

CHROMSYMP. 241

## HIGH-PERFORMANCE LIQUID CHROMATOGRAPHY OF CHROMONOID COMPOUNDS

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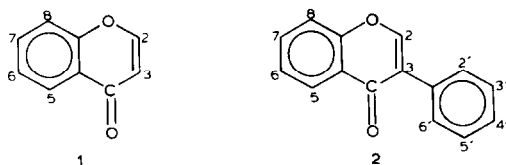
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### SUMMARY

Synthetic chromones and isoflavones have been investigated by high-performance reversed-phase partition chromatography on  $C_8$  and  $C_{18}$  stationary phases in the isocratic mode, using methanol-water and acetonitrile-water eluents. The effect of various substituents on the retention time and capacity ratio was studied. It has been shown that methyl and methoxyl groups increase, whereas a hydroxyl substituent decreases the value of retention time. The effects of these groups on the retention time and on the degree of separation can be predicted for chromones. Of the other substituents the 2-trifluoromethyl, 6-chloro and cyclic groups (e.g. phenyl, benzyl, thiazolyl etc.) increased the retention time while the opposite effect was observed in the case of certain nitrogen-containing (tetrazolyl, cyano, carboxamido) functions.

### INTRODUCTION

The chromatographic behaviour of some 120 chromonoid compounds, comprising a group of chromones (1) and a series of isoflavones (2) prepared in our Institute, has been investigated.



The high-performance liquid chromatographic (HPLC) properties of chromonoid compounds has been relatively little studied; synthetic chromones and isoflavones have been treated only in one<sup>1</sup> and three<sup>2-4</sup> papers, respectively. Helms *et al.*<sup>1</sup> studied the properties of 2-(5-tetrazolyl)chromone, Carlson and Dolphin<sup>2</sup> investigated 4,7-disubstituted isoflavones, Ward and Pelter<sup>3</sup> examined analogous hydroxy- and methoxyisoflavones, whereas the behaviour of isoflavone, 2-methoxy-, 7-methoxy- and 6-methylisoflavone was studied by Schuster<sup>4</sup> with the aid of our group.

We have now used reversed-phase partition chromatography to investigate the relationships between the substituent effect and retention time,  $t_R$ , and capacity ratio,  $k'$ .

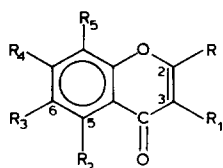
## EXPERIMENTAL

A Hewlett-Packard Model 1082A instrument, operated in the isocratic mode and equipped with an automatic sample injector and an UV-detector (254 nm), was used. The columns applied were as follows: 200 × 4.6 mm I.D. 10- $\mu$ m Hewlett-Packard LiChrosorb RP-8 and 250 × 4.6 mm I.D. 10- $\mu$ m Hewlett-Packard LiChrosorb RP-18.

The examinations were carried out with standards shown to be homogeneous by thin-layer chromatography (TLC). Solvents of analytical purity were applied as eluents.

TABLE I

THE EFFECT OF A METHYL GROUP ON THE ELUTION OF CHROMONES



$$t_0 = 0.85.$$

Compound	$R = H$		$R = CH_3$		$\alpha_{CH_3}$
	$t_R$	$k'$	$t_R$	$k'$	
$R_2 = R_3 = R_5 = H, R_4 = OH, R_1 =$ phthalimido	2.02	1.38	3.17	2.73	1.97
$R_2 = R_3 = R_5 = H, R_4 = OCH_3,$ $R_1 =$ phthalimido	2.78	2.27	3.30	2.88	1.27
$R_2 = R_3 = R_5 = H, R_1 = C_6H_5O, R_4 = OH$	3.29	2.87	4.30	3.82	1.33
	$R_1 = H$		$R_1 = CH_3$		
$R = R_2 = R_3 = R_4 = R_5 = H$	2.41	1.83	3.16	2.71	1.48
$R = R_2 = R_3 = R_5 = H, R_4 = OCH_3$	2.81	2.30	3.82	3.49	1.51
$R = R_2 = R_4 = R_5 = H, R_3 = CH_3$	3.16	2.71	4.20	3.70	1.37
$R = R_2 = R_4 = H, R_3 = R_5 = Cl$	5.78	5.80	8.19	8.63	1.49
	$R_2 = H$		$R_2 = CH_3$		
$R = R_3 = R_5 = H, R_4 = OH,$ $R_1 =$ pyridyl	2.72	2.20	3.98	3.68	1.67
$R = R_3 = R_5 = H, R_4 = OH,$ $R_1 =$ thiazolyl	3.37	2.96	4.97	4.84	1.63
$R = R_3 = R_5 = H, R_4 = OH,$ $R_1 = 2$ -methylthiazolyl	4.99	4.87	6.56	6.71	1.38
	$R_3 = H$		$R_3 = CH_3$		
$R = R_2 = R_4 = R_5 = H, R_1 = NH_2$	2.13	1.50	2.65	2.11	1.40
$R = R_2 = R_4 = R_5 = H, R_1 =$ acetamido	2.59	2.04	2.98	2.50	1.22

## RESULTS AND DISCUSSION

For the separation of natural isoflavones, gradient elution with methanol-water and acetonitrile-water mixtures has previously been used.

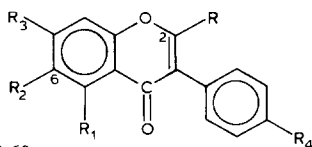
The present experiments were also performed with methanol-water and acetonitrile-water eluents on  $C_8$  and  $C_{18}$  stationary phases. Comparable results were obtained by using  $C_8$  as stationary phase and methanol-water (6:4) as the eluent for chromones and by applying  $C_{18}$  as stationary phase and methanol-water (7:3) eluent in the case of isoflavones (flow-rate  $3.0 \text{ cm}^3/\text{min}$  in each case).

General effects on the chromatographic behaviour of the derivatives could be determined in the case of methyl, methoxyl and hydroxyl as substituents. Independently of their positions in the molecules, the presence of methyl and methoxyl groups increased the retention times of mono- and polysubstituted chromones and isoflavones. A 7-hydroxyl substituent decreased the retention time of each compound (Tables I-VI). From the data in the tables, the effects of these three groups on the retention time and on the degree of separation,  $\alpha$ , can be predicted for both chromones and isoflavones. Also, from a given change in the retention time, the nature of the substituent attached to the molecule can be determined. Under the conditions used, the presence of a methoxyl group increased the retention time is a generally smaller degree than did that of a methyl group.

The effects of other substituents were also investigated over a more limited range. Thus, a 2-trifluoromethyl and 6-chloro substituents resulted in a significant increase in the retention time of the derivative compared to that of the parent molecule. In the case of chromones, cyclic substituents (phenyl, benzyl, thiazolyl, etc.) increased the retention time, but the opposite effect was observed with several nitrogen-containing substituents (tetrazolyl, cyano, carboxamido), Fig. 1. These effects

TABLE II

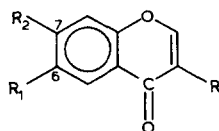
THE EFFECT OF 2- AND 6-METHYL GROUPS ON THE ELUTION OF ISOFLAVONES

 $t_0 = 0.60$ .

Compound	$R = H$		$R = CH_3$		$\alpha_{CH_3}$
	$t_R$	$k'$	$t_R$	$k'$	
$R_1 = R_2 = R_3 = R_4 = H$	2.92	3.86	3.01	4.01	1.04
$R_1 = R_2 = R_4 = H, R_3 = OAc$	2.56	3.26	2.69	3.48	1.07
	$R_2 = R_4 = H$		$R_2 = CH_3, R_4 = H$		
$R = R_1 = R_3 = H$	2.92	3.86	4.23	6.05	1.56
$R_1 = R_3 = H, R = CH_3$	3.01	4.01	4.57	6.61	1.65
$R_1 = R_3 = H, R = CF_3$	4.90	7.16	7.33	11.21	1.56

TABLE III

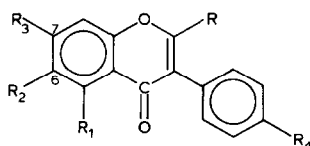
THE EFFECT OF 6- AND 7-METHOXYL GROUPS ON THE ELUTION OF CHROMONES

 $t_0 = 0.85$ .

Compound (R)	$R_1 = R_2 = H$		$R_1 = OCH_3, R_2 = H$		$\alpha_{OCH_3}$
	$t_R$	$k'$	$t_R$	$k'$	
NH <sub>2</sub>	2.13	1.50	2.38	1.80	1.20
Acetamido	2.59	2.04	2.98	2.50	1.22
CH <sub>3</sub>	3.16	2.71	3.62	3.25	1.20
	$R_1 = R_2 = H$		$R_2 = OCH_3, R_1 = H$		
CN	2.03	1.39	2.39	1.81	1.30
H	2.41	1.83	2.81	2.30	1.25
CH <sub>3</sub>	3.16	2.71	3.82	3.49	1.28
C <sub>6</sub> H <sub>5</sub> O	5.07	4.96	6.13	6.21	1.25
C <sub>6</sub> H <sub>5</sub> S	6.83	7.03	8.95	9.53	1.35

TABLE IV

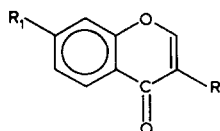
THE EFFECT OF A METHOXYL GROUP FOR THE ELUTION OF ISOFLAVONES

 $t_0 = 0.60$ .

Compound	$R_2 = R_4 = H$		$R_4 = OCH_3, R_2 = H$		$\alpha_{OCH_3}$
	$t_R$	$k'$	$t_R$	$k'$	
R = R <sub>1</sub> = R <sub>3</sub> = H	2.92	3.86	3.10	4.16	1.08
R = CH <sub>3</sub> , R <sub>1</sub> = R <sub>3</sub> = OAc	2.28	2.80	2.35	2.91	1.04
R = CH <sub>3</sub> , R <sub>1</sub> = H, R <sub>3</sub> = OAc	2.69	3.48	2.75	3.58	1.03
R = R <sub>1</sub> = H, R <sub>3</sub> = OCH <sub>3</sub>	3.37	4.61	3.92	5.53	1.20
	$R_3 = H$		$R_3 = OCH_3$		
R = R <sub>1</sub> = R <sub>2</sub> = R <sub>4</sub> = H	2.92	3.86	3.37	4.61	1.19
R = CF <sub>3</sub> , R <sub>1</sub> = R <sub>2</sub> = R <sub>4</sub> = H	4.90	7.16	5.80	8.78	1.08
	$R_2 = H$		$R_2 = OCH_3$		
R = R <sub>1</sub> = R <sub>3</sub> = R <sub>4</sub> = H	2.92	3.86	3.67	5.11	1.32
R = CF <sub>3</sub> , R <sub>1</sub> = R <sub>3</sub> = R <sub>4</sub> = H	4.90	7.16	6.60	10.00	1.39

TABLE V

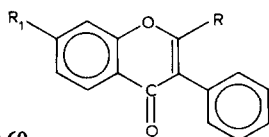
THE EFFECT OF A 7-HYDROXYL GROUP ON THE ELUTION OF CHROMONES

 $t_0 = 0.85$ .

Compound (R)	$R_1 = H$		$R_1 = OH$		$\alpha_{OH}$
	$t_R$	$k'$	$t_R$	$k'$	
CN	2.03	1.39	1.54	0.81	0.58
CH <sub>3</sub>	3.16	2.71	2.24	1.63	0.60
C <sub>6</sub> H <sub>5</sub> O	5.07	4.96	3.29	2.87	0.58
C <sub>6</sub> H <sub>5</sub> S	6.83	7.03	4.32	4.08	0.58

TABLE VI

THE EFFECT OF A 7-HYDROXYL GROUP ON THE ELUTION OF ISOFLAVONES

 $t_0 = 0.60$ .

Compound (R)	$R_1 = H$		$R_1 = OH$		$\alpha_{OH}$
	$t_R$	$k'$	$t_R$	$k'$	
H	2.92	3.86	1.16	0.93	0.24
CH <sub>3</sub>	3.01	4.02	1.30	1.16	0.28
CF <sub>3</sub>	4.90	7.16	1.24	1.07	0.15

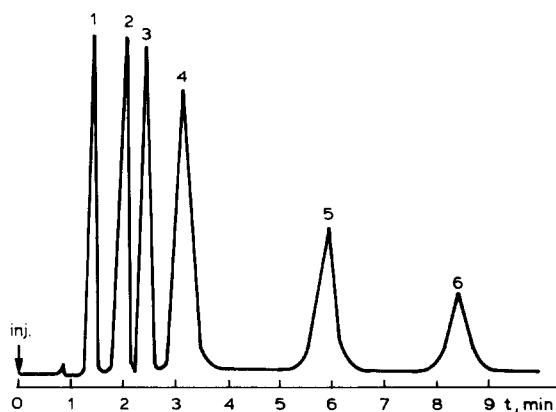


Fig. 1. Separation of C3-monosubstituted chromones on C<sub>8</sub> stationary phase with methanol-water (6:4) as eluent. Flow-rate: 3.0 cm<sup>3</sup>/min. Column: 200 × 4.6 mm I.D. 10- $\mu$ m LiChrosorb RP-8. Peaks: 1 = 3-carboxychromone; 2 = 3-nitrilochromone; 3 = chromone; 4 = 3-methylchromone; 5 = 3-phenylchromone; 6 = 3-benzylchromone.

cannot be regarded as general because of the limited number of chromone derivatives examined.

However, it can be concluded that the nature and extent of the observed substituent effects are determined by the change in the polarity—and consequently in the solubility— of the parent compound, caused by that substituent.

Experiments were carried out also with silica gel as stationary phase, using cyclohexane-isopropanol and chloroform-methanol as eluents. In these cases, no simple relationships involving the substituent effects were observed.

#### REFERENCES

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