CHROM. 18 004

INFLUENCE OF THE MOBILE PHASE COMPOSITION ON THE REVERSED-PHASE THIN-LAYER CHROMATOGRAPHIC BEHAVIOUR OF A SERIES OF PROSTAGLANDINS

COMPARISON OF THE EXTRAPOLATED R_M VALUES

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(Received June 27th, 1985)

SUMMARY

The R_M values of a series of prostaglandins were determined in two reversed-phase thin-layer chromatographic systems, the mobile phase being an aqueous buffer alone or mixed with various amounts of methanol or acetone. The linear relationship between the chromatographic behaviour and the mobile phase composition yielded very similar extrapolated R_M values at 0% organic solvent in both systems. This shows that the extrapolated R_M values are independent of the nature of the organic solvent. In other words, the extrapolated R_M values should be related to the partitioning of the compounds between water and silicone oil in a standard system where all the compounds can be compared.

INTRODUCTION

The lipophilic character of drugs plays a significant rôle in their biological activity[1,2].

The R_M values in reversed-phase thin-layer chromatography (TLC) have been shown to be a reliable measure of the hydrophobicity of molecules[3]. The linear relationship between the chromatographic behaviour and the composition of the mobile phase yields extrapolated R_M values at 0% of organic solvent. Such values might be related to the partitioning of the compounds between water and silicone oil, the latter being the medium impregnating the silica gel G layer in the reversed-phase TLC system[4].

Although few papers have dealt with the relationship between R_M and the mobile phase composition[5–7], this is a very important aspect of chromatography. The

extrapolation technique might be a way of obtaining R_M values in a standard system, *i.e.*, independent of the nature of the organic solvent in the mobile phase. In a previous study the R_M values of a series of dermorphin-related oligopeptides were determined in two reversed-phase TLC systems[8]. The mobile phase was an aqueous buffer alone or mixed with various amounts of methanol or acetone. The extrapolated R_M values at 0% of organic solvent were shown to be very similar in the two systems.

The purpose of the present study was to measure the R_M values of a series of prostaglandins in the above reversed-phase TLC systems as a further contribution to the assessment of the reliability of the extrapolation technique.

MATERIALS AND METHODS

Compounds investigated
The structures of the prostaglandins are shown in Table I. The compounds were a generous gift from Carlo Erba.

Determination of R_M values
The TLC technique employed has been described previously[4,8]. Glass plates (20 × 20 cm) were coated with silica gel G; in order better to control the pH of the stationary phase a slurry of silica gel G was prepared with 0.09 M sodium hydroxide solution. A non-polar stationary phase was obtained by impregnating the silica gel G layer with silicone DC 200 (350 cSt) (Applied Science Labs.). The impregnation was carried out by developing the plates in a 5% silicone solution in diethyl ether. Eight plates could be impregnated in a single chromatographic chamber, containing 200 ml of the silicone solution. The plates were left in the chamber for 12 h, *i.e.*, for several hours after the silicone solution had reached the top of the plates. The chromatographic chamber was saturated with the vapour of the mobile phase.

A migration of 10 cm was obtained on all plates by cutting the layer at 12 cm and spotting the compounds on a line 2 cm from the lower edge of the plate. The mobile phase saturated with silicone was an aqueous buffer (sodium acetate–Veronal buffer, 1/7 M at pH 7.0), alone or mixed with various amounts of acetone or methanol.

Two plates were developed simultaneously in a chromatographic chamber containing 200 ml of mobile phase. The dermorphin-related derivatives were dissolved in methanol (1–2 mg/ml) and 1 μl of solution was spotted randomly on the plates in order to avoid any systematic error. The developed plates were dried and sprayed with an alkaline solution of potassium permanganate. After a few minutes at 120°C, yellow spots appeared on an intense pink background. The R_M values were calculated by means of the equation:

$$R_M = \log\left(\frac{1}{R_F} - 1\right)$$

log P values
Experimental log P values for compounds 1, 2 and 6 have been reported by Hansch and Leo[9]. These were used in order to calculate the log P values of the remaining compounds, by taking advantage of the additive property of the π values.

RESULTS AND DISCUSSION

R_M values

On the basis of our previous work with reversed-phase TLC or high-performance liquid chromatography (HPLC) and in agreement with data from the literature, we pointed out that the relationship between R_M values and mobile phase composition can generally be described by an S-shaped curve[10]. In fact at the lower organic solvent concentrations the compounds tend not to move from the starting line, while at the higher concentrations they tend to move with the solvent front. It has been suggested that the extrapolation from the linear part of the curve should yield the theoretical R_M values at 0% organic solvent in the mobile phase. In this way one should avoid the physical limitations of the chromatographic system represented by the upper and lower parts of the S-shaped curve. The extrapolated R_M values at 0% could be considered as a measure of the partitioning of the compounds between water or an aqueous buffer and the hydrophobic stationary phase, *i.e.*, in a standard system where all the compounds could be compared on the basis of their lipophilic character. Hydrophilic compounds are supposed to show deviations from linearity only at higher organic solvent concentrations, since even at 0% organic solvent in the mobile phase their chromatographic behaviour yields reliable R_M values. As regards the present work, the test compounds did not move from the starting line when the mobile phase was the aqueous buffer alone. In order to obtain suitable R_M values it was necessary to add an organic solvent to the mobile phase.
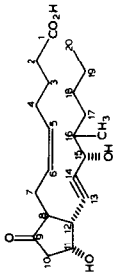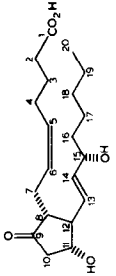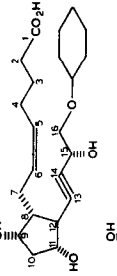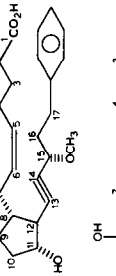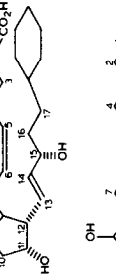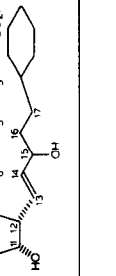
In the methanol system the R_M values reported in Table II were obtained. The plots in Fig. 1 and the equations of Table II show that for each compound the R_M values bear a very good linear relationship to the mobile phase composition over the full range of methanol concentrations yielding reliable R_M values. In Fig. 1 the upper and lower parts of the curves were not reported. At those methanol concentrations the compounds remained so close to the starting line or moved so close to the solvent front that the measurement of the R_M values was unreliable. The intercepts of the equations in Table II represent the theoretical R_M values at 0% methanol in the mobile phase.

The R_M values obtained similarly in the acetone system are given in Table III. The plots in Fig. 1 show that at acetone concentrations higher than 32–36% all the compounds tend to migrate with the solvent front, *i.e.*, in the lower part of the S-curve. The R_M values obtained at acetone concentrations higher than 48% are not reported. Because of the deviations from linearity, the equations in Table III were calculated by means of the R_M values obtained at acetone concentrations only up to 32–36% as shown in Fig. 1. The theoretical R_M values at 0% acetone are very close to those at 0% methanol.

The analysis of variance did not show any significant difference between the two sets of R_M values. This should mean that the extrapolated R_M values are not dependent on the nature of the organic solvent in the mobile phase. In other words, the extrapolated R_M values should be a measure of the partitioning in the same standard system, *i.e.*, between water and the silicone oil impregnating the silica gel G layer. As a consequence the equation describing the correlation between the R_M values in the two chromatographic systems should be characterized by an intercept and slope close to 0 and 1 respectively. This seems to be the case:

TABLE I

STRUCTURES AND log P VALUES OF PROSTAGLANDIN DERIVATIVES

No.	Compound	Structure	Empirical formula	Molecular weight	log P
1	Prostaglandin E_1 (PGE$_1$)		$C_{20}H_{34}O_5$	354.492	2.00
2	Prostaglandin E_2 (PGE$_2$)		$C_{20}H_{32}O_5$	352.476	1.69
3	16δ-Methyl-13,14-didehydro-8,12-diisoprostaglandin E_2		$C_{21}H_{32}O_4$	348.487	3.35
4	13,14-Didehydro-ω-trinor-16-methyl-16-butoxyprostaglandin E_2		$C_{22}H_{34}O_6$	394.514	1.72
5	13,14-Didehydro-ω-trinor-16-methyl-16-pentoxyprostaglandin E_2		$C_{23}H_{38}O_6$	410.557	2.22
6	Prostaglandin $F_{2\alpha}$ (PGF$_{2\alpha}$)		$C_{20}H_{34}O_5$	354.492	1.60

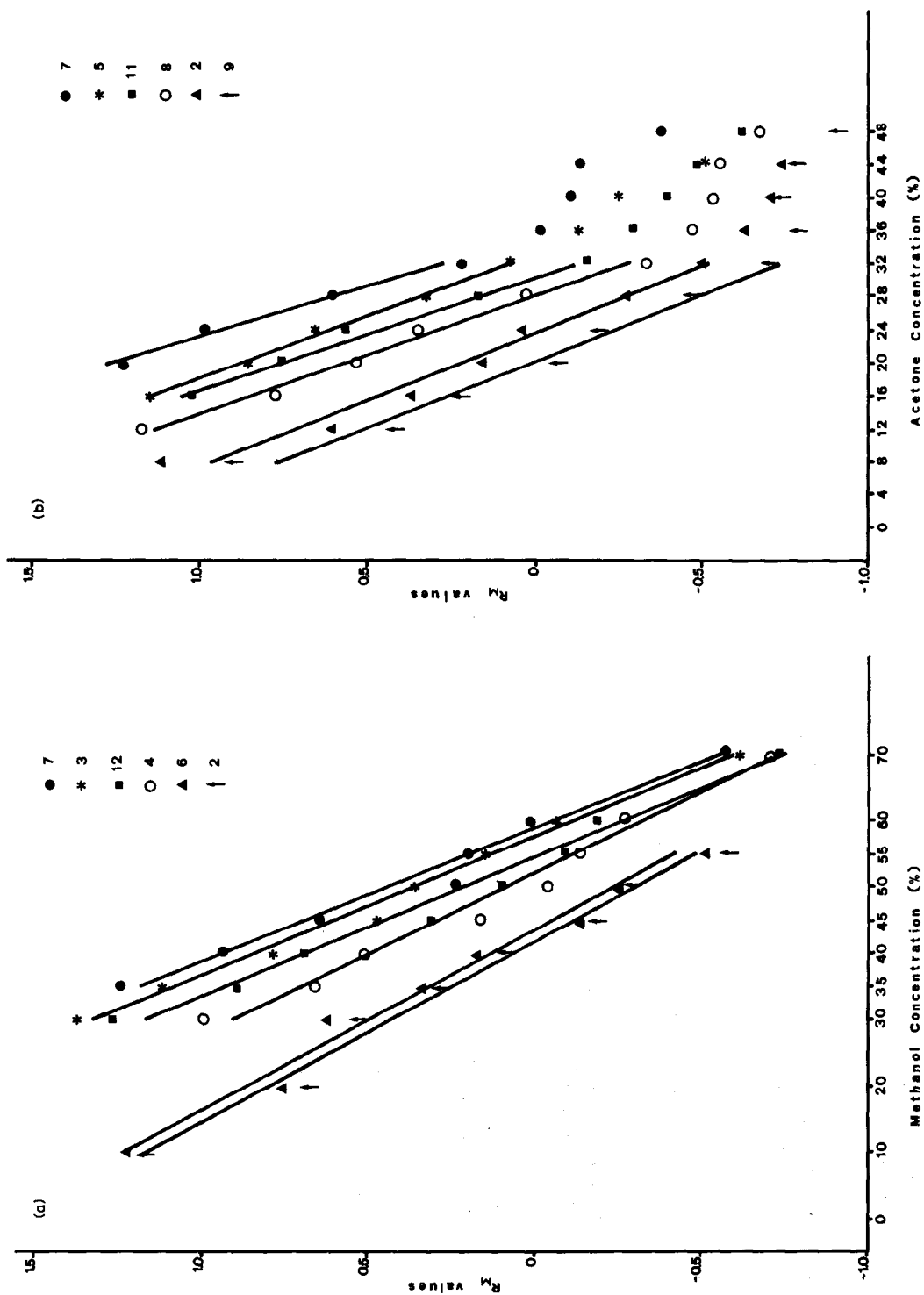| No.	Name	Structure	Formula	MW	R_F
7	ω-Trinor-17-cyclopentyl-9-deoxy-$\Delta^{9,10}$-prostaglandin $F_{2\alpha}$		$C_{22}H_{34}O_4$	362.514	3.10
8	13,14-Didehydro-16R-methyl-15-epiprostaglandin $F_{2\alpha}$		$C_{21}H_{34}O_5$	366.503	2.10
9	13,14-Didehydro-ω-tetranor-16-cyclohexyloxyprostaglandin $F_{2\alpha}$		$C_{22}H_{34}O_5$	394.514	1.14
10	13,14-Didehydro-ω-trinor-17-phenyl-15-methoxyprostaglandin $F_{2\alpha}$		$C_{24}H_{32}O_5$	400.520	2.92
11	ω-Trinor-17-cyclohexyl-8,12-diisoprostanglandin $F_{2\beta}$		$C_{23}H_{38}O_5$	394.557	2.61
12	ω-Trinor-17-cyclohexyl-8,12-diiso-15-epiprostaglandin $F_{2\beta}$		$C_{23}H_{38}O_5$	394.557	2.61

Fig. 1. Relationship between R_M values and methanol (a) or acetone (b) concentrations in the mobile phase.

TABLE II

RELATIONSHIP BETWEEN R_M VALUES OF PROSTAGLANDIN DERIVATIVES AND METHANOL CONCENTRATION IN THE MOBILE PHASE

Compound	Methanol concentration (%)													TLC equation		
	0	5	10	20	30	35	40	45	50	55	60	70	75	$R_M = a$	b	r
1	–	–	1.31	0.93	0.69	0.38	0.18	-0.13	-0.26	-0.54	–	–	–	1.788	-0.041	0.994
2	–	–	1.17	0.69	0.54	0.29	0.09	-0.18	-0.27	-0.57	–	–	–	1.542	-0.037	0.992
3	–	–	–	–	1.38	1.12	0.79	0.49	0.37	0.15	-0.06	-0.61	–	2.769	-0.048	0.996
4	–	–	–	–	1.01	0.67	0.52	0.17	-0.03	-0.13	-0.27	-0.70	–	2.136	-0.041	0.990
5	–	–	–	–	1.24	1.01	0.79	0.40	0.12	0.00	-0.13	-0.68	–	2.644	-0.048	0.993
6	–	–	1.22	0.76	0.63	0.34	0.18	-0.12	-0.24	-0.51	–	–	–	1.613	-0.037	0.991
7	–	–	–	–	–	1.25	0.93	0.65	0.24	0.19	0.02	-0.59	–	2.937	-0.050	0.989
8	–	–	–	–	1.03	0.75	0.51	0.12	-0.04	-0.21	-0.26	-0.73	–	2.215	-0.043	0.985
9	–	–	–	0.63	0.55	0.43	0.18	-0.16	-0.24	-0.36	-0.45	-0.80	–	1.377	-0.031	0.981
10	–	–	–	–	–	0.95	0.59	0.10	-0.06	-0.15	-0.43	-0.76	–	2.424	-0.047	0.974
11	–	–	–	–	1.24	0.91	0.56	0.20	0.05	-0.12	-0.21	-0.73	–	2.506	-0.047	0.984
12	–	–	–	–	1.28	0.90	0.70	0.31	0.10	-0.09	-0.18	-0.73	–	2.610	-0.048	0.991

TABLE III

RELATIONSHIP BETWEEN R_M VALUES OF PROSTAGLANDIN DERIVATIVES AND ACETONE CONCENTRATION IN THE MOBILE PHASE

Compound	Acetone concentration (%)													TLC equation		
	0	4	8	12	16	20	24	28	32	36	40	44	48	$R_M = a$	b	r
1	—	—	1.41	0.81	0.57	0.28	0.12	−0.28	−0.49	−0.68	−0.76	—	—	1.778	−0.071	0.988
2	—	—	1.12	0.61	0.38	0.16	0.04	−0.28	−0.50	−0.62	−0.71	−0.74	—	1.465	−0.062	0.985
3	—	—	—	—	—	1.30	0.96	0.63	0.30	0.11	0.11	0.05	−0.32	2.788	−0.076	0.995
4	—	—	—	1.09	0.90	0.57	0.40	0.07	−0.26	−0.35	−0.42	−0.57	—	1.940	−0.067	0.995
5	—	—	—	—	1.15	0.86	0.66	0.33	0.07	−0.12	−0.25	−0.50	—	2.228	−0.067	0.998
6	—	—	1.27	0.61	0.48	0.21	0.08	−0.29	−0.43	−0.53	−0.72	−0.90	—	1.579	−0.065	0.975
7	—	—	—	—	—	1.23	0.99	0.61	0.23	−0.03	−0.11	−0.13	−0.37	2.962	−0.084	0.995
8	—	—	—	1.18	0.78	0.54	0.35	0.03	−0.33	−0.47	−0.53	−0.55	−0.67	1.995	−0.071	0.995
9	—	—	0.91	0.44	0.24	−0.06	−0.18	−0.46	−0.71	−0.78	−0.72	−0.77	−0.90	1.286	−0.063	0.990
10	—	—	—	—	1.03	0.86	0.46	0.11	−0.24	−0.46	−0.48	−0.56	−0.72	2.418	−0.082	0.994
11	—	—	—	—	1.03	0.76	0.58	0.17	−0.15	−0.28	−0.39	−0.49	−0.62	2.248	−0.074	0.992
12	—	—	—	—	1.14	0.81	0.56	0.19	−0.16	−0.28	−0.45	−0.53	−0.59	2.440	−0.080	0.998

$$R_{M_{CH_3OH}} = (0.171 \pm 0.177) + (0.975 \pm 0.082)R_{M_{(CH_3)_2CO}} \tag{1}$$

$$n = 12, r = 0.966, \text{S.D.} = 0.141, F = 141.16, P < 0.005$$

Another interesting point arises from the comparison of the slopes of the straight lines describing the relationship between R_M values and the mobile phase composition. The slopes for the methanol and acetone systems have mean values of -0.042 and -0.071, respectively (Tables II and III). The more negative slope in the acetone system is due to the higher eluting power of acetone, when compared with that of methanol. The ratio of 1.69 between the above mean values is very close to the ratio of 1.70 between the solvent-strength parameters, E_0, of methanol (0.95) and acetone (0.56), in a reversed-phase chromatographic system. A very similar result was obtained previously with a series of dermorphin-related oligopeptides[8,11].

Relationship between R_M and log P values
The correlation of the extrapolated R_M values with the log P values in Table I is expressed as

$$R_{M_{(CH_3)_2CO}} = (0.438 \pm 0.171) + (0.734 \pm 0.073) \log P \tag{2}$$

$$n = 12, r = 0.954, \text{S.D.} = 0.163, F = 101.05, P < 0.005$$

$$R_{M_{CH_3OH}} = (0.652 \pm 0.261) + (0.692 \pm 0.111) \log P \tag{3}$$

$$n = 12, r = 0.891, \text{S.D.} = 0.249, F = 38.56, P < 0.005$$

The confidence limits of the intercepts and slopes of eqns. 2 and 3 show that there is no reason to reject the hypothesis that they are from the same population. This is in agreement with eqn. 1, showing that the two sets of R_M values used for calculating eqns. 2 and 3 are not significantly different. Therefore, if in both chromatographic systems the extrapolation yields R_M values which can be considered as a measure of the partitioning of the compounds between water and the hydrophobic stationary phase, *i.e.*, in a standard system, an average R_M value can be calculated for each compound from the extrapolated R_M values in the two different chromatographic systems. Eqn. 4 describes the correlation between the average R_M values and the log P values:

$$R_M = (0.545 \pm 0.209) + (0.713 \pm 0.090) \log P \tag{4}$$
$$n = 12, r = 0.930, \text{S.D.} = 0.199, F = 64.21, P < 0.005$$

The present data seem to provide a further contribution to the use of reversed-phase TLC as a standard system for the measurement of R_M values. Two or three organic solvents might provide extrapolated R_M values for compounds covering a wider range of lipophilicity. In this way one might avoid, at least partially, one of the major disadvantages of the R_M values, *i.e.*, their narrower range when compared with that of the log P values.

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