations can be achieved in non-aqueous systems of the non-polar hydrocarbon–medium-polarity solvent (ethyl acetate or chloroform)–organic acid type (Fig. 2). The running time with these systems is 15–20 min. A combination of these types of systems gives a fairly good separation of Dns-amino acids in a relatively short time (Fig. 3). As far as the mechanism of separation is concerned, in an aqueous system a reversed-phase partition takes place while in a non-aqueous system the separation is presumably based on an adsorption effect. This is supported, *e.g.*, by the reversed order of the members of the homologous

MeOH	75	MeOH	75	Benzene	50	Heptane	60
2% AcOH	25	0.01 M Na_2HPO_4	25	$CHCl_3$	48	EtAc	38
				AcOH	2	AcOH	2

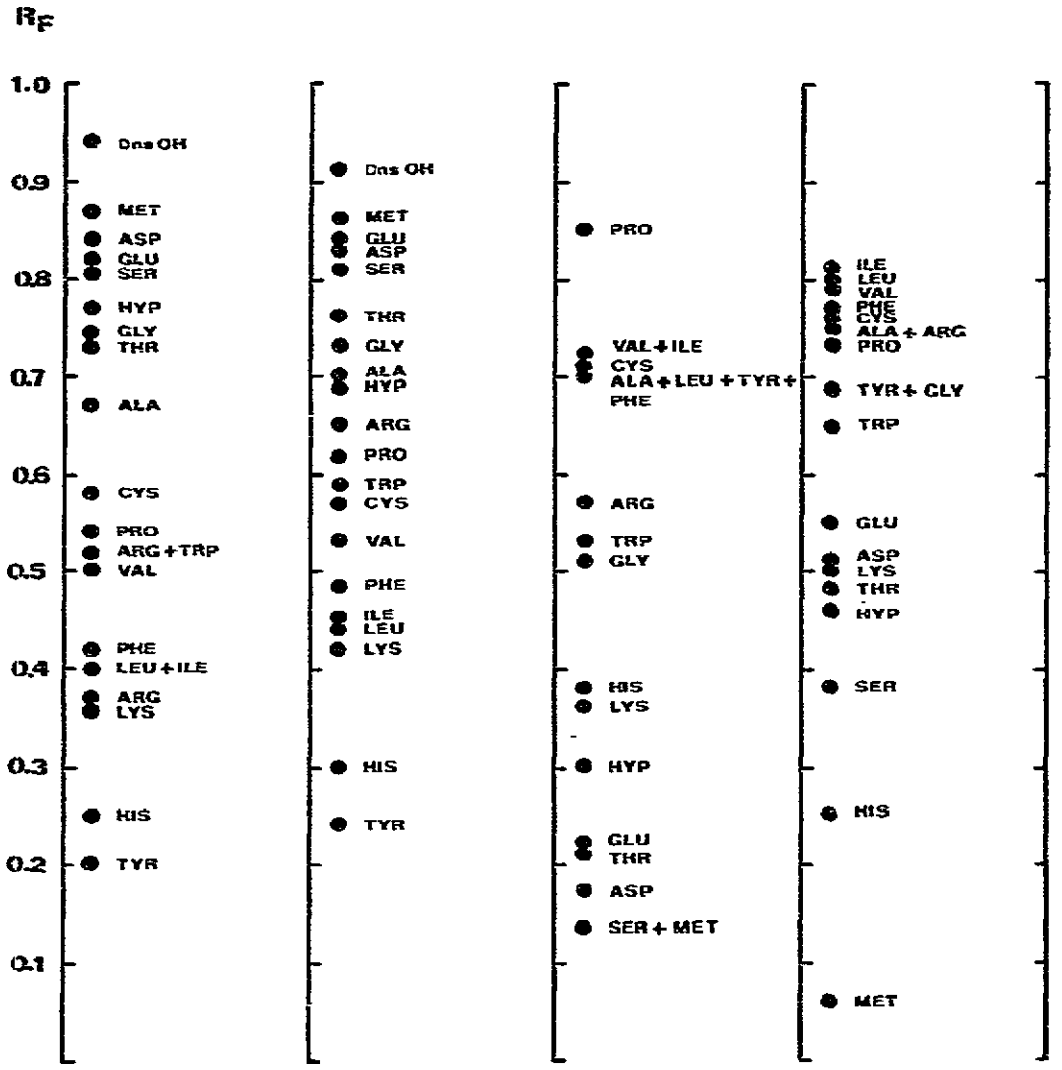
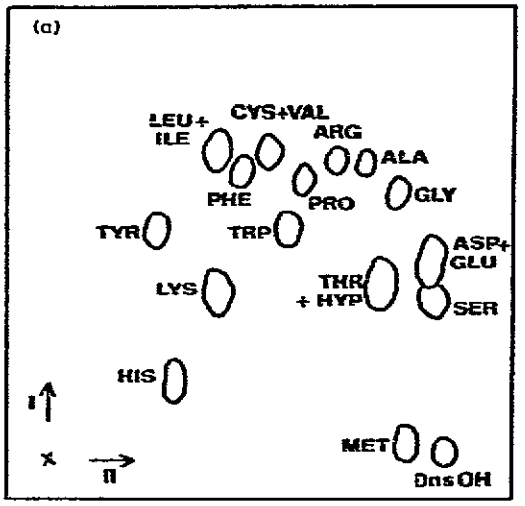


Fig. 2. R_F values of Dns-amino acids in different mobile phases on RP-8 HPTLC plates.

series (Gly, Ala, Val, Leu). Other interactions, however, cannot be ruled out, as is demonstrated by the anomalous mobilities of some Dns-amino acids.

The results obtained are in general agreement with those obtained by Woods and Wang[3] (including the reversed order of the individual Dns-amino acids) on polyamide sheets. Other similar systems were described by Metrione[11]. It was not



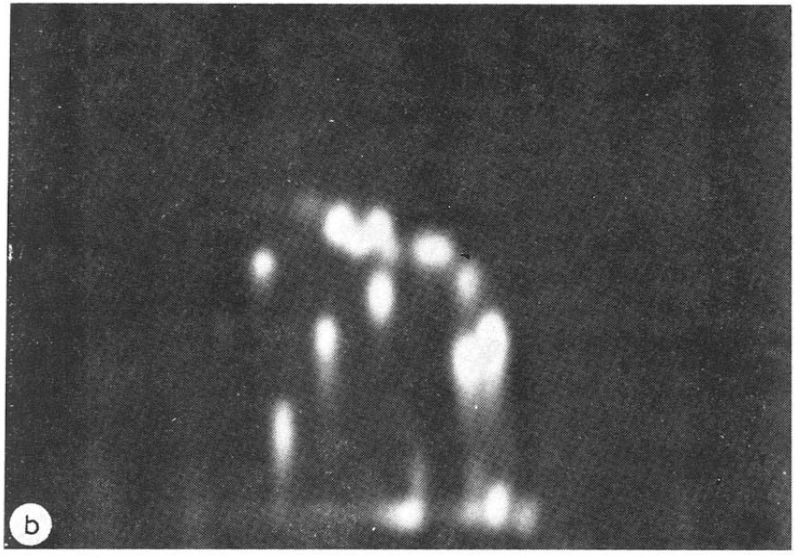


Fig. 3. (a) Schematic representation of a two-dimensional chromatogram of Dns-amino acids on an RP-8 plate. Mobile phases: first dimension, S4; second dimension, S2. (b) Photograph of the same separation.

possible to use aqueous systems with reversed-phase silica gel, and aqueous mobile phase with a high proportion of methanol had to be used. It is unlikely that the described method would replace the more complete separation on polyamide described recently[12], but the exploitation of sorbents with chemically bonded reversed-phases not only for reversed-phase partition but also for adsorption chromatography has a possibility of being widely applied in the chromatography of many types of compounds.

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