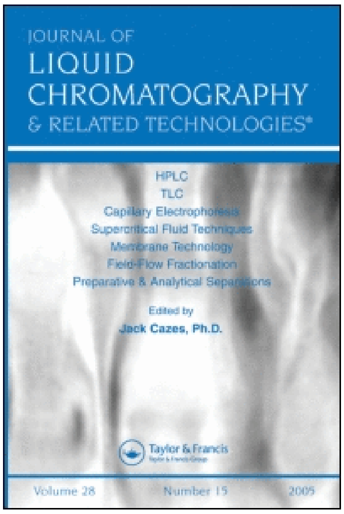

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RETENTION BEHAVIOR OF COUMARIN COMPOUNDS ON SILICA AND FLORISIL TLC LAYERS

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RETENTION BEHAVIOR OF COUMARIN COMPOUNDS ON SILICA AND FLORISIL TLC LAYERS

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ABSTRACT

This paper describes the retention behavior on silica and Florisil layers of ten coumarin compounds identified and isolated from the fruits of *Libanotis dolichostyla* Schischkin (*Umbelliferae*). The variation of retention as a function of the solvent composition has been studied. The linear relationships between retention factors (R_F and R_M) of coumarins and log *c* of various polar organic modifiers in the non-polar mobile phase were established. The elaborated model of retention was used for further optimization of separation conditions for preparative isolation of some coumarins contained in crude fractions derived from a petroleum extract obtained from the fruits of *L. dolichostyla*.

INTRODUCTION

Thin-layer chromatography (TLC) is a routine method, which has been used for years for a broad number of applications, including phytochemistry for isolation of numerous types of natural compounds.[1] In recent years, various mod-

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ern planar chromatographic techniques (including the "PRISMA" system) have been reported for coumarins.[2-5]

In the present study, an attempt was made to investigate the process of adsorption and separation of various classes of coumarin compounds (simple coumarins, furocoumarins, and pyranocoumarins) on silica and Florisil TLC layers.

Florisil, an adsorbent consisting of magnesium silicate, is mainly used in laboratory TLC practices or conventional column chromatography because of its special selectivity, different from classical silica. This special selectivity results from the specific distribution and chemical characteristics of active centers (silanol groups and electron-donor groupings) on the Florisil surface.[6]

Soczewiński and co-workers[7,8] established that the molecular model of adsorption elaborated for silica can be used for describing the adsorption behaviour of organic compounds on Florisil. Some information on the mechanism of adsorption from solution in polar solvents can be obtained from relationships between R_M (log k) and log (eluent composition). Systematic studies of these relationships have been performed earlier for a number of model compounds belonging to different chemical groups using TLC as a separation method and Florisil as an adsorbent.[6,9-12]

In this work, ternary mobile phases, consisting of a (1:1, v/v) hexane-dichloromethane mixture and polar modifiers of ester, ketone, or ether type, were applied to the analysis of the retention behavior of coumarins on silica and Florisil. The optimized chromatographic conditions were utilized in the preparative chromatography of coumarin compounds derived from plant material.

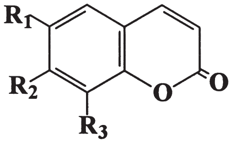
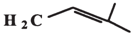
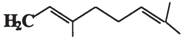
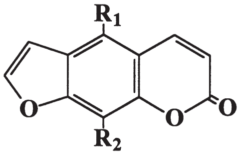
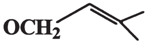
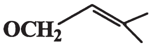
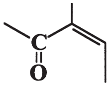
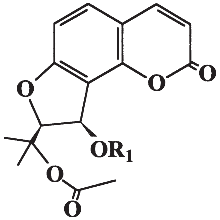
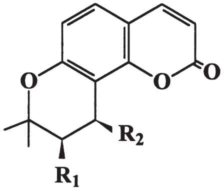
EXPERIMENTAL

Reagents and Chemicals

The molecular structures of the coumarins investigated are shown in Table 1. Xanthotoxin (5), bergapten (6), scopoletin (2), and umbelliferone (1) were purchased from Sigma (St. Louis, Missouri, USA). Imperatorin (7), isoimperatorin (8), edultin (9), osthol (3), ostruthin (4), and (+) *cis*-khellactone (10) were obtained from our collection of coumarin compounds, which were isolated from some species in the *Umbelliferae* family and identified in the Department of Pharmacognosy, Medical University of Lublin, Poland.

Dichloromethane, hexane, dioxane (Dx), tetrahydrofuran (THF), were of chromatographic grade (Merck, Darmstadt, Germany). Diethyl ether (Et_2O), diisopropyl ether (iPr_2O), methyl ethyl ketone (MeCOEt), were obtained from Merck (Darmstadt, Germany); ethyl acetate (EtAcet), n-butyl acetate (BuAcet),

Table 1. Chemical Structures of Coumarins Investigated

No.	Name	R_1	R_2	R_3	Structure
1	umbelliferone	H	OH	H	
2	scopoletin	OCH_3	OH	H	
3	osthol	H	OCH_3		
4	ostruthin		OH	H	
5	xanthotoxin	H	OCH_3	-----	
6	bergapten	OCH_3	H	-----	
7	imperatorin	H		-----	
8	isoimperatorin		H	-----	
9	edultin		-----	-----	
10	(+) *cis*- khellactone	OH	OH	-----	

ethyl formate (EtForm), acetone (MeCOMe), were from POCh (Gliwice, Poland); ethyl butyl ketone (EtCOBu) from Riedel-De Haën (Hannover, Germany), and di-n-propyl ketone (Pr_2CO) from Fluka (Switzerland). All these solvents were of analytical grade.

Plant Material and Extraction Procedure

Fruits of *L. dolichostyla* (300 g), collected in the Botanical Garden of the Dept. of Pharmacognosy (Medical University, Lublin, Poland), were dried at room temperature (20°C), powdered, and then extracted exhaustively with light petroleum in a Soxhlet apparatus. The solvent was partially evaporated under reduced pressure, and the residue left in the refrigerator (4°C) for 48 hr. The crude crystalline fraction of coumarin compounds was removed by filtration, washed with methanol, dried, and then used for preparative TLC separation.

TLC Procedure

Glass plates (10 x 20 cm) were coated with adsorbent slurry made by mixing 30 g of Florisil for TLC (Fluka, Switzerland) with 60 ml of distilled water. The plates with 0.25 mm or 0.5 mm (for micropreparative purpose) layers were dried in air, then activated in 110°C for 2 hr. Similarly, preparative (0.5 mm) silica plates were prepared using Kieselgel 60 G for TLC (Merck, Darmstadt, Germany).

In the first step for the optimization procedure, 0.1 % (w/v) standard solutions of the coumarins investigated were prepared in methanol and spotted onto Florisil and silica plates (Kieselgel 60, 10 x 20 cm, Merck, Darmstadt, Germany). The chromatograms were developed in TLC flat chambers (CHROMDES, Lublin, Poland) for a distance of 9 cm. Mobile phases were prepared as ternary mixtures containing 5, 10, 15, and 20% (v/v) of eleven polar modifiers, in hexane - dichloromethane (1 : 1, v/v).

The same chromatographic equipment was used for preparative chromatography. Methanolic aliquots, containing crude coumarin fractions, were subjected to TLC analysis on silica and Florisil plates (10 x 20 cm). Samples (100 μL) of 0.1%-0.2% (w/v) solutions of the solutes in methanol were spotted 1 cm from the shorter edge of the plate, and developed over a distance of 19 cm in horizontal chambers, using the optimized mobile phases. A 1:1 (v/v) mixture of hexane - dichloromethane, containing 20% (v/v) of ethyl acetate or di-n-propyl ketone was used on silica layers, and the same two-component diluent, containing 20% (v/v) of n-butyl acetate or methyl ethyl ketone on Florisil.

Chromatograms, with spots (or zones) of coumarins, were examined under UV light at $\lambda = 365$ nm. Zones were scraped off the plate, then sorbents, with adsorbed compounds, were washed with acetone - methanol (1 : 1, v/v). After evaporation of the solvent, the residues were re-dissolved in methanol and re-chromatographed.

RESULTS AND DISCUSSION

The investigation of retention behaviour of coumarin compounds identified in *L. dolichostyla* fruits, was performed using eleven polar modifiers possessing various chemical characteristics in a non-polar binary mobile phase, and two adsorbents (Florisil and silica) as a stationary phase.

The experimental results are presented in Tables 2-6 and in Figs 1-5. They were obtained following the Soczewiński-Snyder displacement model.[13,14] For normal-phase chromatographic systems it is expressed in the linear equation:

$$R_M = \log k = \text{const} - m \log c,$$

where k is the retention factor, m - the slope, c - the concentration (in %, v/v) of a polar modifier in the mobile phase.

Table 2. Absolute Slopes of the R_M vs. Log c Plots of Coumarins Determined Using Florisil and Silica TLC-Layers and Various Ester Type Polar Modifiers

	Silica			Florisil		
	Polar modifier:			Polar modifier:		
Compound No*	EtForm	EtAcet	BuAcet	EtForm	EtAcet	BuAcet
1	0.41	0.32	0.35	0.54	0.41	0.45
2	0.36	0.24	0.26	0.34	0.37	0.08
3	0.26	0.24	0.23	0.28	0.33	0.24
4	0.36	0.36	0.35	0.50	0.45	0.40
5	0.26	0.23	0.22	0.36	0.35	0.39
6	0.25	0.23	0.21	0.33	0.34	0.28
7	0.29	0.26	0.25	0.36	0.40	0.27
8	0.29	0.30	0.31	0.33	0.35	0.26
9	0.42	0.35	0.36	0.53	0.48	0.57
10	0.55	0.42	0.26	0.59	0.44	0.13

*Coumarin compounds numbered as in Table 1.

Table 3. Absolute Slopes of the R_M vs. Log c Plots of Coumarins Determined Using Florisil and Silica TLC-Layers and Various Ketone Type Polar Modifiers

	Silica				Florisil			
	Polar modifier:				Polar modifier:			
Compound No*	MeCOMe	MeCOEt	EtCOBu	Pr_2CO	MeCOMe	MeCOEt	EtCOBu	Pr_2CO
1	0.36	0.37	0.38	0.35	0.38	0.39	0.33	0.34
2	0.38	0.29	0.24	0.25	0.37	0.13	0.08	0.08
3	0.24	0.23	0.17	0.18	0.34	0.34	0.19	0.14
4	0.38	0.41	0.35	0.37	0.32	0.34	0.41	0.29
5	0.25	0.24	0.18	0.20	0.36	0.35	0.17	0.17
6	0.24	0.24	0.18	0.20	0.32	0.28	0.18	0.18
7	0.24	0.26	0.20	0.22	0.40	0.37	0.20	0.20
8	0.24	0.23	0.23	0.24	0.30	0.38	0.24	0.20
9	0.31	0.37	0.26	0.33	0.43	0.37	0.40	0.38
10	0.49	0.45	0.20	0.20	0.54	0.37	0.13	0.08

*Coumarin compounds numbered as in Table 1.

Table 4. Absolute Slopes of the R_M vs. Log c Plots of Coumarins Determined Using Florisil and Silica TLC-Layers and Various Ether Type Polar Modifiers

	Silica				Florisil			
	Polar modifier:				Polar modifier:			
Compound No*	Et_2O	iPr_2O	THF	Dx	Et_2O	iPr_2O	THF	Dx
1	0.26	0.28	0.32	0.30	0.38	0.45	0.31	0.42
2	0.15	0.21	0.30	0.22	0.08	0.08	0.13	0.08
3	0.17	0.13	0.21	0.22	0.19	0.13	0.20	0.26
4	0.27	0.36	0.33	0.35	0.39	0.39	0.36	0.48
5	0.19	0.16	0.19	0.20	0.33	0.14	0.19	0.23
6	0.17	0.13	0.19	0.20	0.34	0.13	0.14	0.17
7	0.18	0.17	0.20	0.22	0.24	0.14	0.24	0.29
8	0.20	0.18	0.21	0.22	0.22	0.15	0.23	0.31
9	0.28	0.25	0.31	0.26	0.45	0.26	0.45	0.54
10	0.34	0.13	0.44	0.40	0.13	0.13	0.55	0.57

*Coumarin compounds numbered as in Table 1.

Table 5. ΔR_M Values for Some Pairs of Coumarin Compounds. The Results Obtained on Silica Layers Using Various Types of Polar Modifiers in the Concentration of 20% (v/v) in a Mobile Phase

	ΔR_M (silica)							
Compound No*	EtForm	EtAcet	BuAcet	MeCOEt	Pr_2CO	Et_2O	THF	Dx
1 and 2	0.00	0.20	0.20	0.18	0.24	0.13	0.14	0.03
1 and 4	0.19	0.30	0.34	0.36	0.32	0.15	−0.34	0.35
2 and 10	0.17	0.45	0.63	0.35	0.72	0.56	0.26	0.09
5 and 7	−0.19	−0.20	0.21	−0.19	0.17	0.09	−0.14	−0.11
6 and 8	−0.20	−0.34	−0.40	−0.14	−0.26	−0.16	−0.10	−0.05
3 and 9	0.47	−0.40	0.56	−0.23	0.58	0.32	−0.13	−0.05

*Coumarin compounds numbered as in Table 1.

The comparison of the absolute values of the slopes presented in Tables 2-4 indicates that the molecular mechanism of adsorption of coumarins strictly depends on polar groups in compounds examined. For example, the presence of hydroxyl groups in the molecules of umbelliferone (1), scopoletin (2), ostruthin

Table 6. Δ R_M Values for Some Pairs of Coumarin Compounds. The Results Obtained on Florisil Layers Using Various Types of Polar Modifiers in the Concentration of 20% (v/v) in a Mobile Phase

	Δ R_M (Florisil)							
Compound No*	EtForm	EtAcet	BuAcet	MeCOEt	Pr_2CO	Et_2O	THF	Dx
1 and 2	0.80	0.73	0.91	1.24	0.95	1.03	1.44	1.32
1 and 4	0.32	0.60	0.48	0.55	0.50	0.61	0.54	0.66
2 and 10	0.61	0.17	0.13	0.57	0.00	0.13	1.00	1.19
5 and 7	0.42	0.68	0.61	−0.58	0.66	0.43	0.60	0.66
6 and 8	0.50	0.65	0.63	−0.63	0.67	0.80	0.90	1.02
3 and 9	0.51	−0.66	0.79	−0.52	0.79	0.98	−0.19	−0.23

*Coumarin compounds numbered as in Table 1.

(4), and (+) *cis*-khellactone (10), caused additional increase of the slope in relation to more hydrophobic compounds, such as furocoumarins: xanthotoxin (5), bergapten (6), imperatorin (7), or isoimperatorin (8). The direct reason of this phenomenon is the strong adsorption of coumarins with polar hydroxy groups in the molecules, especially at low concentrations of mobile phase modifiers.

The second problem was the analysis of Δ R_M values for some pairs of coumarin compounds. As one can see, they changed with the modifiers used due to the differences in the polarity of the coumarin substituents and in their interaction with the modifier molecules, and the active centers of both adsorbents. The data presented in Table 5 and 6, indicate that most of the solutes investigated are better separated on Florisil than on silica, which is expressed in higher Δ R_M values using the former adsorbent.

Changing the polar modifier also resulted in differences in selectivity. Generally, the highest variability in Δ R_M values and the better separation for some pairs of coumarins (Table 5 and 6), were achieved using ester (EtAcet, BuAcet) and ketone (MeCOEt, Pr_2CO) type polar modifiers.

The best results for the optimization of chromatographic separation for ten coumarin compounds are presented in Figs 1-5 as the R_M and R_F values plotted against the log c of the polar modifiers in the non-polar diluent (hexane - dichloromethane, 1 : 1 v/v). It can be seen, that these relationships for all compounds examined in established chromatographic conditions are linear. The results obtained were very useful for planning the separation of complex coumarin mixtures isolated from *L. dolichostyla* fruits. A satisfactory selectivity was achieved for hydrophobic coumarins in systems containing 20% of ethyl acetate (Fig. 1) or butyl acetate (Fig. 2) in the mobile phase.

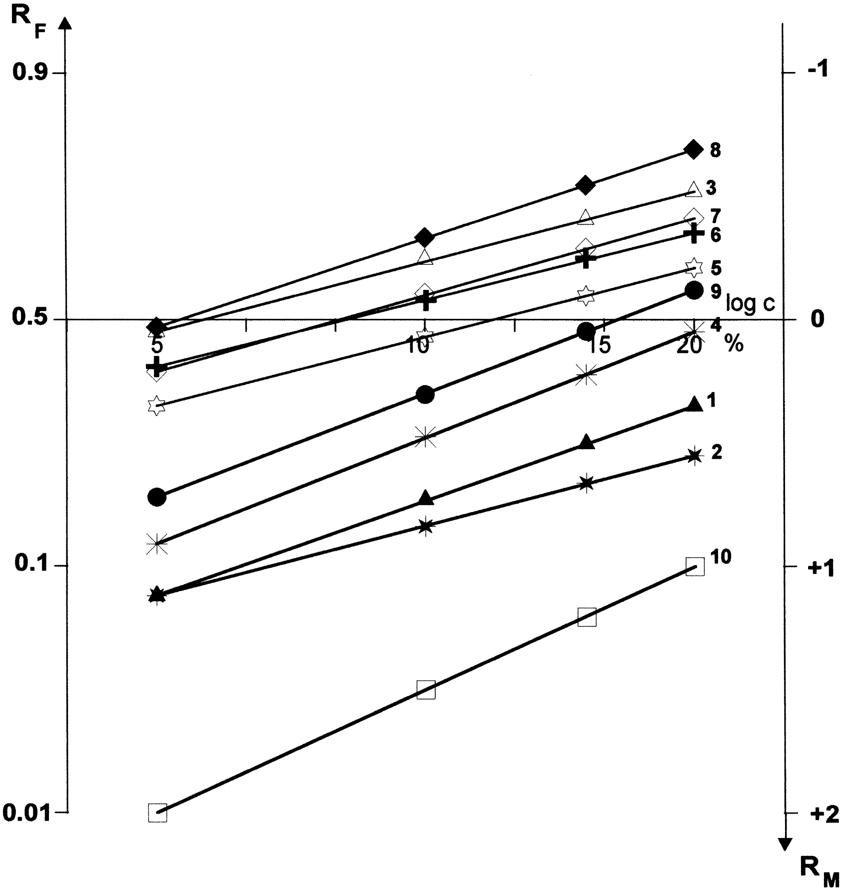


Figure 1. R_F and R_M (log k) values of coumarins plotted against the concentration (vol. %) of ethyl acetate in hexane-dichloromethane (1:1, v/v); a stationary phase: silica. Coumarins numbered as in Table 1.

The first system was utilized for micropreparative isolation of six coumarins: isoimperatorin (8), osthol (3), imperatorin (7), bergapten (6), xanthotoxin (5), and edultin (9). The latter one enabled additional isolation of the first three coumarins. Polar coumarin compounds, such as: umbelliferone (1), scopoletin (2), and (+) *cis*-khellactone (10), were isolated using 20% of methyl ethyl ketone or di-n-propyl ketone in the mobile phase (Fig. 3 and 4). Simultaneously, the application of the second modifier enabled the isolation of ostruthin (4).

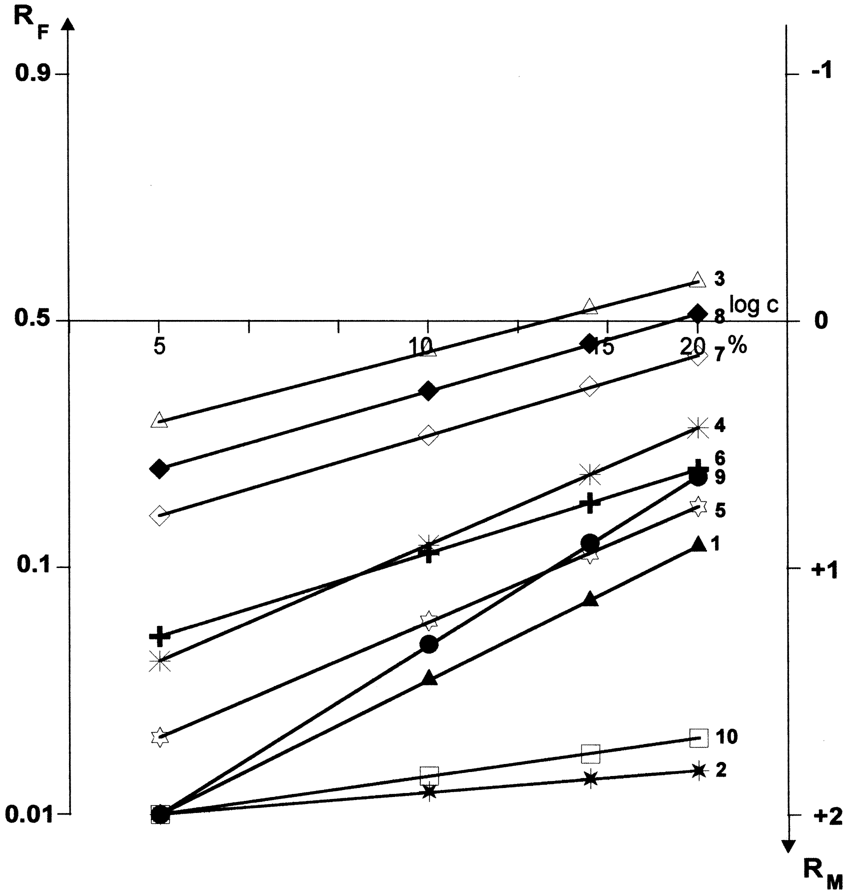


Figure 2. R_F and R_M (log k) values of coumarins plotted against the concentration (vol. %) of n-butyl acetate in hexane-dichloromethane (1:1, v/v); a stationary phase: Florisil. Coumarins numbered as in Table 1.

Successful separation of all ten coumarin compounds was also achieved using dioxane as a polar modifier. Based on the scientific literature,[15,16] it is known that this solvent possesses bifunctional characteristics connected with its molecular structure, especially the presence of two ether oxygens. One of them is bound to the adsorbent surface, and the second preserves electron-donor properties. Because of it, both coadsorption of solutes investigated on the monolayer

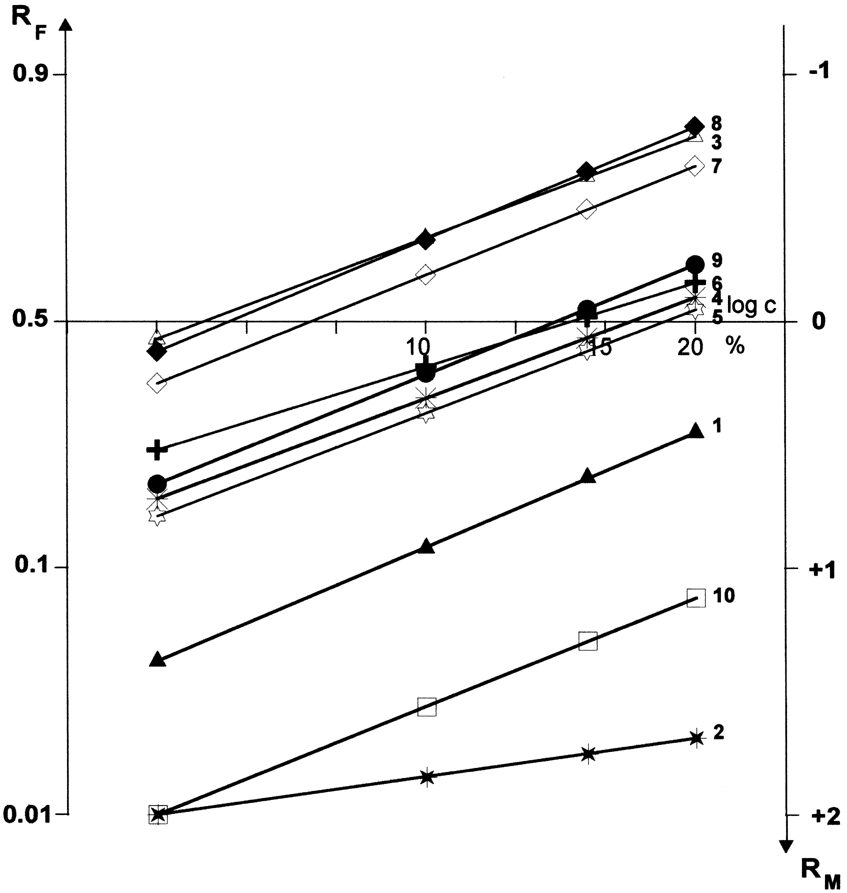


Figure 3. R_F and R_M (log k) values of coumarins plotted against the concentration (vol. %) of methyl ethyl ketone in hexane-dichloromethane (1:1, v/v); a stationary phase: Florisil. Coumarins numbered as in Table 1.

of dioxane and adsorption with displacement and solvation of molecules is possible, resulting in better separation of compounds examined. In this work; 10% dioxane was used in the mobile phase as the optimum concentration of a polar modifier, for satisfactory separation of coumarins on Florisil (Fig. 5). This chromatographic system was utilized for the control of the purity of isolated coumarin compounds.

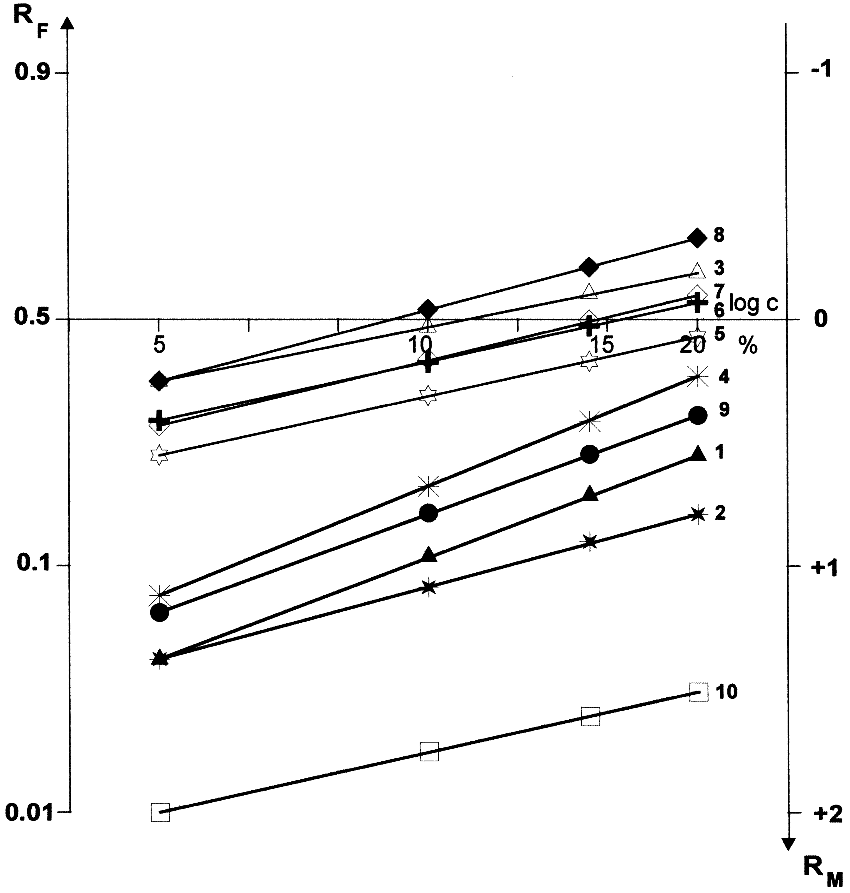


Figure 4. R_F and R_M (log k) values of coumarins plotted against the concentration (vol. %) of di-n-propyl ketone in hexane-dichloromethane (1:1, v/v); a stationary phase: silica. Coumarins numbered as in Table 1.

CONCLUSIONS

Systematic studies on the retention of various classes of coumarins, carried out using a broad spectrum of polar modifiers in a binary mobile phase and two adsorbents (Florisil and silica) as a stationary phase, led to successful and quick optimization of chromatographic separation of the compounds examined. Scaling-up the experimental results enabled the isolation of ten coumarins from

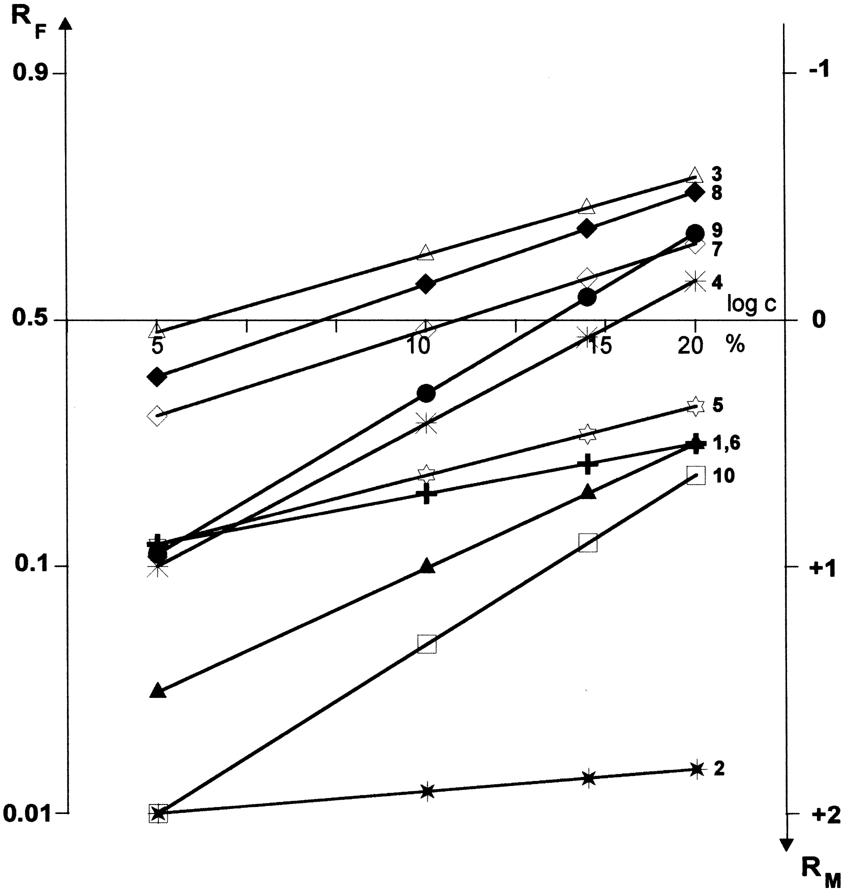


Figure 5. R_F and R_M (log k) values of coumarins plotted against the concentration (vol. %) of dioxane in hexane-dichloromethane (1:1, v/v); a stationary phase: Florisil. Coumarins numbered as in Table 1.

crude extracts, obtained from *L. dolichostyla* fruits, using preparative TLC-analysis both on silica and Florisil layers.

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