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EFFECTS OF MODIFIER AND MOLECULAR STRUCTURE OF SOME COUMARINS ON RETENTION IN REVERSED-PHASE HIGH-PERFORMANCE THIN-LAYER AND COLUMN CHROMATOGRAPHY*

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SUMMARY

Coumarins, furocoumarins and pyranocoumarins (29 compounds) were investigated in silanized silica (HPTLC RP-2 and RP-18 and HPLC RP-18)–water organic modifier (methanol, acetonitrile, dioxane, tetrahydrofuran and methanol containing acetic acid) systems. The physico-chemical properties of these compounds were characterized by their hydrophobicity parameters (R_{Mw}), determined by extrapolation of the linear relationships for retention data in binary solvent systems [$R_M = f(\varphi)$] to pure water. The effect of individual substituents on retention was quantified by using the group contribution parameter ΔR_M or $\Delta \log k'$.

INTRODUCTION

Coumarins are interesting biologically active compounds[1–4] and methods for their determination include thin-layer (TLC)[5,6] and column high-performance liquid chromatography (HPLC)[7–14].

In reversed-phase systems with non-polar chemically bonded stationary phases, the retention of solutes is mainly controlled by adjusting the type and concentration of the organic modifier in an aqueous–organic eluent[15–18]. Solvation effects play an important role and the relationship between capacity factor (log k') and the modifier content depends strongly on the molecular structure of the solute[19,20].

The effect of the qualitative and quantitative eluent composition in retention is an important problem owing to the use of mixed solvents to control the eluent strength and selectivity[21–24]. The linear relationship between the logarithm of the capacity factor and the composition of the eluent can be described by the simple equation[25–28]

$$\log k'_{\mathrm{w,\,mod}} = \log k'_{\mathrm{w}} - b\varphi_{\mathrm{mod}}$$

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* Part of this work was presented at the *Vth Danube Symposium on Chromatography, Yalta, November 11–16, 1985.*

where φ_{mod} is the volume fraction of the modifier (0.01 × vol.-%), b is a constant ($b = \log k'_w - \log k'_{mod}$), $\log k'_w$ (lipophilicity index) is the logarithm of the capacity factor for 100% water as eluent and the subscripts w and mod denote components of the eluent (water and organic modifier, respectively). The usefulness of chromatographic R_{Mw} and $\log k'_w$ values as an expression of the lipophilic character of molecules is generally recognized[29-33].

The investigated series of coumarins are increasingly used as taxonomic markers in the chemotaxonomic identification of plant species and as models for a group of drugs undergoing chemical modification *in vivo*, which justifies the systematic study of their retention behaviour in thin-layer and column chromatography[34,35]. In this regard, the aim of this work was to examine how the characteristics of the sorbent, the type of modifier used and the structure of the solutes influence their retention.

Quantitative correlations between R_M (HPTLC) and $\log k'$ (HPLC) values were obtained; the slope of these relationships permits the practical application of HPTLC data to the optimization of HPLC systems, including preparative separations[36-38].

EXPERIMENTAL

TLC was performed on 10 × 10 cm pre-coated HPTLC plates of RP-18 F_{254} or RP-2 F_{254} (E. Merck, Darmstadt, F.R.G.); 1-μl samples of the solutes (1.0 mg cm^{-3} in methanol) were spotted 1 cm from the edge and eluted with appropriate eluents in saturated tanks over a distance of 8.5 cm. The spots were localized under UV light at 254 nm.

Column HPLC was carried out using a liquid chromatograph (produced at the Institute of Physical Chemistry of the Polish Academy of Sciences, Warsaw, Poland) with a 200-ml syringe pump, a 5-μl sample injection valve and a UV detector (254 nm). A stainless-steel column (250 × 4 mm I.D.) was packed with 10 μm LiChrosorb RP-18 (E. Merck). The flow-rate was 1.2 $cm^3\ min^{-1}$. The eluents were degassed in an ultrasonic bath. The temperature was kept at 21 ± 2°C. The column dead volume was determined using an aqueous solution of sodium nitrate as the non-retained compound.

The coefficients in the regression equations were calculated from the experimental data by the multiple regression analysis using the least-squares method.

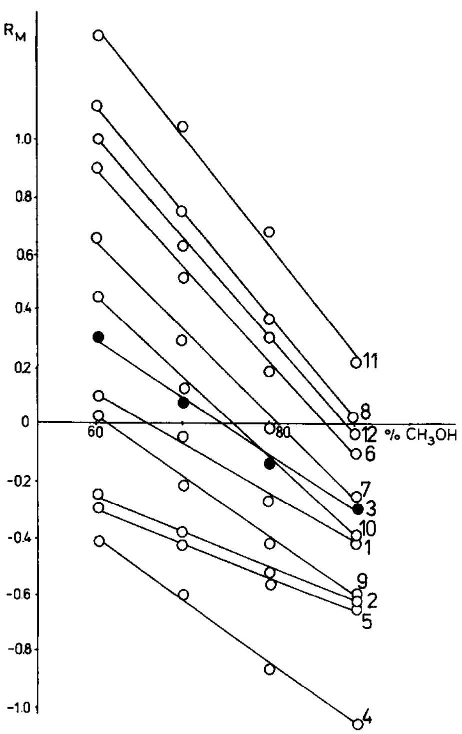
Materials

The coumarins used (Table I) were obtained from the Department of Chemistry, Royal Danish School of Pharmacy, Copenhagen, Denmark. Analytical-reagent grade organic solvents were purchased from E. Merck.

RESULTS AND DISCUSSION

The HPTLC results are shown as plots of R_M *versus* percentage of methanol (Figs. 1 and 2), as R_M *versus* R_M relationships obtained using different systems (Figs. 3–5) as a plot of $\log k'$ *versus* R_M (Fig. 6) and also as parameters of regression equations describing the linear relationships between experimental R_M values and the percentage of concentration of organic modifier in the eluent. The results in Figs. 1

and 2 indicate that in most instances linear relationships were obtained for almost all of the investigated concentration range. The lines sometimes cross (change in the sequence of R_M values) or spread fan-wise. The increase in the selectivity at higher water contents in the eluent is caused by an increasing contribution of hydrophobic interactions, especially for less polar compounds. In reversed-phase systems, in which solvation effects are very important, the retention depends on the molecular structure of the solute; the introduction of small polar or non-polar groups into a large sample molecule influences the retention significantly (Figs. 1 and 2). Compounds with polar hydroxy groups in the molecule, such as umbelliferone, esculetin, scopoletin, dihydrooroselol and *cis*- and *trans*-khellactone, have the weakest retention if an aqueous–organic eluent is used. The methylation of hydroxy groups markedly increased the retention of, *e.g.*, osthol, O-prenylumbelliferone and ostruthin (the spots were always near the start). Similar retentions were obtained for furocoumarins: isoimperatorin, peucenidin, libabotin, athamantin, archangelicin and columbianadin. Of the pyranocoumarins, the greastest hydrophobicity and the longest retention times were shown by visnadin and disenecioyl *cis*-khellactone. In order to elute these non–polar compounds, which possess large hydrophobic fragments in the molecule, the eluent strength must be increased, which can be achieved by increasing the content of organic modifier. At low concentration of the modifier these compounds cannot



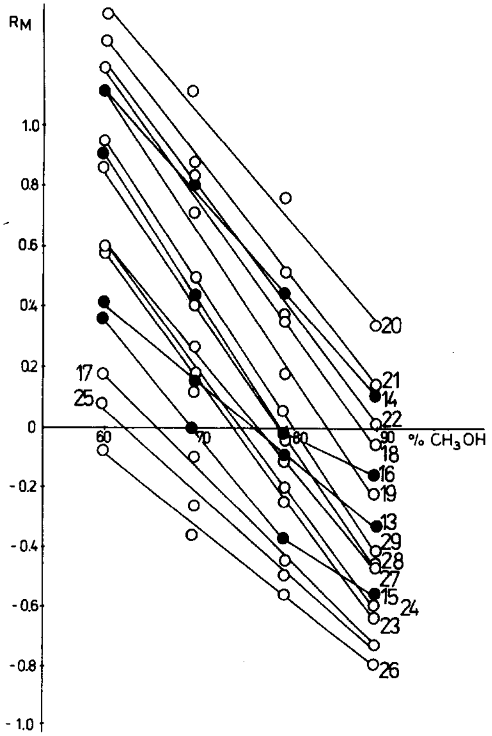
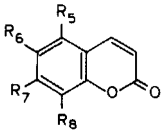
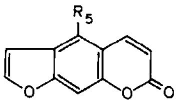


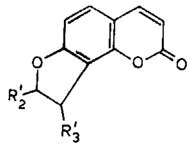
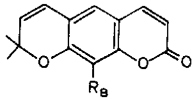
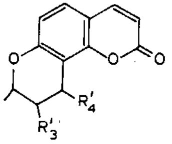
Fig. 1. R_M values plotted against the concentration of methanol in the eluent. Active solid: octadecylsilica. Notation of solutes as in Table I.

Fig. 2. As Fig. 1 for further solutes.

TABLE I

COMPOUNDS INVESTIGATED

Type	*Structure*	*No.*	*Substituents*	*Name*
Simple coumarins		1	None	Coumarin
		2	$R_7 = OH$	Umbelliferone
		3	$R_7 = OCH_3$	Herniarin
		4	$R_6 = R_7 = OH$	Esculetin
		5	$R_6 = OCH_3, R_7 = OH$	Scopoletin
		6	$R_6 = CH_2CH{=}C(CH_3)_2, R_7 = OH$	Demethylsuberosin
		7	$R_7 = OH, R_8 = CH_2CH{=}C(CH_3)_2$	Osthenol
		8	$R_7 = OCH_3, R_8 = CH_2CH{=}C(CH_3)_2$	Osthol
		9	$R_7 = OCH_3, R_8 = CH_2\underset{OH}{C}H\underset{OH}{C}(CH_3)_2$	Merazin hydrate
		10	$R_7 = OCH_3, R_8 = CH_2\underbrace{CH{-}C}_{O}(CH_3)_2$	Merazin
		11	$R_7 = OCH_2CH{=}C(CH_3)_2$	O-prenylumbelliferone
		12	$R_6 = OCH_2CH{=}C(CH_3)CH_2CH_2CH{=}C(CH_3)_2, R_7 = OH$	Ostruthin
Furano-coumarins		13	None	Psoralen
		14	$R_5 = OCH_2CH{=}C(CH_3)_2$	Isoimperatorin
		15	$R_5 = OCH_2\underset{OH}{C}H{-}\underset{OH}{C}(CH_3)_2$	Oxypeucedanin hydrate
		16	$R_5 = OCH_2\underbrace{CH{-}C}_{O}(CH_3)_2$	Oxypeucedanin

Dihydro-furano-coumarins		17	$R'_2 = C(OH)(CH_3)_2$	Dihydrooroselol
		18	$R'_2 = C[OCOCH_2CH(CH_3)_2](CH_3)_2$, $R'_3 = OCOCH_3$	Peucenidin
		19	$R'_2 = C[OCOC(CH_3){=}CHCH_3](CH_3)_2$, $R'_3 = OCOCH_3$	Libanotin
		20	$R'_2 = C[OCOCH_2CH(CH_3)_2](CH_3)_2$, $R'_3 = OCOCH_2CH(CH_3)_2$	Athamantin
		21	$R'_2 = C[OCOC(CH_3){=}CHCH_3](CH_3)_2$, $R'_3 = OCOC(CH_3){=}CHCH_3$	Archangelicin
		22	$R'_2 = C[OCOC(CH_3){=}CHCH_3](CH_3)_2$	Columbianadin
Pyrano-coumarins		23	None	Xanthyletin
		24	$R_8 = OCH_3$	Luvangetin
Dihydro-pyrano-coumarins		25	$R'_3 = R'_4 = OH$	*cis*-Khellactone
		26	$R'_3 = R'_4 = OH$	*trans*-Khellactone
		27	$R'_3 = OCOCH{=}C(CH_3)_2$, $R'_4 = OCOCH_3$	Samidin
		28	$R'_3 = OCOCH(CH_3)CH_2CH_3$, $R'_4 = OCOCH_3$	Visnadin
		29	$R'_3 = R'_4 = OCOCH{=}C(CH_3)_2$	Disenecioyl *cis*-khellactone

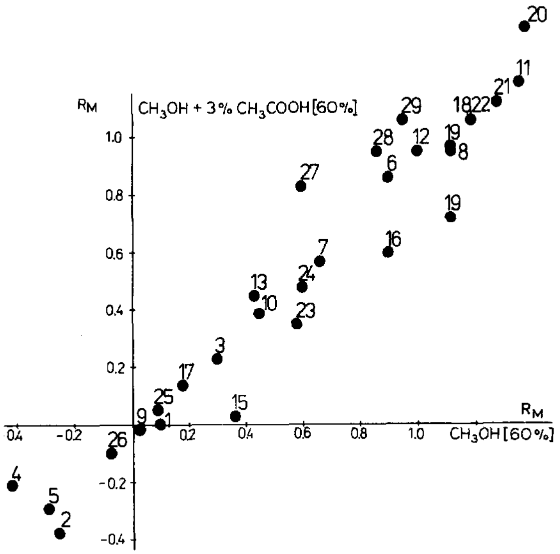


Fig. 3. R_M *vs.* R_M relationships. Active solid: octadecylsilica. Notation of solutes as in Table I. Eluent compositions indicated on the axes.

be separated. In these instances the range of measurable data of coumarins whose retentions (log *k'*) show a linear dependence on the concentration of the modifier can be extended by extrapolation of experimental $R_M = f(\varphi)$ relationships to a pure water medium.

It should be pointed out that extrapolation over a wide concentration range

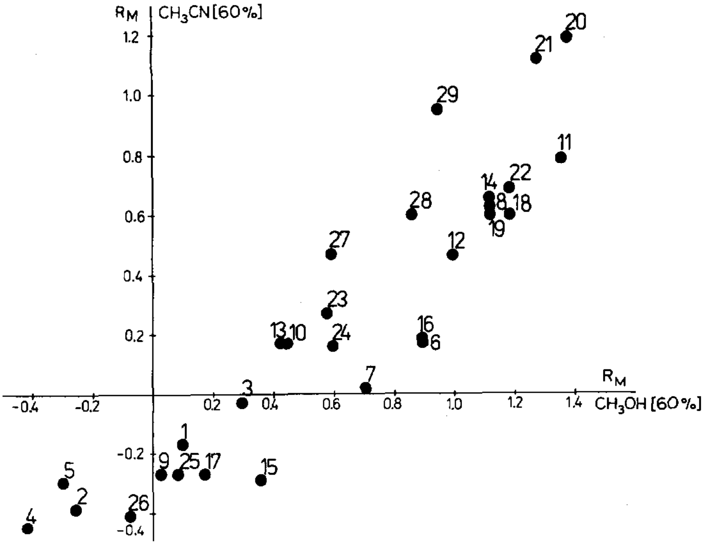


Fig. 4. As Fig. 3.

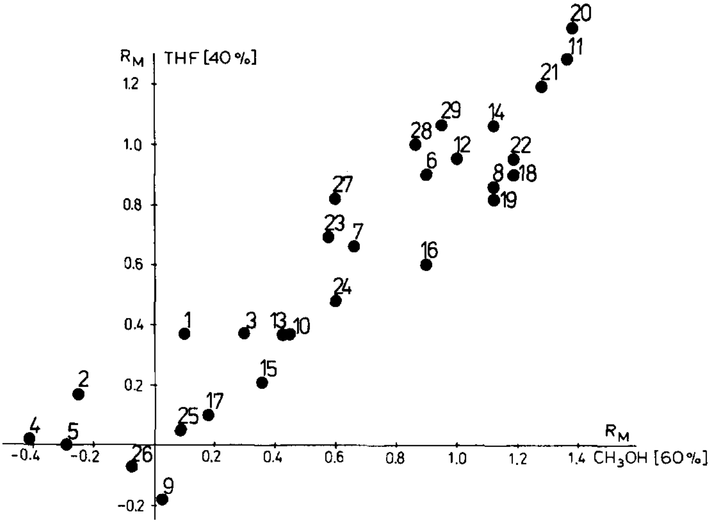


Fig. 5. As Fig. 3.

(φ_{mod} = 50–0%), assuming linear relationships over the whole concentration range, may give unreliable results. The structure of the stationary "brush" phase changes for water-rich eluents, which is also reflected by increased zone spreading[39]. Therefore, extrapolated (idealized) log k'_w or R_{M_w} values, although more or less different from the real parameters obtained for the system RP sorbent–100% water[40], may provide better characteristics of the lipophilicity of the solutes for the given water–modifier systems. However, the extrapolated R_{M_w} values may differ for the individual modifiers.

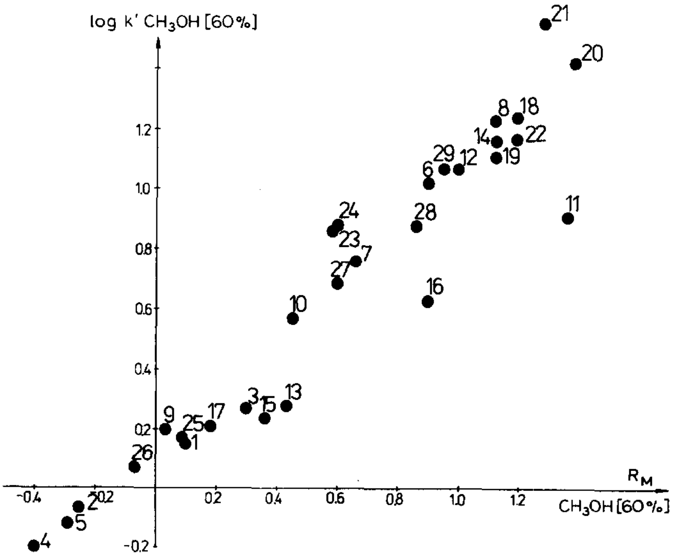


Fig. 6. Log k' *vs.* R_M relationships.

The theoretical capacity factors $R_{M\mathrm{w}}$ thus estimated are summarized in Table II as the parameter a in the equation

$$R_M = a - b\varphi \tag{1}$$

Theoretically, the $R_{M\mathrm{w}}$ value of an individual solute should be content for a given stationary phase, independent of the organic modifier used in an aqueous–organic phase; however, for the investigated coumarins the $R_{M\mathrm{w}}$ values derived from different solvent systems depend on the nature of the organic modifier and also on the composition range used for determining the experimental data. The extrapolated $R_{M\mathrm{w}}$ values (a) obtained using the methanol–water system were correlated with $R_{M\mathrm{w}}$ values

TABLE II

PARAMETERS a (INTERCEPT) b (SLOPE) AND r (CORRELATION COEFFICIENT) OF THE LINEAR RELATIONSHIPS IN EQN. 1

*Compound**	*System I (RP-18; methanol concentration range 60–30%)*			*System II (RP-18; methanol + 3% acetic acid, concentration range 50–90%)*			*System III (RP-18; tetrahydrofuran concentration range 40–70%)*		
	a	b	r	a	b	r	a	b	r
1	1.17	1.77	0.992	1.74	2.82	0.994	1.40	2.56	0.999
2	0.56	1.35	0.997	1.38	2.87	0.998	1.02	2.09	0.998
3	1.49	2.00	0.996	2.19	3.23	0.998	1.40	2.56	0.999
4	0.92	2.21	0.998	0.65	1.45	0.999	0.88	2.17	0.999
5	0.41	1.18	0.986	0.99	2.16	0.998	0.90	2.25	0.999
6	2.88	3.34	0.997	3.18	3.85	0.996	2.48	3.98	0.997
7	2.38	2.92	0.995	2.60	3.45	0.998	2.02	3.34	0.997
8	2.76	2.84	0.967	2.71	2.96	0.999	2.35	3.80	0.996
9	1.27	2.09	0.998	1.72	2.92	0.999	0.64	2.03	0.999
10	2.01	2.66	0.995	2.45	3.41	0.998	1.40	2.50	0.999
11	3.69	3.82	0.994	2.84	2.70	0.998	3.26	4.91	0.997
12	2.80	3.22	0.997	2.71	2.96	0.999	2.51	4.02	0.996
13	1.95	2.55	0.999	2.28	3.05	0.999	1.04	1.76	0.998
14	3.19	3.43	0.999	2.02	2.15	0.997	2.63	4.01	0.997
15	2.18	3.10	0.999	2.12	3.47	0.996	1.05	2.14	0.997
16	3.68	4.65	0.999	3.39	4.68	0.998	1.77	2.94	0.997
17	1.94	2.98	0.998	1.88	2.91	0.996	0.79	1.69	0.994
18	3.73	4.02	0.999	3.55	4.19	0.998	2.41	3.82	0.997
19	3.85	4.53	0.997	2.82	3.25	0.972	2.16	3.38	0.999
20	3.47	3.40	0.999	3.65	3.76	0.998	3.32	4.88	0.999
21	3.53	3.77	0.999	3.01	3.14	0.999	2.93	4.40	0.998
22	3.66	4.07	0.996	3.88	4.68	0.996	2.11	3.70	0.999
23	2.94	3.98	0.998	2.96	4.31	0.997	1.84	2.93	0.997
24	2.96	3.96	0.998	2.29	2.96	0.995	1.42	2.36	0.999
25	1.66	2.64	0.979	1.83	2.93	0.998	0.71	1.64	0.997
26	1.31	2.34	0.995	1.50	2.65	0.997	0.53	1.49	0.993
27	2.73	3.54	0.999	2.92	3.49	0.996	2.05	3.17	0.996
28	3.48	4.39	0.999	3.01	3.45	0.998	2.44	3.66	0.997
29	3.69	4.58	0.999	3.07	3.35	0.998	2.56	3.80	0.998

* See Table I.

for other investigated systems (solvent systems denoted by Roman numerals as in Table II):

$$R_{Mw}\,(\mathrm{I}) = 1.17\,R_{Mw}\,(\mathrm{II}) - 0.39 \qquad (n = 29, r = 0.884) \tag{2}$$

$$R_{Mw}\,(\mathrm{I}) = 1.03\,R_{Mw}\,(\mathrm{III}) + 0.66 \qquad (n = 29, r = 0.838) \tag{3}$$

$$R_{Mw}\,(\mathrm{I}) = 1.06\,R_{Mw}\,(\mathrm{V}) + 0.15 \qquad (n = 29, r = 0.856) \tag{4}$$

The moderate correlation coefficients obtained for systems with methanol containing acetic acid (II), tetrahydrofuran (III) or dioxane (V) as modifier showed that the molecular properties of the solute in both of the compared systems contribute

System IV (RP-18; acetonitrile concentration range 50–90%)			*System V (RP-18; dioxane concentration range 40–80%)*			*System VI (RP-2; methanol concentration range 40–80%)*			*System VII (RP-18 methanol 60%), log k*
a	*b*	*r*	*a*	*b*	*r*	*a*	*b*	*r*	
1.56	2.82	0.997	1.18	2.48	0.997	0.87	1.55	0.992	0.15
1.16	2.64	0.998	1.31	3.15	0.991	1.25	2.47	0.996	−0.07
1.74	2.93	0.999	2.19	4.02	0.998	1.94	3.14	0.996	0.27
1.12	2.64	0.999	1.00	3.18	0.998	1.15	2.77	0.999	−0.20
1.21	2.35	0.998	0.67	2.08	0.998	1.22	2.31	0.999	−0.12
2.24	3.38	0.996	3.00	4.57	0.999	2.79	3.84	0.997	1.02
2.15	3.50	0.998	2.61	4.37	0.998	2.59	3.79	0.997	0.76
2.51	3.12	0.998	3.14	4.43	0.996	1.37	2.25	0.986	1.23
1.55	2.98	0.997	1.14	2.88	0.998	1.90	3.32	0.996	0.20
2.06	3.12	0.998	2.42	4.17	0.996	2.37	3.64	0.996	0.57
2.85	3.38	0.997	2.95	3.66	0.999	1.65	2.74	0.997	0.91
2.33	3.06	0.998	2.39	3.27	0.998	1.21	2.31	0.997	1.07
1.90	2.09	0.999	1.15	2.03	0.998	1.93	2.90	0.999	0.28
2.33	2.80	1.000	2.45	3.91	0.991	1.75	2.70	0.994	1.16
0.87	1.97	0.993	1.04	2.18	0.996	1.81	2.88	0.999	0.24
1.59	2.38	0.995	2.11	3.28	0.989	1.64	2.73	0.987	0.63
1.39	2.81	0.998	1.58	2.85	0.998	1.74	2.74	0.998	0.21
2.29	2.82	0.997	2.88	4.11	0.999	3.42	4.39	0.997	1.24
2.43	3.04	0.999	2.87	4.14	0.999	3.63	4.54	0.996	1.11
3.25	3.43	0.992	3.55	4.30	0.999	3.70	4.43	0.997	1.42
3.28	3.63	0.999	3.35	4.40	0.998	3.78	4.61	0.996	1.55
2.52	3.06	0.999	3.00	4.17	0.997	3.61	4.78	0.998	1.17
1.52	2.13	0.993	2.45	3.94	0.987	1.90	2.68	0.998	0.86
1.62	2.51	0.990	2.17	3.35	0.999	3.42	4.39	0.997	0.88
1.39	2.81	0.998	1.34	2.56	0.998	1.58	2.56	0.998	0.17
0.94	2.33	0.985	1.04	2.22	0.998	1.25	2.22	0.996	0.07
2.16	2.84	0.997	2.70	3.72	0.997	3.40	4.26	0.998	0.69
2.26	2.80	0.998	3.10	4.13	0.977	2.99	3.72	0.999	0.88
2.95	3.37	0.998	3.00	3.96	0.998	3.64	4.47	0.998	1.07

TABLE III

LOG k' (HPLC), R_M (HPTLC), ΔLOG k' AND ΔR_M VALUES OF COUMARINS

Selectivities relative to coumarin (1) for solutes 2–13 and 23, to psoralen (3) for solutes 14–22 and to xanthyletin (23) for solutes 24–29.

*Compound**	*HPLC RP-18, 60% methanol*		*HPTLC RP-18, 60% of modifier in the eluent*												*From System I in Table II, ΔR_{M_w}*
	log k'	*Δlog k'*	*Methanol*		*Methanol + 3% acetic acid*		*Acetonitrile*		*Dioxane*		*Tetrahydrofuran*		*RP-2, methanol*		
			R_M	ΔR_M	R_M	ΔR_M	R_M	ΔR_M	R_M	ΔR_M	R_M	ΔR_M	R_M	ΔR_M	
1	0.15	0.00	0.10	0.00	0.00	0.00	−0.17	0.00	−0.31	0.00	−0.14	0.00	−0.07	0.00	0.00
2	−0.07	−0.22	−0.25	−0.35	−0.37	−0.37	−0.39	−0.22	−0.60	−0.29	−0.23	−0.09	−0.19	−0.12	−0.61
3	0.27	0.12	0.30	0.20	0.23	0.23	−0.03	0.14	−0.19	0.12	−0.14	0.00	0.10	0.17	0.32
4	−0.20	−0.35	−0.41	−0.51	−0.21	−0.21	−0.45	−0.28	−0.90	−0.59	−0.43	−0.29	−0.50	−0.43	−0.25
5	−0.12	−0.27	−0.29	−0.39	−0.29	−0.29	−0.30	−0.13	−0.57	−0.26	−0.45	−0.31	−0.17	−0.10	−0.76
6	1.02	0.87	0.90	0.80	0.86	0.86	0.17	0.34	0.23	0.54	0.05	0.19	0.48	0.55	1.71
7	0.76	0.61	0.66	0.56	0.57	0.57	0.02	0.19	−0.05	0.26	0.02	0.16	0.29	0.36	1.21
8	1.23	1.08	1.12	1.02	0.95	0.95	0.63	0.80	0.43	0.74	0.02	0.16	−0.05	0.02	1.59
9	0.20	0.05	0.03	−0.07	−0.02	−0.02	−0.27	−0.10	−0.60	−0.29	−0.57	−0.43	−0.05	0.02	0.10
10	0.57	0.42	0.45	0.35	0.39	0.39	0.17	0.34	−0.10	0.21	−0.14	0.00	0.19	0.26	0.84
11	0.91	0.76	1.36	1.26	1.19	1.19	0.79	0.96	0.73	1.04	0.27	0.41	0.05	0.12	2.52
12	1.07	0.92	1.00	0.90	0.95	0.95	0.47	0.64	0.43	0.74	0.09	0.23	−0.18	−0.11	1.63
13	0.28	0.13	0.43	0.33	0.45	0.45	0.17	0.34	−0.07	0.24	0.00	−0.14	0.27	0.34	0.00
14	1.16	0.88	1.12	0.69	0.72	0.27	0.65	0.48	0.41	0.48	0.19	−0.19	0.12	0.15	1.24
15	0.24	−0.04	0.36	−0.07	0.03	−0.42	−0.29	−0.46	−0.31	−0.24	−0.25	−0.25	0.09	−0.18	0.23
16	0.63	0.35	0.90	0.47	0.60	0.15	0.18	0.01	0.02	0.09	0.02	0.02	−0.02	−0.29	1.75
17	0.21	−0.07	0.18	−0.25	0.14	−0.31	−0.27	−0.44	−0.10	−0.03	−0.25	−0.25	0.09	−0.18	−0.01
18	1.24	0.96	1.19	0.76	1.06	0.61	0.60	0.43	0.43	0.50	0.07	0.07	0.83	0.56	1.78
19	1.11	0.83	1.12	0.69	0.96	0.51	0.60	0.43	0.37	0.44	−0.12	−0.12	0.95	0.68	1.90
20	1.42	1.14	1.38	0.95	1.38	0.93	1.19	1.02	0.79	0.86	0.37	0.37	1.06	0.79	1.52
21	1.55	1.27	1.28	0.85	1.12	0.67	1.12	0.95	0.72	0.79	0.25	0.25	1.06	0.79	1.58
22	1.17	0.89	1.19	0.76	1.06	0.61	0.69	0.52	0.48	0.55	0.18	0.18	0.72	0.45	1.71
23	0.86	0.71	0.58	0.48	0.35	0.35	0.27	0.44	0.09	0.40	0.05	0.19	0.27	0.34	0.00
24	0.88	0.02	0.60	0.02	0.48	0.13	0.16	−0.11	0.18	0.09	0.00	0.05	−0.29	−0.56	0.02
25	0.17	−0.69	−0.09	−0.49	0.05	−0.30	−0.27	−0.54	−0.17	−0.26	−0.29	−0.34	0.05	0.22	−1.23
26	0.07	−0.79	−0.07	−0.65	−0.10	−0.45	−0.41	−0.68	−0.31	−0.40	−0.39	−0.44	−0.09	−0.36	−1.63
27	0.69	−0.17	0.60	0.02	0.83	0.48	0.47	0.20	0.50	0.41	0.14	0.09	0.75	0.48	−0.21
28	0.88	0.02	0.86	0.28	0.95	0.60	0.60	0.33	0.79	0.70	0.21	0.16	0.79	0.52	0.54
29	1.07	0.21	0.95	0.37	1.06	0.71	0.95	0.68	0.66	0.57	0.25	0.20	1.00	0.73	0.75

differently to the retention for various modifier systems.

A significantly worse correlation was obtained for the RP-2 sorbent as the stationary phase:

$$R_{M\text{w}}\ (\text{I}) = 1.95\ R_{M\text{w}}\ (\text{VI}) + 0.81 \qquad (n = 29, r = 0.695) \tag{5}$$

indicating that the characteristics of the support also influence the retention.

The best correlation coefficient was obtained for the relationship R_M *vs.* log k' values at 60% of methanol in the eluent in both of the compared techniques:

$$R_M = 1.01 \log k' - 0.06 \qquad (n = 29, r = 0.955) \tag{6}$$

the slope being close to unity and the intercept nearly zero. The mechanism of the separation processes in the two systems was the same and the R_M values easily determined in the HPTLC system can be used in the optimization of HPLC systems.

The HPLC selectivity parameter $\alpha = k_2/k_1$ corresponding to $\Delta R_M = \log k'_2/k'_1$ shows the effect of individual substituents on the retention and the selectivity of the developing systems.

Table III summarizes the log k', Δlog k', R_M and ΔR_M values obtained for 60% organic modifier in the eluent. The ΔR_M values are differentiated for the various investigated modifiers owing to the differences in the polarity of the substituents and in the interaction with the eluent and the stationary phase.

With 60% of methanol as modifier (Table III), $\Delta R_M(\text{OH}) = -0.35$ (compounds 1 and 2). A methoxy group in the molecule increases the hydrophobic properties, $\Delta R_M(\text{OCH}_3) = 0.20$ (compounds 1 and 3). Prenylation of umbelliferone (2) to O-prenylumbelliferone (11) causes a large increase in retention: ΔR_M (O-prenyl) = 1.61. ΔR_M (geranyloxy) = 1.25 for compounds 2 and 12. Also, the addition of a condensed furan or pyran ring to the basic coumarin structure increases the retention; ΔR_M for compounds 1 and 13 is 0.33 and for compounds 1 and 23 it is 0.48.

Comparison of dihydroxyfuranocoumarins possessing large radicals shows that all the investigated compounds give high retention times, except dihydrooroselol (17) with a hydrophilic isopropanol group in the molecule. Methanol–water and methanol-containing acetic acid are the most selective eluents with respect to substituent effects on retention. For other investigated modifiers the ΔR_M values are significantly lower. The lowest ΔR_M values were obtained for 60% of tetrahydrofuran as eluent (see Table III).

The selectivity of the systems used was also illustrated by R_M *vs.* R_M correlations taking 60% of methanol as the reference modifier (Figs. 3–5). The compounds with R_M values lying on the same vertical line cannot be resolved when methanol is used and the compounds with R_M values lying on a horizontal line cannot be separated in the second correlated system. As shown in Fig. 3, the addition of 3% acetic acid to the methanol–water eluent significantly improved the separation of compounds 8, 14 and 19 and of 6 and 16, which cannot be separated with 60% methanol, but the selectivity for compounds 8, 12, 19 and 20 deteriorates. The pair of positional [demethylsuberosin (6) and osthenol (7)] and *cis-trans* isomers [*cis*-khellactone (25) and *trans*-khellactone (26)] were well separated in the systems used.

The results indicate that for the investigated modifiers the eluent strength changes more or less depending on the molecular structure of the solutes[41]. For coumarins, tetrahydrofuran and dioxane were found to be the solvents with greatest eluent strength, but were less selective.

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