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Note

Substitutent effect of polymethoxylated flavones in high-performance liquid chromatography

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Polymethoxylated flavones are found in high concentrations in citrus peel and in low amounts in citrus juice[1]. Because of their important physiological response in higher animals[2,3], the separation and quantitation of these compounds has been done via a thin-layer chromatographic (TLC)–spectrophotometric procedure[1,4]. A high-performance liquid chromatographic (HPLC) procedure for the analysis of polymethoxylated flavones offers more advantages in speed and accuracy for their identification and quantitation. An HPLC method has been used for the separation[5,6] and quantitation[7,8] of numerous polymethoxylated flavones, but several minor compounds remain to be identified.

This paper reports an HPLC method for the separation of sixteen polymethoxylated flavones.

EXPERIMENTAL

The materials and chromatographic method used were essentially the same as described previously[5,7]. Three flavones, tangeretin (14), nobiletin (15), heptamethoxyflavone (16), were obtained from orange peel oil using preparative TLC. The others were synthesized from various 2-hydroxylated acetophenones and anisoyl or veratroyl chloride using a modified Baker–Venkataraman procedure[9,10]. We used home-built equipment consisting of an Orlita (Giessen, G.F.R.) DMPAE 1044 dual-stroke pump and an ISCO (Lincoln, NE, U.S.A.) Model UA5 dual-beam UV spectrophotometer at 280 nm. The columns (25 cm × 4 mm I.D.) were slurry-packed with LiChrosorb Si 60 (60 μm) purchased from Merck (Darmstadt, G.F.R.). Separations were performed by isocratic elution with two isocratic solvent systems, heptane–isopropanol (60:40) and heptane–isopropanol (70:30) containing 0.1% of water, by Karl Fisher determinations. For each flavone, retention times and capacity factors were determined.

RESULTS AND DISCUSSION

Retention data for polymethoxylated flavones are listed in Table I. Generally, separations are slightly affected by the percentage of water present[7]. The selectivity

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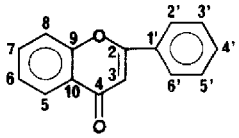
TABLE I

CAPACITY FACTORS OF POLYMETHOXYLATED FLAVONES



Compound	Substituent*					k'	
	3	3'	5	6	8	Heptane–isopropanol (60:40)	Heptane–isopropanol (70:30)
1	H	H	OCH_3	H	H	2.1	2.45
2	H	H	OCH_3	H	OCH_3	5.8	6.3
3	H	OCH_3	OCH_3	H	OCH_3	9.2	9.8
4	OCH_3	H	OCH_3	H	OCH_3	4.0	4.45
5	OCH_3	OCH_3	OCH_3	H	OCH_3	6.5	7.0
6	H	H	OCH_3	OCH_3	H	1.05	1.20
7	H	OCH_3	OCH_3	OCH_3	H	2.4	2.65
8	OCH_3	H	OCH_3	OCH_3	H	0.84	0.95
9	OCH_3	OCH_3	OCH_3	OCH_3	H	1.8	2.1
10	H	H	H	OCH_3	OCH_3	0.69	0.82
11	H	OCH_3	H	OCH_3	OCH_3	1.4	1.55
12	OCH_3	H	H	OCH_3	OCH_3	0.54	0.63
13	OCH_3	OCH_3	H	OCH_3	OCH_3	1.1	1.25
14	H	H	OCH_3	OCH_3	OCH_3	0.72	0.85
15	H	OCH_3	OCH_3	OCH_3	OCH_3	1.50	1.70
16	OCH_3	OCH_3	OCH_3	OCH_3	OCH_3	1.20	1.30

* 4' and 7: OCH_3.

is improved by using larger amounts of polar solvent, but the capacity factors decreased and the increasing viscosity of the mobile phase required the use of lower flow-rates in order that the mass transfer resistance did not increase the height equivalent to a theoretical plate (HETP) too much[11].

As can be clearly seen from Table I, there is a correlation between the capacity factors of flavones and the position of the methoxy groups on the flavone skeleton. The plot of log k' *versus* log $\Delta k'$ ($\Delta k' = k'_x/k'_{10}$, with $x = 10$, 11, 12 and 13) given in Fig. 1 for the solvent system heptane–isopropanol (60:40) shows that fifteen flavones (2–16) are on four lines, D, E, F and G, defining four flavone groups having the same number and position of methoxy groups on the A ring (5, 7, 8; 5, 6, 7; 5, 6, 7, 8; and 6, 7, 8 patterns, respectively). With the flavones 1–16, it is possible to know the effect on the retention time of the presence of one or more methoxy groups on the A, B and C rings of a flavone. Introduction of a methoxy group in the 3-position reduces the capacity factor whereas introduction in the 3'-position increases the capacity factor. Simultaneous introduction in the 3'- and 3-positions increases the capacity factor because the 3'-effect is higher than the 3-effect. If one compares the capacity factor of compound 1 with those of flavones of the D series (compounds 2, 3, 4 and 5) and the E series (compounds 6, 7, 8 and 9), one may check that the introduction of a methoxy group in the 8-position sharply increases the capacity

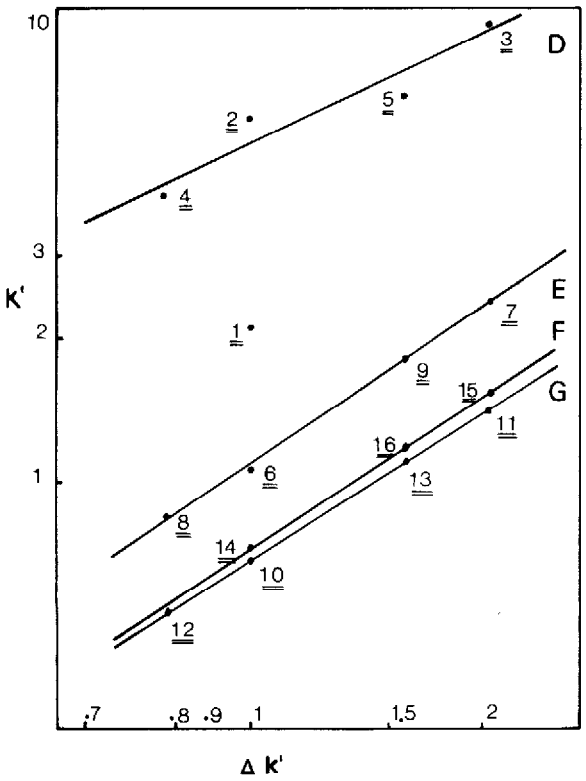


Fig. 1. Effect of methoxy substituents on the retention of flavones. Logarithmic plot of k' *vs.* $\Delta k'$ ($\Delta k' = k_x - k_{10}$ with x = 10, 11, 12 and 13) for the solvent mixture heptane–isopropanol (60:40). D, 5,7,8-Trimethoxyflavone series; E, 5,6,7-trimethoxyflavone series; F, 5,6,7,8-tetramethoxyflavone series; G, 6,7,8-trimethoxyflavone series.

factor whereas introduction in the 6-position reduces the capacity factor. In series F, the introduction of a methoxy group in the 5-position weakly increases the capacity factor *versus* the G homologues.

The correlation between chromatographic behaviour and chemical structure of flavonoids was first dealt with by Bate-Smith and Westall[12] and has been reviewed by Harbone[13]. The R_m value is generally known to be a linear combination of a constant of the skeleton of the molecule and constants related to substituents[14]:

$$R_m = G_0 + mG_x + nG_y + \ldots \quad (1)$$

Roberts and co-workers[15,16] have shown that a straight-line relationship exists between the R_m values of flavonoids and the numbers of their hydroxy and sugar substituents. It was shown by Wong and Taylor[17] that the order of R_F values in flavones, isoflavones and flavanones is the same in each class, indicating that the effects of structural variation on R_F values are similar in all classes investigated. The greater lyophilic effect of the methoxy group is also apparent in the previous results of Simpson and Garden[18] and Shaw and Simpson[19].

As individual groups may influence each other, the eqn. 1 is not often observed as it does not take into account intramolecular effects[20]. The relative retentions of the various flavones are difficult to determine because the geometry of the molecules fixed on the LiChrosorb Si 60 surface cannot be easily described. In this instance, however, the contribution of one substituent proved to be the same whatever the position of the other substituents. For example, substitution by a methoxy group in the 3′-position is independent of the number and position of other substituents on the A and B rings. The additivity of substituents effect is thus true in this instance.

Hence it clearly appears that the order of elution of polymethoxylated flavones is related not only to the number of methoxy groups fixed but, above all, to the position of these groups on the flavone skeleton. These results permit, in quantitative analysis[7], a better choice of the column and the eluting system in the separation of not only the five principal flavones found in citrus [tetra-O-methoxyl-scutellarein (7), sinensetin (8), tangeretin (14), nobiletin (15) and heptamethoxyflavone (16)], but also the minor ones.

REFERENCES

1 M. K. Veldhuis, L. J. Swift and W. C. Scott, *J. Agr. Food Chem.*, 18 (1970) 590.
2 S. M. Kupchan, J. R. Knox and M. S. Udaya Murthy, *J. Pharm. Sci.*, 54 (1965) 929.
3 R. C. Robbins, *Int. J. Vitam. Nutr. Res.*, 47 (1977) 373.
4 L. J. Swift, *J. Agr. Food Chem.*, 13 (1965) 431.
5 J. P. Bianchini and E. M. Gaydou, *J. Chromatogr.*, 190 (1980) 233.
6 S. V. Ting, R. L. Rousseff, M. H. Dougherty and J. A. Attaway, *J. Food Sci.*, 44 (1979) 69.
7 J. P. Bianchini and E. M. Gaydou, *J. Chromatogr.*, 211 (1981) 61.
8 R. L. Rousseff and S. V. Ting, *J. Chromatogr.*, 176 (1979) 75.
9 E. M. Gaydou and J. P. Bianchini, *Bull Soc. Chim. Fr.*, (1978) 43.
10 E. M. Gaydou and J. P. Bianchini, *Ann. Chim. Fr.*, 2 (1977) 303.
11 L. R. Snyder and J. J. Kirkland, *Introduction to Modern Liquid Chromatography*, Wiley, New York, 1974.
12 E. C. Bate-Smith and R. G. Westall, *Biochim. Biophys. Acta*, 4 (1950) 427.
13 J. B. Harbone, *J. Chromatogr.*, 2 (1959) 581.
14 A. J. P. Martin, *Annu. Rev. Biochem.*, 19 (1950) 518.
15 E. A. H. Roberts and D. J. Wood, *Biochem. J.*, 53 (1953) 332.
16 E. A. H. Roberts, R. A. Cartwright and D. J. Wood, *J. Sci. Food Agr.*, 7 (1956) 637.
17 E. Wong and A. O. Taylor, *J. Chromatogr.*, 9 (1962) 449.
18 T. H. Simpson and L. Garden, *J. Chem. Soc.*, (1952) 4638.
19 B. L. Shaw and T. H. Simpson, *J. Chem. Soc.*, (1952) 5027.
20 L. R. Snyder, *Principles of Adsorption Chromatography*, Marcel Dekker, New York, 1968, pp. 185ff.