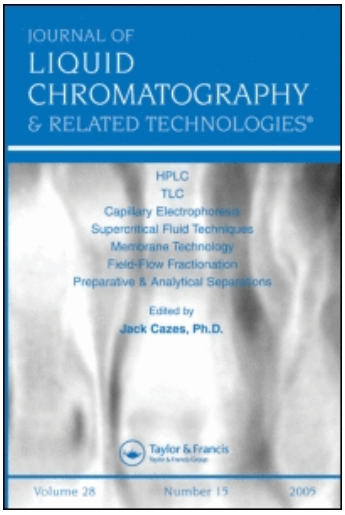

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DISPLACEMENT THIN-LAYER CHROMATOGRAPHY OF SOME ECDYSTEROIDS

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ABSTRACT

The essential steps of displacement thin-layer chromatography and its applications for separation of ecdysteroids are outlined. Finding adequate conditions of displacement thin-layer chromatography for plant ecdysteroids is detailed including the optimisation of mobile phase composition, mobile phase flow rate, and preelution before displacement development. Application of preelution before displacement chromatography has an importance in the case of planar chromatography, both exploring and achieving the displacement separations by HPLC.

INTRODUCTION

Chromatography with elution type of developments is generally performed at the linear parts of the Langmuir isotherms, while displacement chromatography operates with high load, that is at concentrations that are at the non-linear parts of the isotherms [1].

Displacement chromatography has served to improve both analytical and preparative separations. Modern variations of chromatography with displacement type of developments include high performance (column liquid) displacement chromatography [1, 2] and planar (thin-layer) displacement chromatography [3, 4].

Using displacement chromatography, there are two mobile phases. One of them is the carrier which is adequate for non-movement (or very slow movement) of the compounds to be purified. The other one is the displacer, that is displacing the sample components from the stationary phase, therefore, pushing them forward. In the case of displacement thin-layer chromatography (D-TLC), the displacer is dissolved in the carrier. There are two fronts of the mobile phase running forward, the carrier front (first front) and the displacer front (the second one). The sample components to be purified have to move in front of the displacer. As the thin-layer chromatogram has been developed, the displaced component forms a well defined, very sharp zone before the displacer front, it can be easily detected and removed for preparative purposes [4].

Ecdysteroids are insect hormones found in insects, in various other animals and also in plants. In insects, ecdysteroids are moulting hormones, in plants their role is not well explained. However, some plants can be the raw materials for the isolation of ecdysteroids because of their high concentration (up to 3.3% which is much higher than in insects). Among several other types of organic compounds (amines, phenyl alkyl compounds, steroids, etc.), ecdysteroids have also been the subject of our displacement chromatographic separations using planar arrangements of the stationary phase [5-8].

New methods for displacement thin-layer chromatography of plant ecdysteroids have been recently developed [6, 7]. In this paper, optimization of conditions of displacement development by using various carriers, displacers, as well as multiple developments will be detailed.

MATERIALS AND METHODS

Pre-coated TLC plates silica gel 60 F-254 (Merck, Darmstadt, Germany), solvents and chemicals from commercial sources were used.

2-Deoxy-20-hydroxyecdysone (**db**) and 20-hydroxyecdysone (**b**) were the kind gift of Dr. D. H. S. Horn (Acherone, Victoria, Australia). The extraction of *Silene otites (L.) Wib.* (**ex**), and the isolation of 2-deoxyecdysone (**a**), 20-hydroxyecdysone-22-acetate (**ac**) and integristerone (**i**) have been described elsewhere [9].

TLC plates were developed in Desaga (Heidelberg, Germany) chambers using non-saturated vapour phase. Solvent systems are detailed in Table 1.

Chrompres 10 (forced-flow TLC equipment) was purchased from Laberte (Budapest, Hungary).

TABLE 1.

SOLVENT SYSTEMS FOR DEVELOPMENT OF TLC PLATES

dichloromethane--i.propanol--3-dimethylaminopropylamine (dkm--i.PrOH-DAPA)	(220:20:5)
dichloromethane--i.propanol--3-dimethylaminopropylamine (dkm--i.PrOH-DAPA)	(160:20:5)
dichloromethane--i.propanol--3-dimethylaminopropylamine (dkm--i.PrOH-DAPA)	(140:20:5)
dichloromethane--i.propanol--3-dimethylaminopropylamine (dkm--i.PrOH-DAPA)	(140:30:5)
dichloromethane--i.propanol--3-dimethylaminopropylamine (dkm--i.PrOH-DAPA)	(110:40:5)
dichloromethane--i.propanol--3-dimethylaminopropylamine (dkm--i.PrOH-DAPA)	(80:30:5)
dichloromethane--i.propanol (dkm--i.PrOH)	(140:20)
ethyl acetate--methanol--ammonia (EtAc--MeOH--NH_3)	(85:10:5)

RESULTS

Fig. 1 presents the alteration of displacement chromatogram when the ratio of dichloromethane was changed from 140:20 to 110:40, and thereby the 2-deoxy-20-hydroxyecdysone left the displacement front and became eluted by the carrier itself, however, the 20-hydroxyecdysone became part of the displacement train. For comparison, the extract of *Silene otites (L.) Wib.* was also spotted, that extract contained both 2-deoxy-20-hydroxyecdysone and 20-hydroxyecdysone.

Preelution before displacement TLC can improve the separation. Fig. 2 shows the TLC chromatogram after preelution but before displacement (left side) and after performing the displacement separation (right side). With preelution, 20-hydroxyecdysone can be well separated from the overwhelming majority of other components of *Silene otites (L.) Wib.* extract, including the removal of 2-deoxy-20-hydroxyecdysone.

Other arrangements, such as using dichloromethane--i.propanol-3-dimethylaminopropylamine (220:20:5) ratio makes possible the selective concentration of 2-deoxy-20-hydroxyecdysone in the displacement train, as it is demonstrated in Fig. 3.

Also, preelution with ethyl acetate--methanol--ammonia (85:10:5) followed with displacement chromatography (using dichloromethane--i.propanol--3-dimethylaminopropylamine (160:20:10)) makes possible the concentration of both 2-deoxy-20-hydroxyecdysone and 20-hydroxy-ecdysone in the displacement train (Fig. 4.).

Results of displacement thin-layer chromatography with forced-flow developments depend on the flow rate of the mobile phase. This phenomenon is given in Figs. 5 and 6 where the plates were developed with 0.7 and 0.45 ml/min flow rate, thereby both the eluent and the displacer fronts showed peculiar characteristics.

DISCUSSION

Although preparative separation of ecdysteroids is generally done by a combination of various chromatographic procedures [9], efforts have

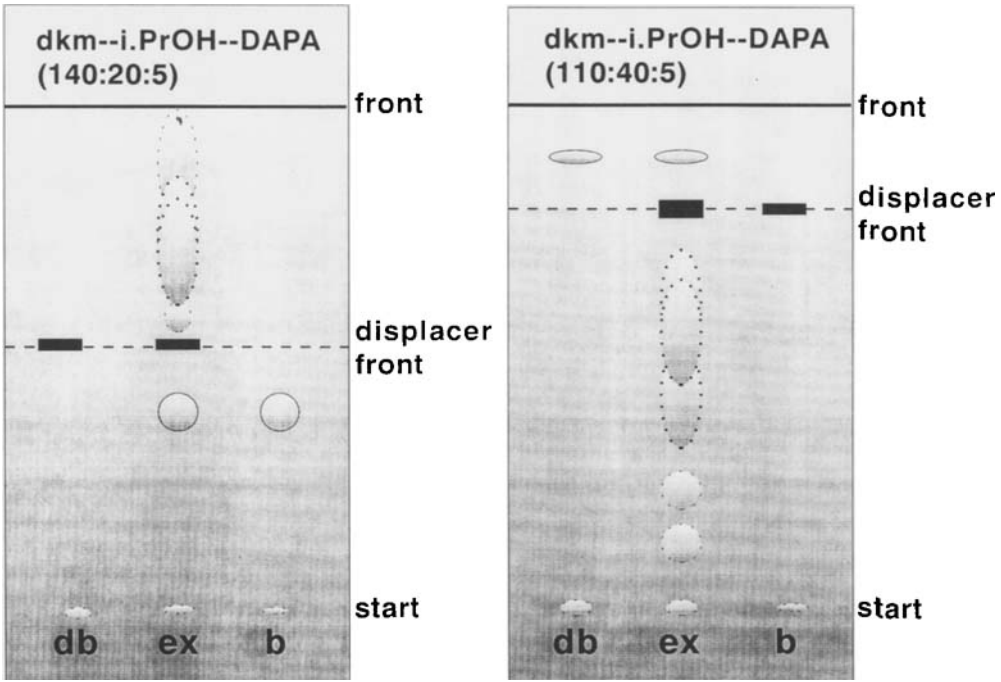

Figure 1.
Displacement thin-layer chromatography of 2-deoxy-20-hydroxyecdysone (**db**), 20-hydroxyecdysone (**b**) and the extract of Silene otites (L.) (**ex**) is given on TLC plates silica gel 60 F-254 using the mobile phases such as dichloromethane--i.propanol--3-dimethylaminopropylamine (140:20:5) and dichloromethane--i.propanol--3-dimethylaminopropylamine (110:40:5) on the left side plate and on the right side plate, respectively.

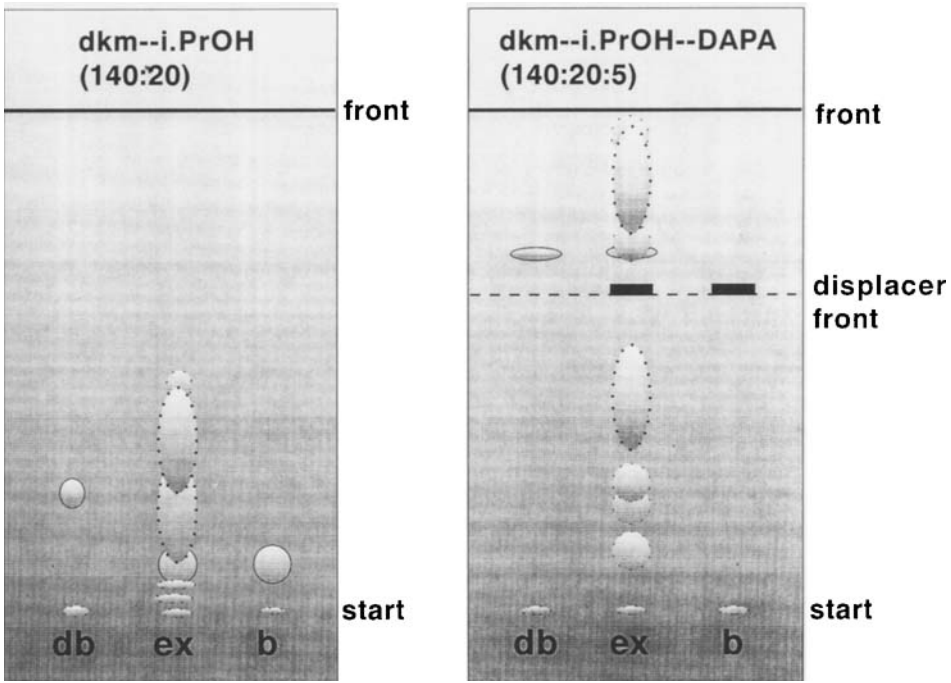

Figure 2.
Thin-layer chromatography followed by displacement thin-layer chromatography of 2-deoxy-20-hydroxyecdysone (**db**), 20-hydroxyecdysone (**b**) and the extract of Silene otites (L.) (**ex**) is given on TLC plate silica gel 60 F-254 using the mobile phases such as dichloromethane--i.propanol (140:20) and dichloromethane--i.propanol--3-dimethylamino-propylamine (140:20:5) on the left side plate and on the right side plate, respectively.

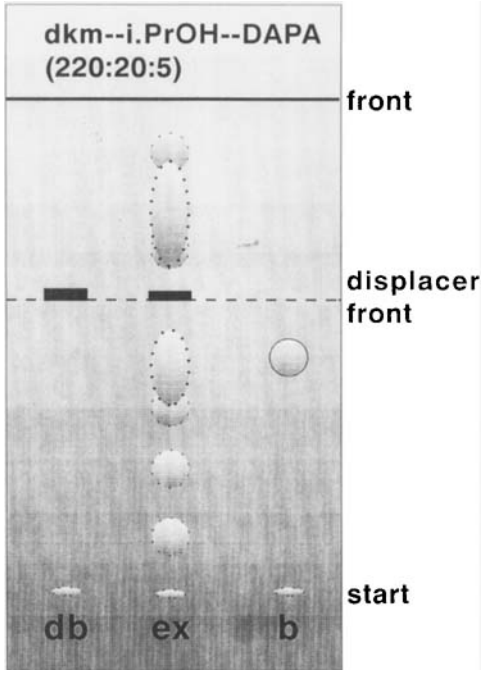

Figure 3.
Displacement thin-layer chromatography of 2-deoxy-20-hydroxyecdysone (**db**), 20-hydroxyecdysone (**b**) and the extract of Silene otites (L.) (**ex**) is given on TLC plate silica gel 60 F-254 using the mobile phase dichloromethane--i.propanol--3-dimethylaminopropylamine (220:20:5).

been made to circumvent the difficulties of the multistep separations. One of these methods is the high-performance displacement chromatography [1] and its variation, the displacement thin-layer chromatography [2-8]. Elution-type developments work with concentrations where the so called adsorption isotherms are linear, thereby the load is limited. At the same time, displacement chromatography works at higher concentration (several mg/ml) which allows the separation of amines, amino acids, peptides, proteins, steroids, generally with good yield.

Displacement thin-layer chromatography of ecdysteroids have been described in our earlier publications, when the influence of the saturation of the chamber and other conditions were investigated [6, 7].

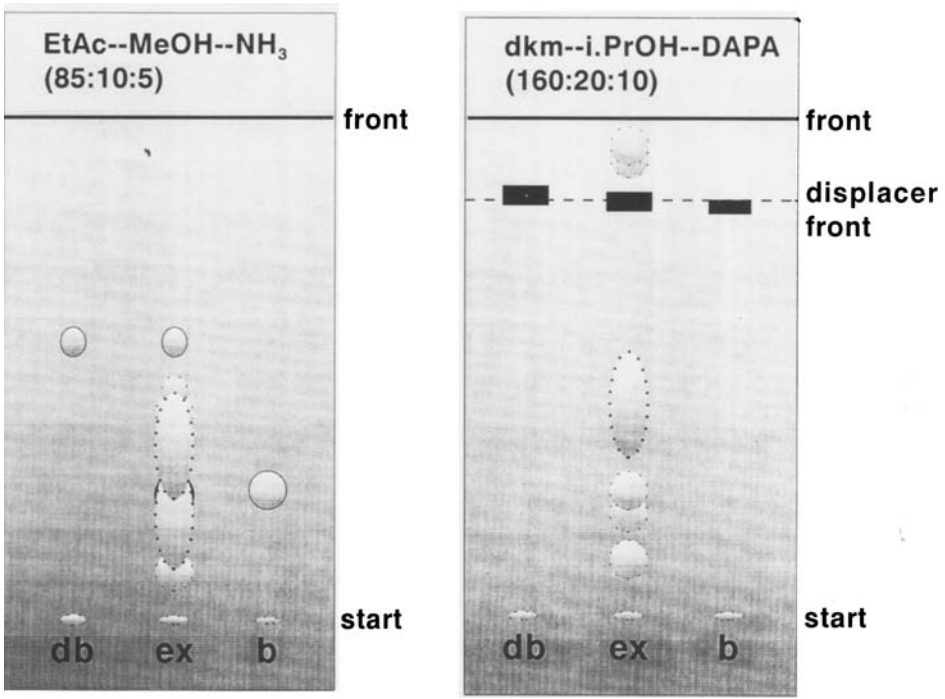

Figure 4.
Thin-layer chromatography followed by displacement thin-layer chromatography of 2-deoxy-20-hydroxyecdysone (**db**), 20-hydroxyecdysone (**b**) and the extract of Silene otites (L.) (**ex**) is given on TLC plate silica gel 60 F-254 using the mobile phases such as ethyl acetate--methanol--ammonia (85:10:5) and dichloromethane--i.propanol--3-dimethylaminopropylamine (160:20:10) are given on the left side and on the right side, respectively.

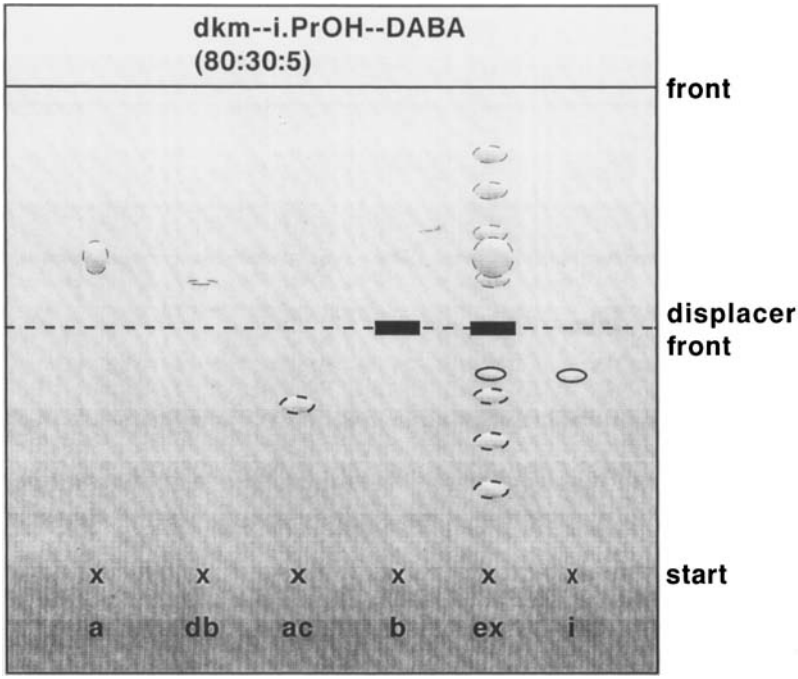

Figure 5.
Forced-flow displacement thin-layer chromatography of 2-deoxyecdysone (**a**), 2-deoxy-20-hydroxyecdysone (**db**), 20-hydroxyecdysone-22-acetate (**ac**), 20-hydroxyecdysone (**b**), the extraction of Silene otites (L.) (**ex**), and integristerone (**i**) is given on TLC plate silica gel 60 F-254 using the mobile phase dichloromethane--i.propanol--3-dimethylaminopropylamine (80:30:5) with flow rate of 0.7 ml/min.

Effective separations were found when two-dimensional (elution-displacement) chromatography was used.

In this paper displacement separations are described, when the preelution and displacement chromatography are performed in the same direction, but elution-type of development precedes displacement, thereby, effective removal of contaminants is possible. Moreover, preelution also influences the development of displacement train. The composition of the system, used for preelution, also determines the

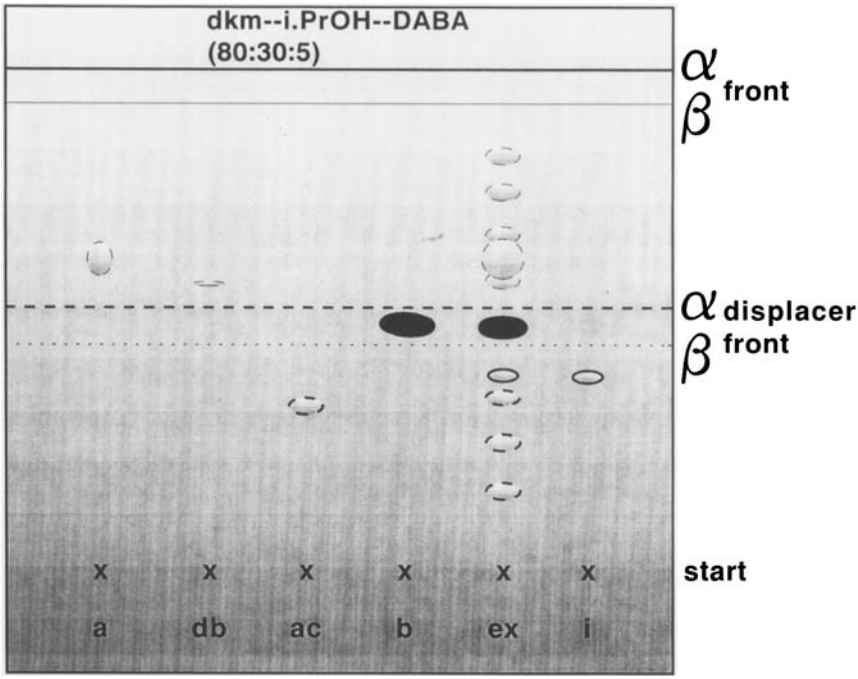

Figure 6.
Forced-flow displacement thin-layer chromatography of 2-deoxyecdysone (**a**), 2-deoxy-20-hydroxyecdysone (**db**), 20-hydroxyecdysone-22-acetate (**ac**), 20-hydroxyecdysone (**b**), the extraction of Silene otites (L.) (**ex**), and integristerone (**i**) is given on TLC plate silica gel 60 F-254 using the mobile phase dichloromethane--i.propanol--3-dimethylaminopropylamine (80:30:5) with flow rate of 0.45 ml/min.

members of the displacement train. These results can give the basis of ecdysteroid separations by displacement HPLC, as has been shown before [6, 9, 11-13].

Thin-layer displacement chromatography can also be performed in a forced-flow system [7]. While the movement of developing solvents in classical planar chromatography is propagated by capillary forces, forced-flow TLC uses pumps to deliver the mobile phase. Thereby, the speed of development can be regulated [7, 10, 11], just as it has been done in the

case of HPLC. For optimal separations, the flow rate should be chosen over a certain limit (Figs. 5, 6). The displacement thin-layer chromatography requires a definite speed of development to reach optimised and reproducible separations.

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