

## Comparison of selectivity of silica and Florisil in the separation of natural pyranocoumarins

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### ABSTRACT

Natural pyranocoumarin standards were separated using thin layers of Florisil (magnesium silicate) or silica and a sandwich chamber. Binary and ternary solvents containing a polar modifier (diisopropyl ether, ethyl acetate, acetonitrile or 2-propanol) as a mobile phase were used. Based on the relationship between  $R_M$  values and the concentration of polar modifier in the eluent, the optimum conditions were chosen for the separation of the isomers (samidin and isosamidin or *cis*- and *trans*-khellactone) and pyranocoumarin components of plant extracts from the fruits of *Libanotis* species. The correlation of retention parameters for silica and Florisil showed individual differences in selectivity.

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### INTRODUCTION

A large number of plant species containing coumarins, furanocoumarins and pyranocoumarins have been investigated using thin-layer chromatography [1,2] and over-pressurized thin-layer chromatography (TLC) [3,4]. The TLC method is useful for chemotaxonomic studies [5,6], analyses of coumarins and their metabolites [7-10], optimization of high-performance liquid chromatographic systems [11,12] and preparative separations by thin-layer, column [9,13] or medium-pressure liquid chromatography [14]. Interesting results for the optimization of the chromatographic system for the separation of furanocoumarins using silica gel and Florisil (magnesium silicate) adsorbents [15] stimulated a comparison of the selectivities of these two adsorbents in the separation of natural pyranocoumarins. The choice of silica and Florisil was due to the different surface structures and characteristics of the adsorption sites, leading to different selectivity characteristics. Systematic studies of the selectivity of the investigated systems (adsorbent-binary or ternary eluent) allows the selection of the optimum conditions for the separation of pyranocoumarin standards and for the chemotaxonomic study of medicinal plant species containing pyranocoumarins and their identification in plant extracts by TLC.

## EXPERIMENTAL

The extraction of powdered ripe fruits from *Libanotis transcaucasica* Schischkin, *Libanotis delichostylos* Schischkin and *Seseli libanotis* (*Libanotis intermedia* Rupr.) was carried out with methanol in a Soxhlet-type extractor at room temperature. Analytical (and micropreparative) TLC was carried out on 100 × 200 mm or 200 × 200 mm glass plates covered with 0.25- or 0.5-mm layers of silica gel Si-60H (E. Merck, Darmstadt, Germany) or Florisil (Fluka, Buchs, Switzerland).

The separation was performed in sandwich-type glass chambers [16] with a glass distributor (Polish Reagents, Poland); 2- $\mu$ l samples of the standard solutes (1.0 mg/cm<sup>3</sup> in dichloromethane) were applied in the form of spots or short bands and eluted with appropriate eluents over a distance of 15 cm. The spots were localized under UV light at 254 nm.

*Materials*

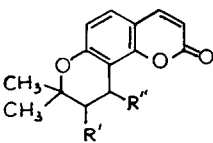
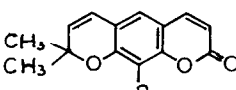
The pyranocoumarin standards (Table I) were obtained from the Department of Chemistry, Royal Danish School of Pharmacy, Copenhagen, Denmark. Organic solvents of analytical-reagent grade were purchased from E. Merck. The plant material was collected in the Pharmacognostic Garden, Medical Academy of Lublin, in 1989.

## RESULTS AND CONCLUSIONS

The results of the optimization of the adsorbent–eluent systems for separation of ten pyranocoumarin standards with binary eluent mixtures are shown in Figs. 1 and 2 as plots of  $R_M$  vs. concentration of acetonitrile in dichloromethane. (% v/v).

Pyranocoumarins with two hydroxy groups in the molecule such as *cis*- (**9**) and *trans*-khellactone (**10**) were strongly adsorbed, giving measurable  $R_M$  values only at higher concentrations of the modifiers (polar solvents added to dichloromethane or *n*-heptane) for both adsorbents examined.

TABLE I  
STRUCTURES OF THE COMPOUNDS INVESTIGATED

Structure	Name	R	R'	R''	No. <sup>a</sup>
	Samidin		OCOH = C(CH <sub>3</sub> ) <sub>2</sub>	OCOCH <sub>3</sub>	1
	Isosamidin		OCOCH <sub>3</sub>	OCOH = C(CH <sub>3</sub> ) <sub>2</sub>	2
	Visnadin		OCOCH(CH <sub>3</sub> )		
			CH <sub>2</sub> CH <sub>3</sub>	OCOCH <sub>3</sub>	3
	Dihydroseselin		H	H	4
	Pterixin		OCOCH <sub>3</sub>	OCOC(CH <sub>3</sub> ) = CHCH <sub>3</sub>	6
	Diseneciyoil- <i>cis</i> -khellactone		OCOCH = C(CH <sub>3</sub> ) <sub>2</sub>	OCOCH = C(CH <sub>3</sub> ) <sub>2</sub>	8
		<i>cis</i> -Khellactone		OH	OH
<i>trans</i> -Khellactone			OH	OH	10
	Xanthyletin	H			5
	Luvangetin	OCH <sub>3</sub>			7

<sup>a</sup> Numbering corresponds to typical sequence and analogy of molecular structure and polarity.

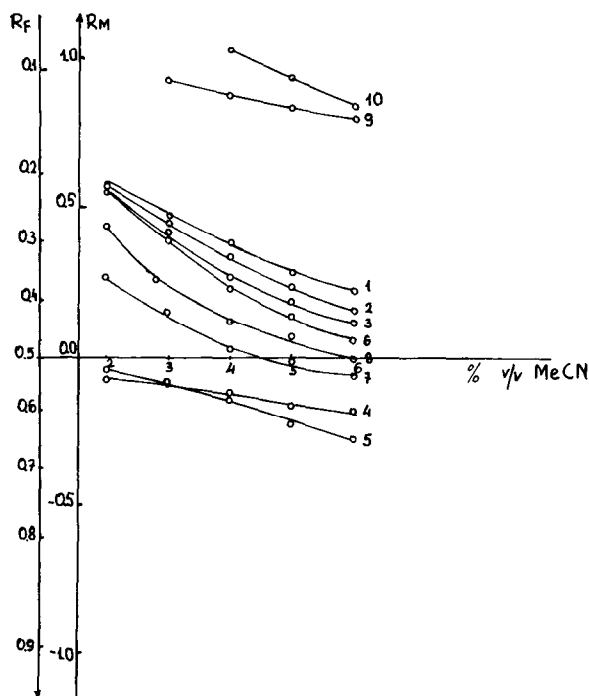


Fig. 1.  $R_M$  values of pyranocoumarins vs. concentration of acetonitrile (MeCN) in dichloromethane as the eluent and silica as the adsorbent. Numbering of solutes as in Table I.

An increase in selectivity at higher acetonitrile concentrations for silica and a decrease for Florisil were observed for both adsorbents: the separation of isomers *cis*-**(9)** and *trans*-khellactone **10** or samidin **(1)** and isosamidin **(2)** is possible, but they have the weakest retention on silica.

The selectivity of the system used is also illustrated by  $R_M$  vs.  $R_M$  correlation plots obtained for silica and Florisil with a few mobile phases chosen in optimization investigations; 6% (v/v) acetonitrile in dichloromethane (Fig. 3), 6% (v/v) ethyl acetate in dichloromethane (Fig. 4), 10% (v/v) diisopropyl ether in dichloromethane (Fig. 5) and 5% (v/v) 2-propanol mixture in *n*-heptane-dichloromethane (1:1) (Fig. 6). The spread of points in the  $R_M$  vs.  $R_M$  correlation plots shows the different selectivities of the two adsorbents. Points lying close to a vertical line (e.g., Fig. 6, **3** and **7**; **1** and **2**) correspond to solutes well separated on Florisil but which have similar  $R_F$  ( $R_M$ ) values on silica.

The selectivity also depends on the eluent used. One can compare of solutes **1** and **2** in Fig. 6 (no separation on either adsorbents) and in Figs. 4 and 5 (good separation on Florisil). For some eluents the spread of points is minimal (Fig. 3), which shows that both adsorbents have similar selectivities.

The less polar pyranocoumarins, such as xantyletin **(5)** and dihydroseselin **(4)** were easily separated in all the systems examined.

Pyranocoumarins possessing the same polar radicals in the molecule, such as samidin **(1)**, isosamidin **(2)** and pterixin **(6)** can be better separated on Florisil than on silica.

TABLE II  
 $R_M$  AND  $\Delta R_M$  VALUES OF PYRANOCOURMARINS

Selectivities for solutes No. 1-4 and 6-10 are relative to xanthyletin (No. 5).

Com- pound <sup>a</sup>	10% (v/v) diisopropyl ether in dichloromethane			5% (v/v) 2-propanol in dichloromethane-heptane (1:1)			6% (v/v) acetonitrile in dichloromethane			6% (v/v) ethyl acetate in dichloromethane						
	Silica	Florisol	$\Delta R_M$	Silica	Florisol	$\Delta R_M$	Silica	Florisol	$\Delta R_M$	Silica	Florisol	$\Delta R_M$				
1	0.30	0.53	0.18	0.25	-0.11	0.25	-0.33	-0.33	0.04	0.64	0.26	0.54	0.38	0.80	0.52	0.67
2	0.28	0.51	0.37	0.44	-0.11	0.25	-0.33	-0.33	-0.06	0.54	0.18	0.46	0.38	0.80	0.68	0.83
3	0.25	0.48	0.31	0.38	-0.10	0.26	0.37	0.37	0.01	0.61	0.15	0.43	-0.30	0.12	0.49	0.64
4	-0.18	0.05	-0.23	-0.16	-0.25	0.11	-0.30	-0.30	-0.50	0.10	-0.18	0.10	-0.36	0.06	-0.30	-0.15
5	-0.23	0.00	-0.07	0.00	-0.36	0.00	0.00	0.00	-0.60	0.00	-0.28	0.00	-0.42	0.00	-0.15	0.00
6	0.21	0.44	0.35	0.42	-0.20	0.16	0.35	0.35	-0.15	0.45	0.08	0.36	-0.20	0.22	0.43	0.58
7	-0.08	0.15	-0.13	-0.06	-0.15	0.21	0.07	0.07	-0.38	0.22	-0.07	0.21	-0.18	0.24	0.28	0.43
8	0.14	0.37	0.22	0.29	-0.30	0.06	0.18	0.18	-0.26	0.34	-0.01	0.27	-0.19	0.23	0.35	0.50
9	1.08	1.31	1.06	1.13	0.17	0.53	1.06	1.06	1.02	1.62	0.81	1.09	1.04	1.46	0.96	1.11
10	1.26	1.49	1.12	1.19	0.30	0.66	1.13	1.13	1.12	1.72	0.86	1.14	1.20	1.62	1.07	1.22

<sup>a</sup> See Table I.

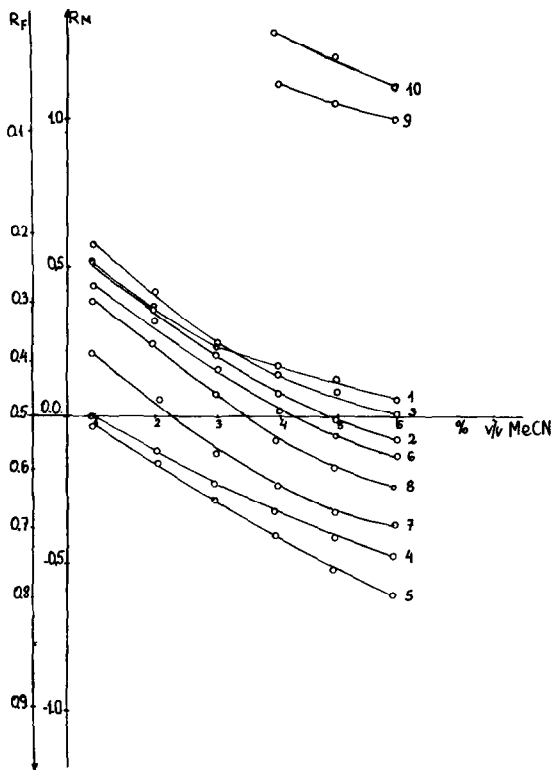


Fig. 2.  $R_M$  values of pyranocoumarins vs. concentration of acetonitrile (MeCN) in dichloromethane as the eluent and Florisil as the adsorbent.

A ternary eluent containing 5% (v/v) 2-propanol in *n*-heptane–dichloromethane (1:1) shows the greatest eluent strength for polar pyranocoumarins *e.g.*, *cis*-(**9**) and *trans*-khellactone (**10**) on silica, but with weaker selectivity for other compounds.

Table II summarizes the  $R_M$  and  $\Delta R_M$  values obtained for the same eluent and both adsorbents, where the  $\Delta R_M$  values are a measure of the selectivity  $\{\Delta R_M = \log \alpha$ , where  $\alpha$  = separation factor;  $R_M = \log[(1 - R_F)/R_F] = \log k'\}$ .

The 6% (v/v) acetonitrile (modifier) shows a similar selectivity on silica and Florisil for almost all the compounds studied. Comparison of pyranocoumarins possessing similar large polar radicals such as samidin (**1**) isosamidin (**2**) visnadin (**3**), pterixin (**6**) and disencioyl-*cis*-khellactone (**8**) shows that 6% ethyl acetate in dichloromethane as a mobile phase and Florisil is the most selective system for their separation. Different selectivities of silica and Florisil with 5% 2-propanol in heptane–dichloromethane (1:1) as eluent for the separation of stereoisomers [*cis*-(**9**) and *trans*-khellactone (**10**)] was established.

The results illustrated above can be utilized in the preparative chromatography of the natural pyranocoumarins in plant material extracts with both adsorbents and 5% diisopropyl ether in dichloromethane as the mobile phase. Fig. 7 shows the separation of coumarin extracts from *Libanotis* species fruits in comparison with

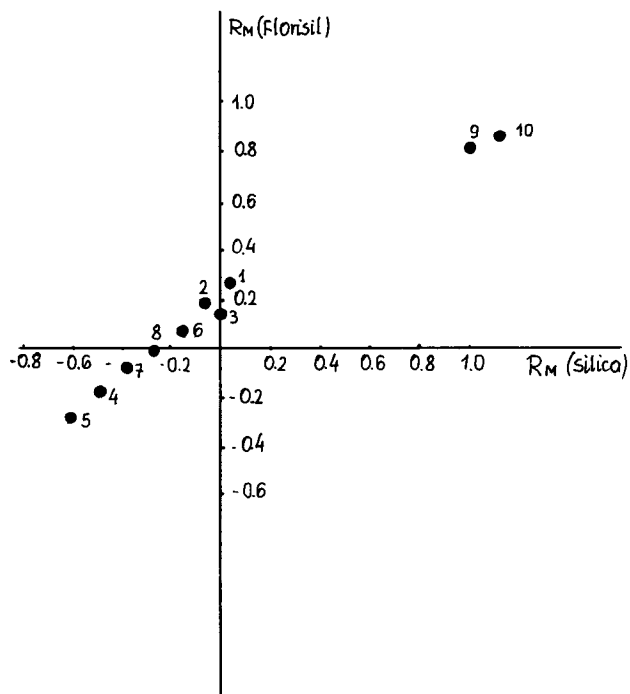


Fig. 3. Correlation of  $R_M$  values obtained on silica and Florisil with 6% (v/v) acetonitrile in dichloromethane.

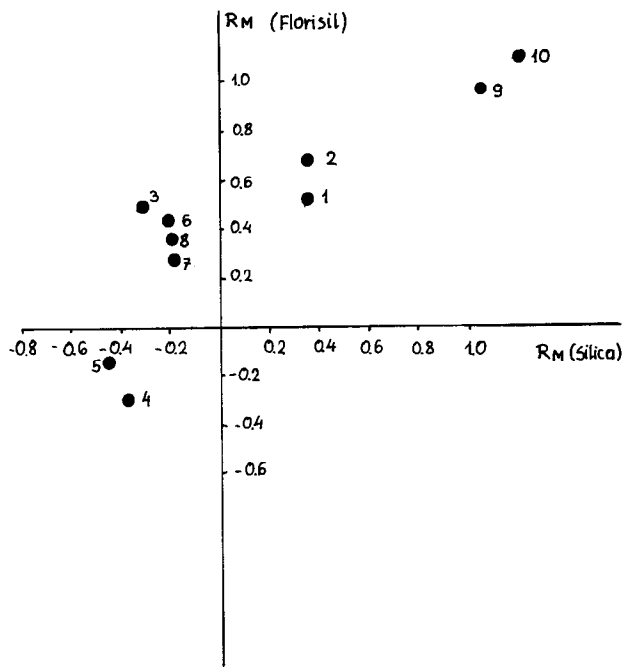


Fig. 4. Correlation of  $R_M$  values obtained on silica and Florisil with 6% (v/v) ethyl acetate in dichloromethane.

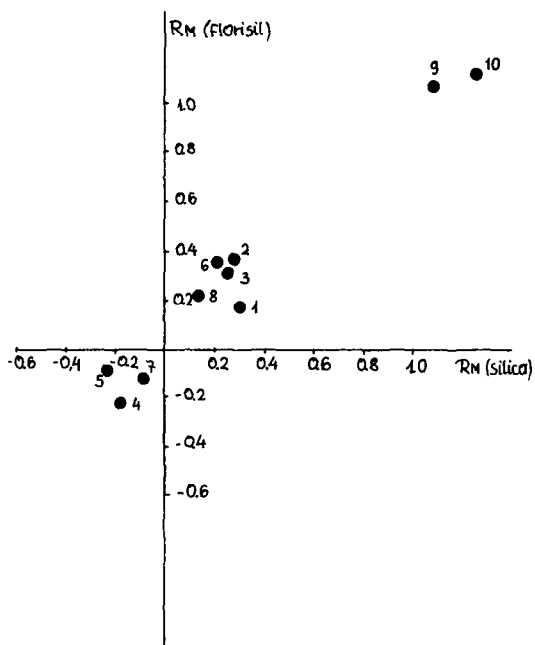


Fig. 5. Correlation of  $R_M$  values obtained on silica and Florisil with 10% (v/v) diisopropyl ether in dichloromethane.

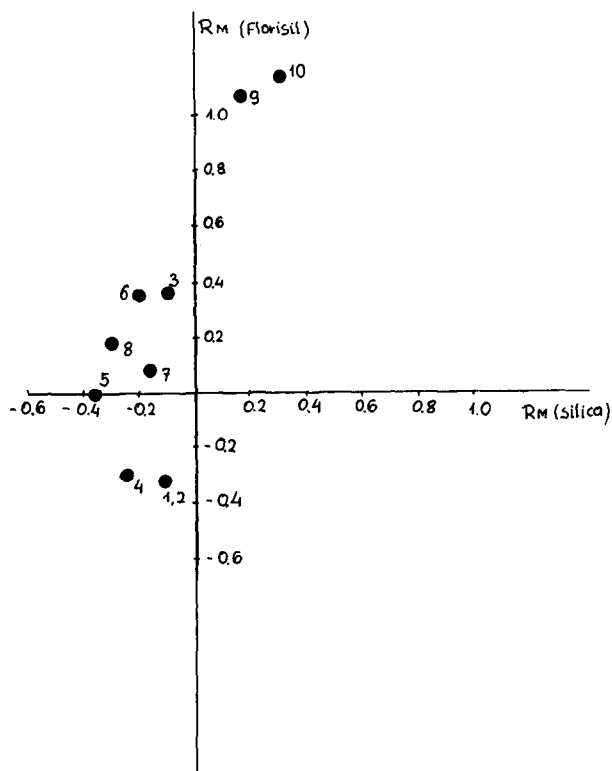


Fig. 6. Correlation of  $R_M$  values obtained on silica and Florisil with 5% (v/v) 2-propanol in *n*-heptane dichloromethane (1:6).

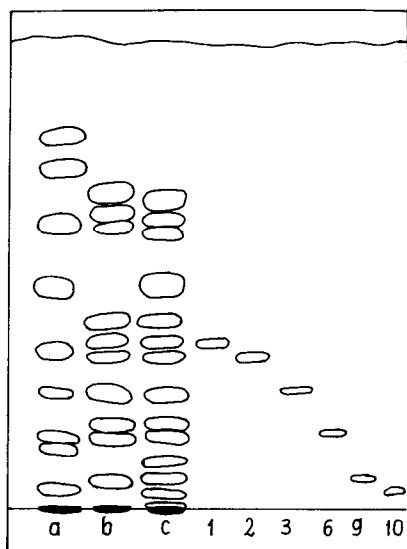


Fig. 7. Separation of extracts from *Libanotis* species fruits. Eluent, 5% (v/v) diisopropyl ether in dichloromethane; adsorbent, silica (a) *Libanotis transcaucausica* Schischkin; (b) *Libanotis delichostylos* Schischkin; (c) *Libanotis intermedia* Rupr. (*Seseli libanotis*).

pyranocoumarin standards. Some of them (samidin, pterixin, *cis*-khellactone) have been reported in the plants examined and others are characteristic of *Libanotis* and *Seseli* species [17]. The good separation of standard and all the coumarin components of the extracts examined revealed the different coumarin compositions.

Chromatography on Florisil and silica layers is useful for chemotaxonomic studies of *Umbelliferous*, *Seseli* and *Libanotis* species plants containing pyranocoumarins and can provide information on the presence of the compounds investigated by rechromatography with standards, and can be applied in screening studies of medicinal plants containing pyranocoumarins.

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