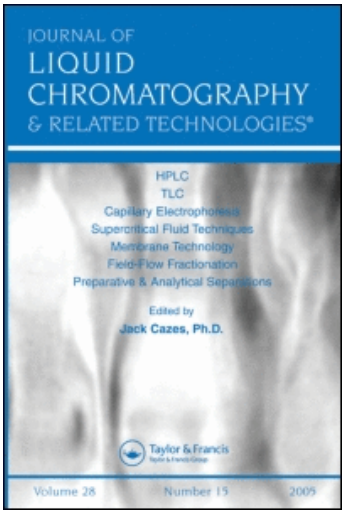

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The Application of Systems with Different Selectivity for the Separation and Isolation of some Furocoumarins

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THE APPLICATION OF SYSTEMS WITH DIFFERENT SELECTIVITY FOR THE SEPARATION AND ISOLATION OF SOME FUROCOUMARINS*

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ABSTRACT

Using thin layer chromatography in flat chamber or liquid column chromatography on the micropreparative scale the separation and isolation of furocoumarins from *Angelica Archangelica off.*(L) and *Pastinaca sativa* (L) fruit extracts was carried out.

The best results were obtained using chromatography and rechromatography in different systems (e.g.,different selectivity of reversed and normal phase systems). Close analogy of polarity and molecular structure of furocoumarins limited the loading of the system to 0.001 g per 1 g of the adsorbent.

INTRODUCTION

Some substances from the furocoumarins group are important drugs in therapy of psoriaris and leucodermy. Especially a rich source of these photoactive compounds

*) Preliminary report on this work was presented at the Sixth Danube Symposium on Chromatography in Varna, Bulgaria, 12-17 October, 1987.

are the fruits of *Archangelica off.* and *Pastinaca sativa*. Due to the similarity of molecular structures of furocoumarins and similar polarity their separation and isolation in the preparative scale is difficult.

The satisfactory results of analytical separation of coumarins were obtained using HPLC (1,2), or combination of thin layer chromatography and reversed-phase HPLC (3). Swager and Cardellina (4) have separated some coumarins in the milligram scale using various chromatographic methods and systems. More recently the preparative isolation of some furocoumarins from plant extracts was performed using overpressured layer-chromatography (OPLC) (5,6). Looking for the simpler and cheaper LCC separation methods the commercial Lobar A Si 60 column (E.Merck) and straight phase systems were applied for the isolation of some components of *Archangelica off.* fruit extracts (7).

In the present investigations reversed phase chromatographic systems for the separation and isolation of components of furocoumarin fraction from plant extracts were optimized using the horizontal sandwich chamber with a glass distributor of eluent. The chamber enables equilibration of the system (as in column chromatography), gradient elution, preconcentration of the sample (8). The formation and separation of zones can frequently be observed under UV light.

The design of the chamber allows also for continuous development and band application of the sample across the plate from the edge of the layer (9-11).With this mode of sampling the size of the sample can be greatly increased and the effect of the system overloading can be examined.

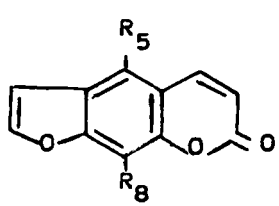
TABLE 1

List of Compounds Investigated

No	Name of compound	Abbreviation	Structure	Substituents
1	Bergapten	B	1	R_5 = OCH_3
2	Phellopterin	Ph	1	R_5 = OCH_3 R_8 = $OCH_2CH{=}C(CH_3)_2$
3	Imperatorin	I	1	R_8 = $OCH_2CH{=}C(CH_3)_2$
4	Isopimpinellin	iP	1	R_5 = R_8 = OCH_3
5	Xanthotoxin	X	1	R_8 = OCH_3
6	Xanthotoxol	Xol	1	R_8 = OH
7	Osthol	Os	3	R_7 = OCH_3 R_8 = $CH_2CH{=}C(CH_3)_2$
8	Umbeliprenin	U	3	R_7 = $O[CH_2CH{=}C(CH_3)CH_2]_2$ $-CH_2CH{=}C(CH_3)_2$
9	Pimpinellin	P	2	R_5 = R_6 = OCH_3

STRUCTURES:

1



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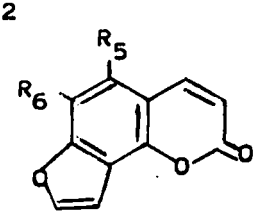

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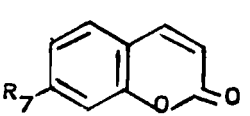

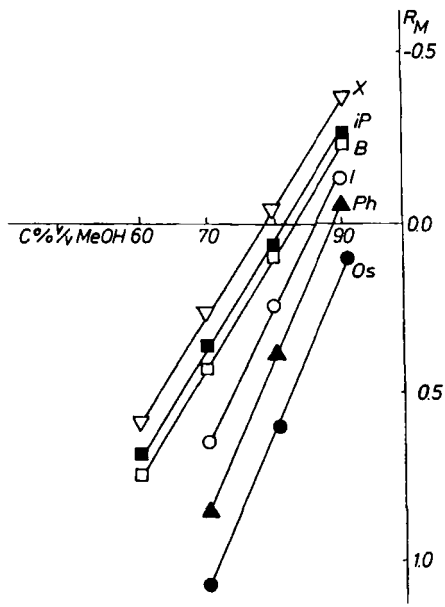

FIGURE 1 Plots of R_M vs concentration(%) of the modifier in mobile phase. Active solid: silanized silica gel.Mobile phase: methanol (MeOH) in water. For the identification of solutes see Table 1.

EXPERIMENTAL

The petroleum ether extracts of *Archangelica off.* and *Pastinaca sativa* fruits were analyzed and separated chromatographically. Analytical thin layer chromatography was carried out on 100 x 200 mm glass plates covered with 0.25 mm silanized silica gel (E.Merck, Darmstadt, F.R.G.). Micropreparative thin layer chromatography was carried out on 100 x 200 mm glass plates covered with 0.75 mm silanized silica for preparative chromatography (E.Merck). The thin layer elution was performed in the sandwich chamber with a glass distributor of the eluent produced by Polish

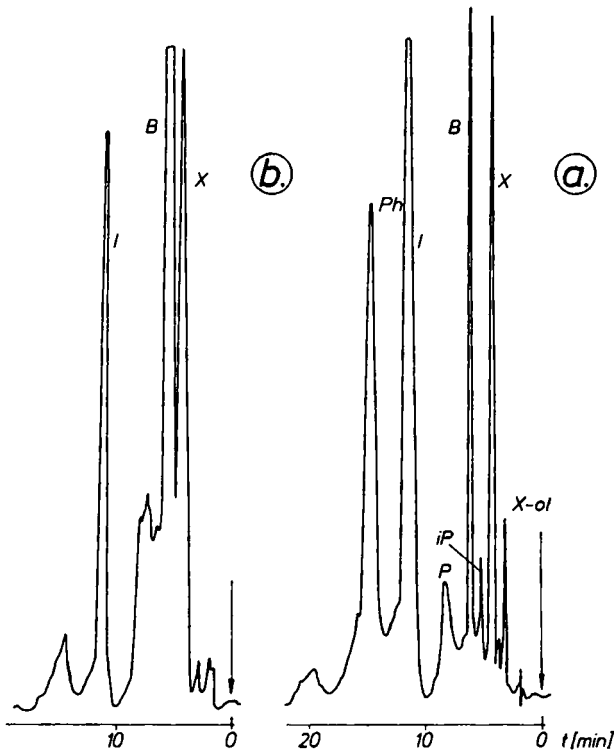

FIGURE 2 Chromatograms (HPLC) of fruit extracts from (a) *Angelica Archangelica off.* L and (b) *Pastinaca sativa* L. Column: LiChrosorb RP-18.Mobile phase: 6:4 methanol-water. For the identification of solutes see Table 1.

Reagents,Lublin (12). The zones and spots were localized under UV light at 366 nm. The localized zones can be scraped off in the conventional way and the fractions isolated by extraction with a strong solvent. The extraction was carried out using glass columns stoppered with glass wool, the upper end of the column forms a funnel to facilitate adsorbent collection.

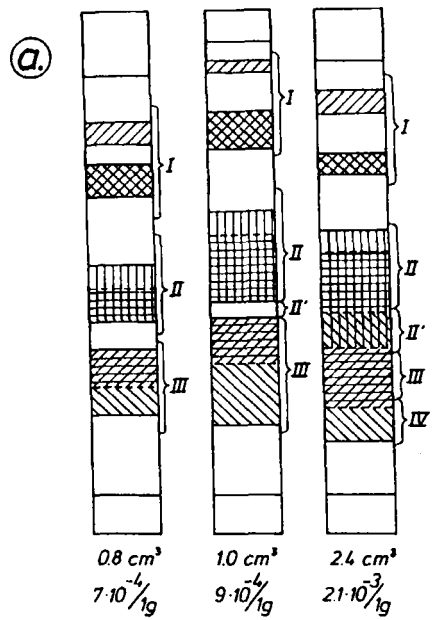

FIGURE 3 (a) Micropreparative thin-layer chromatograms of the extract from *Angelica Archangelica* fruits. Active solid: silanized silica gel. Mobile phase: 6:4 methanol-water. Samples: increasing volumes of 0,5% v/v solution of *Archangelica* extract.
(b) Analytical (HPLC) chromatograms of the fractions eluted from zones indicated on the plates. Column: LiChrosorb RP-18. Mobile phase: 6:4 methanol-water. For the identification of solutes see Table 1.

For micropreparative column separation of the extracts Lobar pre-packed columns: LiChroprep Si 60 (40-63μm), size A (240 x 10 mm i.d.) and LiChroprep RP-8 (40-63 μm), size B (310 x 25 mm i.d.) (E.Merck) were used. The eluent was delivered from a home-made pump at a flow rate of 5-6 ml/min and pressure of 0.3-0.6 MPa. The samples were injected using a calibrated syringe by the stop-flow method.

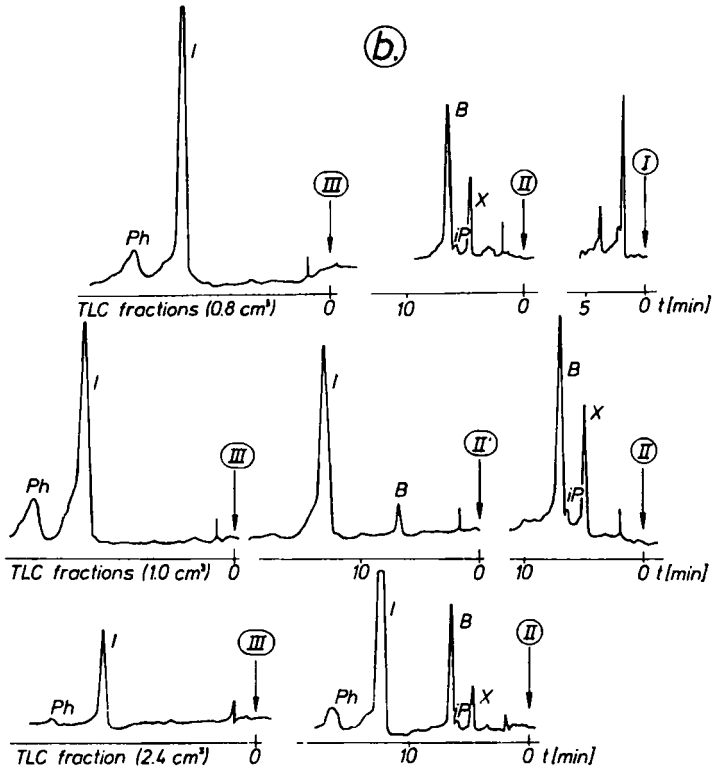

FIGURE 3 (continued)

A photometric detector at 254 nm was used. Mixtures of dichloromethane and heptane (7:3) containing 5% diisopropyl ether and 1:1 or 7:3 mixtures of methanol-water were used as the mobile phase for normal-phase and reversed-phase systems, respectively.

For the analysis of the plant extract and eluted column and TLC fractions HPLC was applied using a liquid chromatograph (produced by the Institute of Physical Chemistry, Warsaw) equipped with a 200 ml

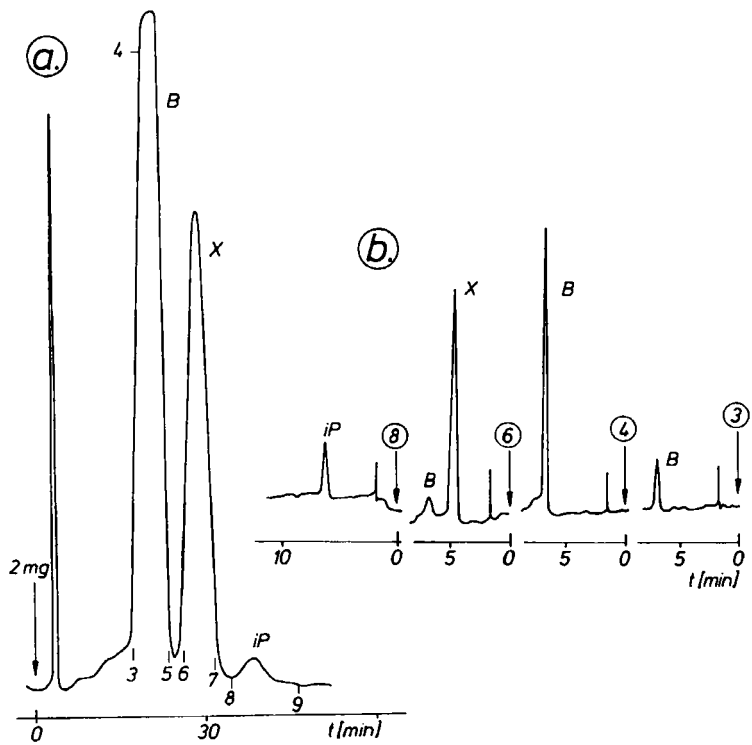

FIGURE 4 (a) Rechromatography on micropreparative scale of the fraction II eluted from TLC plates (see Fig.3a). Column: Lobar A, Si 60. Mobile phase: 7:3 dichloromethane + heptane containing 5% diisopropyl ether. Flow rate 5.8 ml/min. Pressure 0.6 MPa. Numbers indicate fractions.
(b) Analytical (HPLC) chromatograms of the fractions eluted from the Lobar A Si 60 column. Column: LiChrosorb RP-18. Mobile phase: 6:4 methanol-water. For the identification of solutes see Table 1.

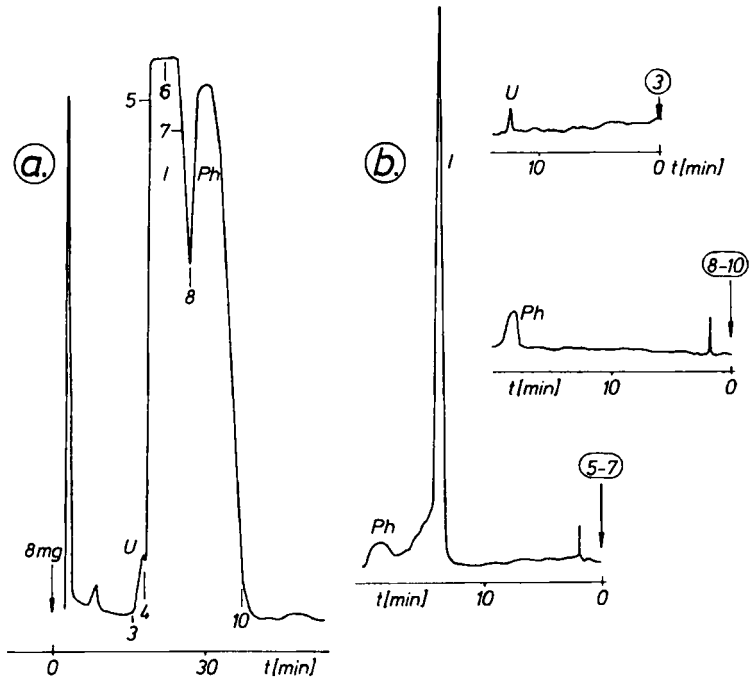

FIGURE 5 (a) Rechromatography of the fraction III eluted from TLC plates (see Fig.3a). Column: Lobar A Si 60. Mobile phase: 7:3 dichloromethane + heptane containing 5% diisopropyl ether. Flow rate 5 ml/min. Pressure 0.6 MPa. Numbers indicate fractions.
(b) Analytical (HPLC) chromatograms of the fractions eluted from the Lobar A Si 60 column. Column: LiChrosorb RP-18. Mobile phase: 6:4 methanol-water. For the identification of solutes see Table 1.

syringe pump and a UV (254 nm) detector. A 250 x 4 mm i.d. stainless-steel column was packed with 10 μm LiChrosorb RP-18 (E.Merck) and 5 μl samples of extract solutions in the eluent were injected. Methanol-water mixtures were used as the mobile phase at a flow rate of 1.2 ml/min. All measurements were carried out at ambient temperature.

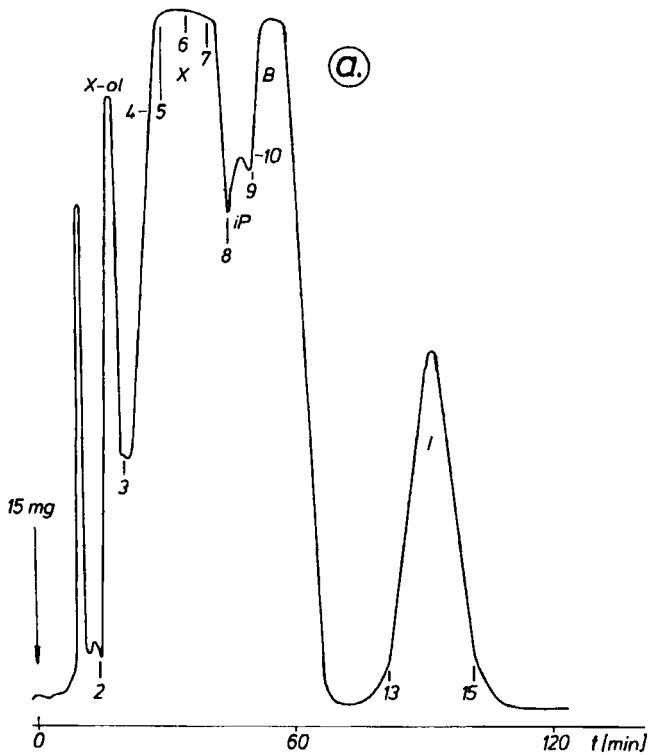

FIGURE 6 (a) Micropreparative separations of the extract from *Pastinaca sativa* fruits. Column: Lobar B RP-8. Mobile phase 7:3 methanol-water Flow rate: 5.5 ml/min. Sample: 1.4 ml of methanolic solution containing 15 mg of the extract. Numbers indicate collected fractions.
(b) Analytical (HPLC) chromatograms of the fractions eluted from column. Column: LiChrosorb RP-18. Mobile phase: 6:4 methanol-water.

MATERIALS

The investigated fruit extracts of *Angelica Archangelica off.* (L) and *Pastinaca sativa* (L) were obtained in a Soxhlet-type extractor with the closed solvent (petroleum ether) cycle (13). The dried

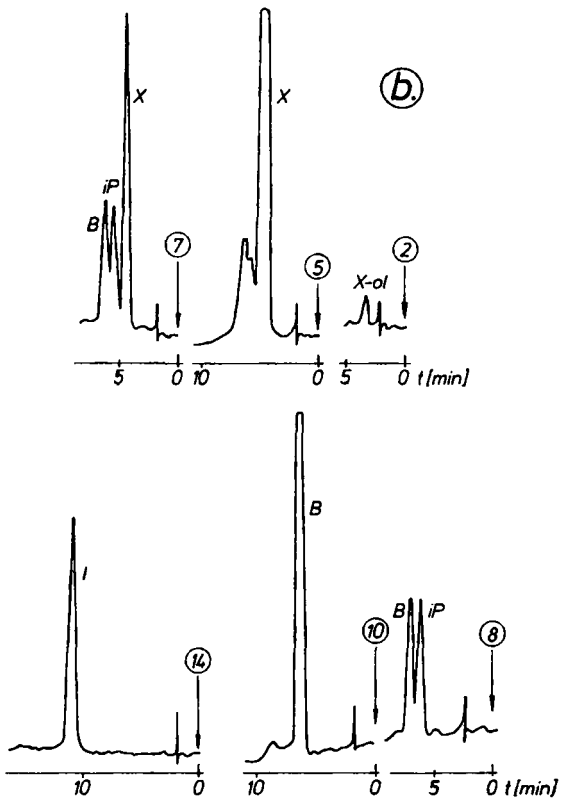

FIGURE 6 (continued)

extracts were dissolved in the mobile phase. Analytical reagent-grade organic solvents (all E.Merck) were applied.

RESULTS AND DISCUSSION

In order to select the optimum separation conditions of the plant extracts a systematic thin layer chromatographic analysis of the standards of the main furocoumarins occurring in *Archangelica off.* and

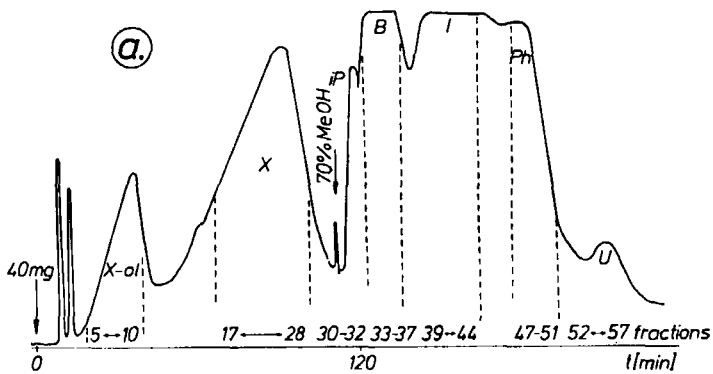

FIGURE 7 (a) Micropreparative two-step gradient separation of the extract from *Angelica Archangelica* fruits. Column: Lobar B RP-8. Mobile phase: 1:1 methanol-water; after 110 min 7:3 methanol-water. Flow rate: 6 ml/min. Sample: 2 ml of methanolic solution containing 40 mg of the extract. Numbers indicate collected fractions.
(b) Analytical (HPLC) chromatograms of the fractions eluted from the Lobar B RP-8 column. Column: LiChrosorb RP-18. Mobile phase: 6:4 methanol-water.

Pastinaca sativa fruits was carried in the binary solvent systems. Typical R_M vs.c% v/v of modifier relationships are presented in Figure 1(see also ref. 14). Using the thin-layer and HPLC RP-18 chromatographic data and basing on the earlier studies on the separation of components of *Heracleum Genus* fruits (14) two systems for preparative separation of extracts were chosen:

1. reversed phase system: 50-70% methanol in water (see Figure 1)

2. 5% v/v diisopropyl ether in binary diluent dichloromethane-heptane (7:3) / silica (7). The combination of these two systems may permit to isolate

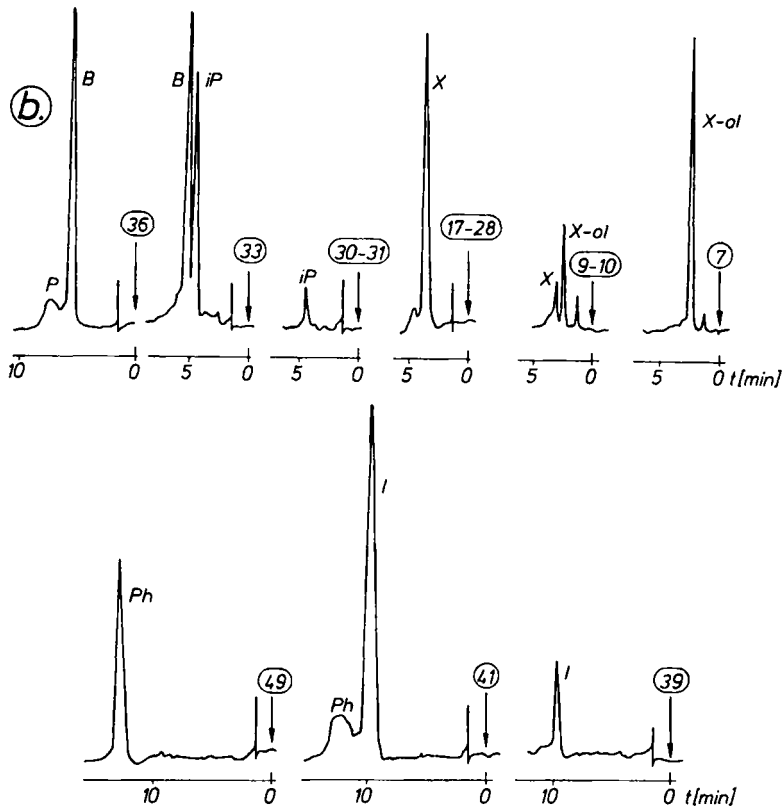

FIGURE 7 (continued)

some pure components of plant extracts, especially the photoactive ones - xanthotoxin and bergapten.

Figure 2 shows the chromatograms obtained for fruit extracts of *Archangelica off.* and *Pastinaca sativa* using reversed-phase HPLC. The main compounds of both extracts are bergapten, xanthotoxin and imperatorin. The highest amounts of bergapten and xanthotoxin were found in *Pastinaca sativa*, and the highest amounts of imperatorin and also phellopterin in *Archangelica off.*

The results of the investigations on the influence of the sample size on separation of *Archangelica* extract are shown in Figure 3. Using the plates covered with 0.75 mm layer of silanized silica, increasing volumes of 0.5 % v/v extract solutions were introduced and then developed with methanol-water (6:4) mixture. Under these conditions three main fractions were obtained - the first with unidentified compounds, the second zone containing xanthotoxin, isopimpinelin and bergapten, and the third - imperatorin and phellopterin.

The rechromatography of the second zone isolated from the thin-layer plates, using another system resulted in base-line separation (see Fig.4); unfortunately,the separation of less polar furocoumarins from the third zone (imperatorin, phellopterin) is not so efficient (see Fig.5). It was also shown that for closely related compounds overloading of this system should not exceed 0.001g sample per 1g of the adsorbent.

Basing on TLC-RP data the micropreparative separation of *Pastinaca sativa* fruit extract was carried out using LOBAR-8 (RP-8) column and 70% solution of methanol in water (Fig.6a).The analytical chromatograms of the collected fractions (see Fig.6b) indicate the necessity of rechromatographing the non-separated fractions.The application of two-step gradient elution improves the isolation of xanthotoxol and xanthotoxin (Fig.7), but other components are only partially separated.

The best results are obtained in two-stage separations using systems of differentiated selectivity, e.g. normal and reversed-phase chromatography.

CONCLUSIONS

1. The compounds occurring in the fruits of some *Umbelliferae* family plants are difficult to separate (especially furocoumarins) due to their similar chemical structure.
2. Two-step gradient elution using reversed-phase system permits to obtain pure xanthotoxol and xanthotoxin.
3. Rechromatography (using a different system) significantly improves the separation. In reversed-phase system imperatorin and phellopterin can be well separated and in normal phase system the more polar compounds (xanthotoxin,bergapten).
4. The similarity of molecular structures of furocoumarins limits the overloading"of the system to about 0.001g/1g adsorbent.

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