Note

Selective liquid–liquid partition systems for the chromatographic analysis of some coumarins and phenolic acids (derivatives of cinnamic acid)

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Phenolic acids that are derivatives of cinnamic acid frequently occur in plants as products of biosynthesis from amino acids (phenylalanine). They play some role in plant physiology as stimulants of plant growth and have antibacterial, antiviral and antifungal activity. It has been reported that some of them accumulate in cancer tissues.

There have been numerous papers on the separation of phenolic acids by paper and thin-layer chromatography, column (gas and liquid) chromatography and electrophoresis[1–11]. However, the methods reported so far do not permit the direct separation of a large number of isomeric phenolic acids and their derivatives (gas chromatography requires derivatization to trimethylsilyl compounds). Therefore, it seemed worthwhile to investigate liquid–liquid systems with minimal mutual solubility of the phases as potential highly selective systems for the separation of the naturally occurring derivatives of cinnamic acid.

EXPERIMENTAL

The "moist paper" technique with controlled impregnation of paper with the aqueous phase was used. Whatman No. 4 paper strips, 7 × 23.5 cm, were impregnated with a 1% aqueous solution of citric acid (to suppress the ionization of the phenolic acids), the excess of the liquid was removed by blotting between two sheets of filter-paper and the phenolic acids and coumarins were spotted as 0.1–0.5% acetone solutions (*ca.* 10 μl). After partial drying to a moisture content corresponding to 0.5 ml of aqueous phase per gram of dry paper, the paper strips were developed in all-glass tanks for descending development[12]. The spots were localized in UV light (at 360 nm) after spraying with a saturated solution sodium of hydrogen carbonate or by coupling with bis-diazotized benzidine.
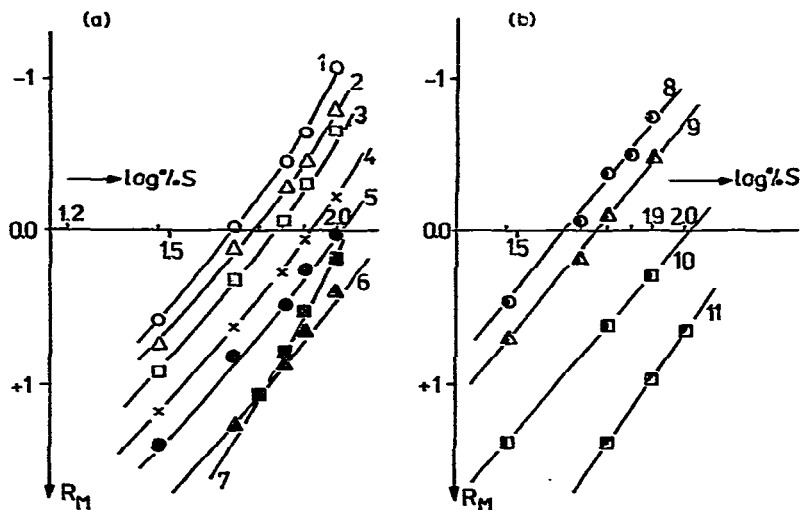
Fig. 1. R_M values of (a) phenolic acids and (b) related coumarins plotted against concentration of diisopropyl ether (S) in the developing solvent. Diluent: cyclohexane. For identification of solutes, see Table I.

RESULTS AND DISCUSSION

The phenolic acids were chromatographed in systems of the type polar solvent diluted with cyclohexane or benzene–1% aqueous solution of citric acid. Electron-donor solvents of various polarities were used as polar components of the mobile phase: diethyl ether, diisopropyl ether, methyl n-propyl ketone, diisobutyl ketone and tri-n-butyl phosphate. The results are presented as graphs of R_M values of the solutes plotted against the volume-per cent concentration of the polar solvent (on a logarithmic scale); such plots provide a quantitative characterization of the partition
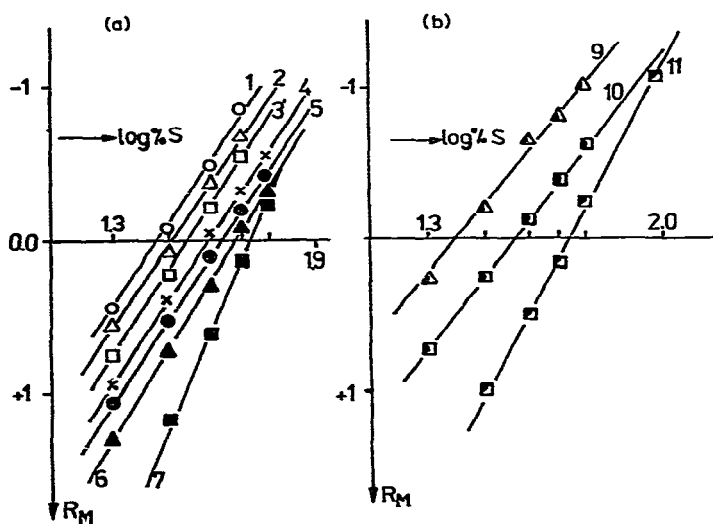

Fig. 2. As Fig. 1; developing solvent, solutions of methyl isopropyl ketone (S) in cyclohexane.
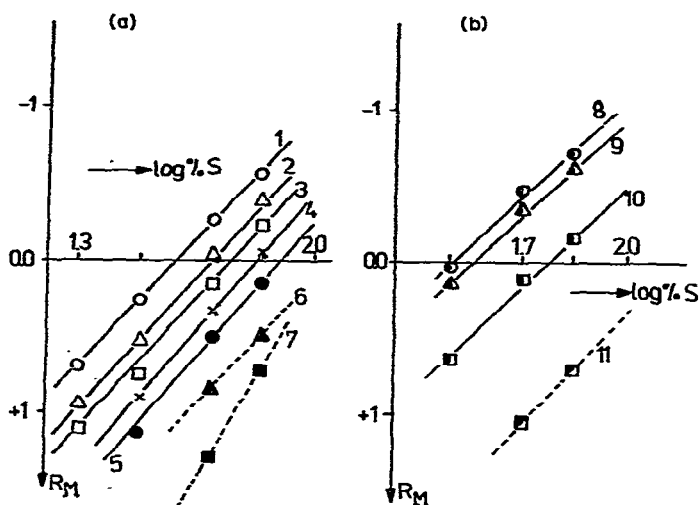
Fig. 3. As Fig. 1; developing solvent, solutions of diisobutyl ketone in cyclohexane.

system and indirect information about the presumed molecular solvation mechanism in the organic phase[13].

Fig. 1 shows that diisopropyl ether is a good extractant of the phenolic acids; a suitable range of k' values is obtained by dilution of the polar solvent with cyclohexane to *ca.* 80% concentration. The R_M *versus* log [iPr$_2$O] lines are approximately parallel, which indicates similar solvation mechanisms. Analogous results were obtained when diethyl ether was used as the polar solvent.

The slopes (apparent solvation numbers) of the R_M *versus* log [solvent] lines given in Table I seem to indicate that coumaric and ferulic acids form solvation complexes with ethers in the ratio 1:2 or even 1:3; for caffeic acid and aesculetin the plots
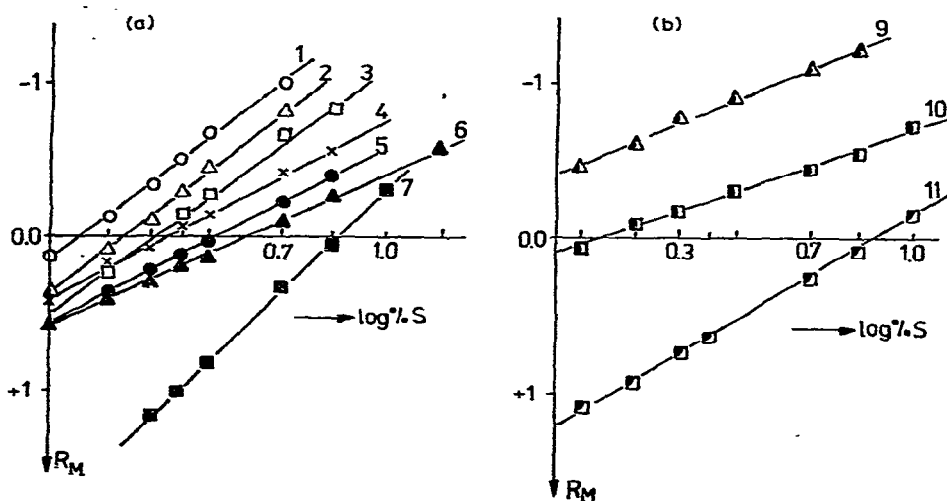

Fig. 4. As Fig. 1; developing solvent, solutions of tri-*n*-butyl phosphate in benzene.

TABLE I

SLOPES (ABSOLUTE VALUES) OF R_M vs. LOG % S PLOTS FOR VARIOUS POLAR COMPONENTS OF THE DEVELOPING SOLVENT

No.	Solute	Et₂O	iPr₂O	MePrCO	iBu₂CO	TBP
1	o-Coumaric acid	2.8	2.8–3.2	3.3	2.4	1.7
2	m-Coumaric acid	2.8	2.7–2.9	3.3	2.5	1.7
3	p-Coumaric acid	2.8	2.6–3.0	3.3	2.4	1.6
4	Ferulic acid	2.8	2.5–2.8	3.3	2.4	1.2
5	Isoferulic acid	2.8	2.6	3.4	2.5	1.2
6	Sinapic acid	3.3	3.0	3.5	2.5	1.0
7	Caffeic acid	5.2	4.0	5.0	4.0	2.1
8	4-Hydroxycoumarin	2.8	2.6	—	2.1	—
9	Umbelliferone	2.8	2.6	2.8	2.0	0.9
10	Scopoletin	2.7	2.5	2.9	2.1	0.8
11	Aesculetin	5.5	3.5	4.0	2.8	1.2

are steeper (3.0–5.5 using diethyl ether, diisopropyl ether and methyl n-propyl ketone), presumably because of larger number of unhindered hydroxyl groups and the formation of higher solvates.

Figs. 2 and 3 illustrate the chromatographic behaviour of the phenolic acids and coumarins in solvent systems containing methyl n-propyl ketone or diisobutyl ketone. For methyl n-propyl ketone the plots are steeper, indicating stronger solvation of the solutes. The solvation ability of the latter solvent is lower owing to its greater molar volume (lower molar concentrations) and steric shielding of the carbonyl group.

Of the five polar solvents investigated, the strongest extraction ability was exhibited by tri-n-butyl phosphate (TBP): even low concentrations of the extractant in benzene caused significant increases in the R_F values of the solutes (Fig. 4). The
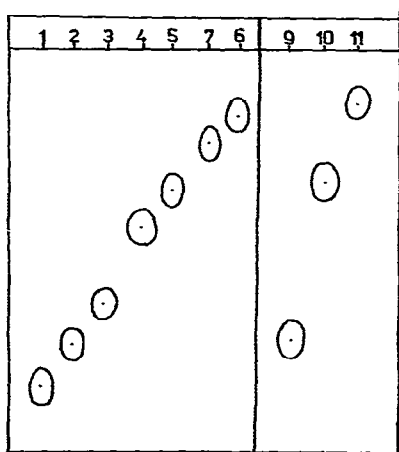

Fig. 5. Chromatogram of the solutes obtained for development with an 80% solution of diisopropyl ether in cyclohexane.
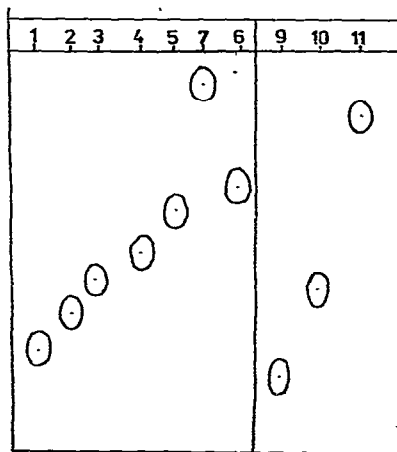
Fig. 6. Chromatogram of the solutes obtained for development with a 2.5% solution of tri-n-butyl phosphate in benzene.

slopes of the plots (Table I) seem to indicate that TBP, in the concentration range studied, forms 1:1 solvation complexes with various contributions of 1:2 complexes, which tend to be formed especially by solutes with unhindered hydroxyl groups (*i.e.*, without vicinal methoxy groups).

R_M *versus* solvent composition relationships enable one to choose partition systems with optimal spacing of the spots on the chromatograms; Figs. 5 and 6 illustrate chromatograms obtained for two solvent systems chosen in this way. The duration of analysis is limited owing to the low viscosities of the developing solvents (*ca.* 30 min for a distance of 16 cm). As the two liquid phases are almost immiscible, the selectivity of the systems is relatively high, as can be seen from a comparison of the chromatographic parameters with those reported in the literature (Fig. 7). The systems of the type investigated are therefore also promising in high-performance liquid chromatography with silica impregnated with water, *e.g.*, by the *in situ* technique[14].
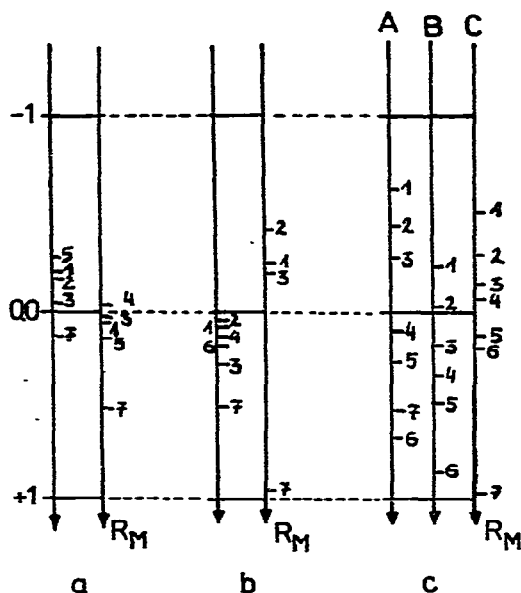

Fig. 7. Comparison of the selectivities of various chromatographic systems: a, ref. 11; b, ref. 2; c, this study; A and C, data from Figs. 5 and 6, respectively; B, chromatogram developed with a 50% solution of diisobutyl ketone in cyclohexane.

The solutes investigated have a wide range of capacity ratios, from the hydrophobic *o*-coumaric acid (a single hydroxyl group tends to form an internal hydrogen bond with the double bond in the side-chain) to the hydrophilic caffeic acid, aesculetin and especially chlorogenic acid. Therefore, the "general elution problem"[15] which may be encountered in the column chromatography of these compounds can be solved by gradient elution based on the R_M (log k') *versus* solvent composition relationships[16].

REFERENCES

1 P. Ribereau-Gayon, *Plant Phenolics*, Oliver and Boyd, Edinburgh, 1972, pp. 90–105.
2 C. F. van Sumere, G. Wolf, H. Teuchy, and J. Kint, *J. Chromatogr.*, 20 (1965) 48.
3 K. vande Casteele, H. de Pooter and C. F. van Sumere, *J. Chromatogr.*, 121 (1976) 49.
4 Z. Grodzińska-Zachwieja, W. Kahl and M. Klimczak, *Diss. Pharm. Pharmacol.*, 23 (1971) 179.
5 J. P. Haluk, C. Duval and M. Metche, *Int. Symp. Chromatogr. Electrophoresis*, Presses Acad. Eur., Brussels, 1971, p. 431.
6 P. A. Hedin, J. P. Minyard, Jr. and A. C. Thompson, *J. Chromatogr.*, 30 (1967) 43.
7 G. Alibert, *J. Chromatogr.*, 80 (1973) 173.
8 H. Smidtlein and K. Herrmann, *Z. Lebensm.-Unters.-Forsch.*, 159 (1975) 139, 213 and 255.
9 E. D. Pellizzari, C.-M. Chuang, J. Kuc and E. B. Williams, *J. Chromatogr.*, 40 (1969) 285.
10 L. W. Wulf and C. W. Nagel, *J. Chromatogr.*, 116 (1976) 271.
11 K. Randerath, *Thin-Layer Chromatography*, Academic Press, New York, 1964, p. 178.
12 E. Soczewiński, *Chem. Anal. (Warsaw)*, 18 (1973) 219.
13 E. Soczewiński and G. Matysik, *J. Chromatogr.*, 111 (1975) 7.
14 B.-A. Persson and B. L. Karger, *J. Chromatogr. Sci.*, 12 (1974) 521.
15 L. R. Snyder, in J. J. Kirkland (Editor), *Modern Practice of Liquid Chromatography*, Wiley, New York, 1971, p. 149.
16 P. Jandera and J. Churáček, *J. Chromatogr.*, 91 (1974) 207.