Journal of Chromatography, 171 (1979) 29–36

CHROM. 11,572

CHROMATOGRAPHIC STUDY OF LOCAL ANAESTHETICS —BASIC ESTERS OF SUBSTITUTED CARBANILIC ACIDS

II. THE RELATIONSHIP BETWEEN CHROMATOGRAPHIC VALUES, OTHER PHYSICO-CHEMICAL PARAMETERS AND ACTIVITY

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(First received January 27th, 1978; revised manuscript received October 27th, 1978)

SUMMARY

R_M values and the increments $\Delta R_{M(CH_2)}$ have been calculated from the chromatographic values R_F of a group of anaesthetics comprising an homologous series of basic esters of substituted carbanilic acids. A linear relationship is demonstrated between R_M values from partition chromatography and the partition coefficient (log P) or the substitution constant π. The chromatographic parameter R_M is also correlated with the pharmacological characteristic log U, the logarithm of the surface anaesthetic activity.

INTRODUCTION

The theoretical basis of the relationship between the R_F values obtained from partition chromatography and chemical structure was elaborated by Martin and Synge[1] and Consden *et al.*[2]. Bate-Smith and Westall[3] introduced the following equation:

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

The R_M values were used as a hydrophobic parameter, replacing the partition coefficient in biological correlations[4]. The linear relationship between the partition coefficient and R_M was derived[5] as follows

$$R_M = a \cdot \log P + b \quad (2)$$

and has been verified for various groups of substances[6–9].

In these correlations the partition coefficient can be replaced by the π-substitution constant, as defined by Hansch *et al.*[10], which characterizes the contribution

of a substituent to the lipophilic nature of the whole molecule. Since the lipophilic nature of a drug is important in the processes which influence the activity of the drug, the R_M value was used instead of log P in correlations with the biological activity[7,11,12]. The applications of R_M values obtained from partition chromatography are various, and many additional examples are summarized in Tomlinson's review[13].

To investigate the relationship between R_M and other physico-chemical properties of local anaesthetics and their biological activity, several series of substances, differing in both the aromatic part and in the character of the basic alcohol, were prepared by Dr. A. Borovanský and co-workers at the Department of Pharmaceutical Chemistry, Comenius University, Bratislava. In our previous study[14] we demonstrated the use of Silufol and Lucefol foils in thin-layer chromatography (TLC) of some groups of these substances.

In the present work, two groups of substances were examined: 2-morpholinoethyl esters of 2-, 3- and 4-alkoxycarbanilic acid, and 2-(N,N-dipropylamino)ethyl esters of 2-, 3- and 4-alkoxycarbanilic acid. In the homologous series (within the chosen systems) a regular increase of R_F values was observed, and thus the R_M values and the group constants $\Delta R_{M(CH_2)}$ for 2-, 3- and 4-derivatives were calculated from the R_F values. We then attempted to confirm the relationship between log P and R_M or π and ΔR_M for these substances. A correlation between chromatographic parameters and biological activity was demonstrated for the substances chosen.

EXPERIMENTAL

For the chromatographic separations, ready-made Silufol® UV 254 foils with Silpearl® silica gel layers and Lucefol® Quick cellulose foils were used. The detection on the silica gel foils was carried out by use of a fluorescent indicator in UV light (UV lamp; Camag, Muttenz, Switzerland) and on the cellulose foils by spraying them with Dragendorff's reagent. The substances under study were prepared as 1% solutions in chloroform; 1–4-μl volumes were applied on the chromatogram. In absorption chromatography using the Silufol UV 254 foils the elution system was light petroleum (b.p. 30–50°)–diethylamine (4:1) for substances I–XIX, and (20:1) for substances XX–XXXVII.

In partition chromatography, Lucefol Quick impregnated with 10% formamide in ethanol was used for substances I–VI. The mobile phase was *n*-heptane. For substances VII–XIX, the same carrier, impregnated with 30% formamide and the developer *n*-heptane–diethylamine (20:1), was used. Silufol UV 254 impregnated with 40% formamide and 1% tris(hydroxymethyl)aminomethane was used for the separation of substances XX–XXXVII in the solvent system *n*-heptane–diethylamine (10:1).

The R_M values were calculated from the mean R_F values (obtained from six chromatograms) using eqn. 1.

The experimental partition coefficient was determined in the system *n*-octanol–water (phosphate buffer, pH 7.0) at 20.0°. An appropriate amount of the substance was dissolved in the phosphate buffer and to 9.5 ml of this solution was added 0.5 ml of *n*-octanol. The liquid was then shaken for 2 h. After separation and stabilization of the phases (1 h), the absorbance of the water layer was measured by a Spectromom 202 spectrophotometer (MOM, Budapest, Hungary). The partition coefficient was

calculated from the molar concentrations using the equation presented in ref. 15; the values of three parallel measurements were taken.

The dissociation constants were determined potentiometrically using Henderson's relationship; the pK_a values of the substances were obtained by determination of the pH of solutions of the examined substance titrated to 50% neutralization with alkali. Owing to the low solubility in water of the base formed during the titration, a mixture of water and 60% methanol had to be used. The pK_a values of the examined substances were then corrected for the volume of methanol used.

Pharmacological evaluation of the substances was carried out by the method of Vrba and Sekera[16]; *i.e.*, effective concentrations of the substances were determined and their anaesthetic effects at the surface of the rabbit cornea were compared with that of a standard 0.01 *M* solution of cocaine.

The calculations were done on the Siemens 4004/150 computer at the ÚVT VŠ (UK Bratislava, Czechoslovakia).

RESULTS AND DISCUSSION

Adsorption chromatography on Silufol UV 254 foils was used to study the physico-chemical parameters of substances from the alkoxy-substituted derivatives of carbanilic acid having local-anaesthetic activity. The choice of the developer and the other conditions were discussed previously[14]. The R_F values of substances I–XIX in light petroleum–diethylamine (4:1) are given in Table I, those for substances XX–XXVII in light petroleum–diethylamine (20:1) are in Table II.

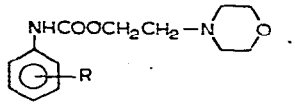
The examined substances represented homologous series, where it could be expected that an incremental increase of the number of methylene groups would result in a regular increase of the R_F values for the successive members of the series. It can be seen from Figs. 1 and 2, that the relationship between the R_M value and the number of carbons, C, in the alkoxyl chain is linear. Table III gives the slopes of such relationships as well as the mean values of the $\Delta R_{M(CH_2)}$ increments in the developers employed.

Conditions for the application of partition chromatography on Lucefol Quick cellulose layers were also sought. *n*-Heptane was chosen as the developer for substances I–VI on a layer impregnated with 10% formamide and *n*-heptane–diethylamine (20:1) as the developer for substances VII–XIX with 30% formamide impregnation. For substances XX–XXVII, Lucefol impregnated with 40% formamide in ethanol and isopropanol as the elution system was useful, but on further study this system was shown not to be convenient because of the very low $\Delta R_{M(CH_2)}$ values. Thus, for this group of substances, Silufol UV 254 impregnated with 40% formamide and 1% tris-(hydroxymethyl)aminomethane in the elution system *n*-heptane–diethylamine (10:1) was used. The mean R_F values are given in Tables I and II together with the standard deviations and calculated R_M values.

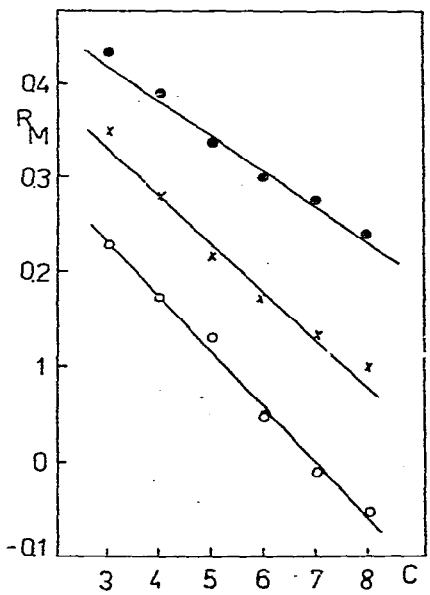
In the case of partition chromatography, the increase in the number of methylene groups in the homologous series also resulted in a regular increase of the R_F values. The relationship between R_M and the number of carbon atoms in the alkoxyl chain is linear (Figs. 3 and 4). The values of the slopes for the series of 2-, 3- and 4-alkoxy-substituted derivatives and the mean values of the incremental increase for the series of positional isomers are shown in Table III.

TABLE I

pK_a, R_F AND R_M VALUES OF MORPHOLINOETHYL ESTERS OF ALKOXY-SUBSTITUTED CARBANILIC ACIDS



Compound	*R*	*pK_a*	*Adsorption chromatography*			*Partition chromatography*		
			R_F	*s*	R_M	R_F	*s*	R_M
I	2-OC_3H_7	5.52	0.369	0.018	0.233	0.752	0.020	−0.482
II	2-OC_4H_9	5.79	0.402		0.173	0.811		−0.633
III	2-OC_5H_{11}	5.60	0.424		0.133	0.858		−0.781
IV	2-OC_6H_{13}	5.69	0.471		0.050	0.877		−0.853
V	2-OC_7H_{15}	5.36	0.504		−0.007	0.901		−0.960
VI	2-OC_8H_{17}	5.37	0.530		−0.052	0.917		−1.043
VII	3-OC_3H_7	5.71	0.310	0.022	0.348	0.330	0.015	0.308
VIII	3-OC_4H_9	5.65	0.344		0.280	0.515		−0.026
IX	3-OC_5H_{11}	5.67	0.377		0.218	0.698		−0.364
X	3-OC_6H_{13}	5.58	0.401		0.174	0.816		−0.647
XI	3-OC_7H_{15}	5.62	0.424		0.132	0.896		−0.935
XII	3-OC_8H_{17}	5.50	0.443		0.099	0.923		−1.079
XIII	4-OC_3H_7	5.68	0.270	0.017	0.432	0.161	0.018	0.717
XIV	4-OC_4H_9	5.65	0.291		0.387	0.324		0.319
XV	4-OC_5H_{11}	5.71	0.315		0.337	0.499		0.002
XVI	4-OC_6H_{13}	5.72	0.335		0.298	0.672		−0.311
XVII	4-OC_7H_{15}	5.71	0.348		0.273	0.809		−0.627
XVIII	4-OC_8H_{17}	5.55	0.366		0.239	0.858		−0.781
XIX	—	—	0.0			0.0		



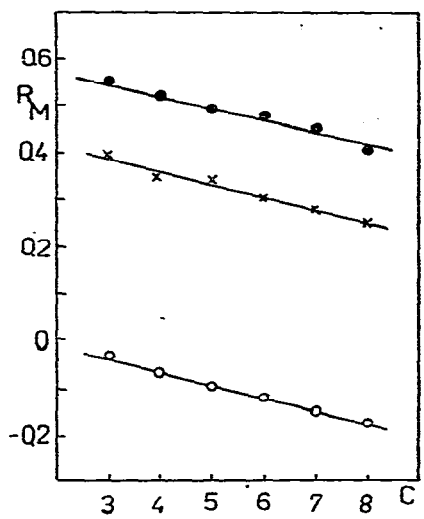
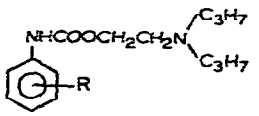


Fig. 1. Dependence of R_M values on the number of carbon atoms in the alkoxy substituent for morpholinoethyl exters of carbanilic acids, using adsorption chromatography. Derivatives: (○), 2-alkoxy; (×), 3-alkoxy; (●), 4-alkoxy.

Fig. 2. Dependence of R_M values on the number of carbon atoms in the alkoxy substituent for 2-(N,N-dipropylamino)ethyl esters of carbanilic acids, using adsorption chromatography. Derivatives as in Fig. 1.

TABLE II

pK_a, R_F AND R_M VALUES OF 2-(N,N-DIPROPYLAMINO)ETHYL ESTERS OF ALKOXY-SUBSTITUTED CARBANILIC ACIDS



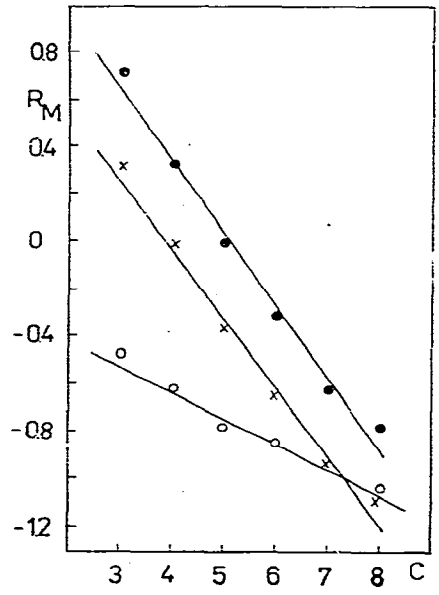
Compound	*R*	*pK_a*	*Adsorption chromatography*			*Partition chromatography*		
			R_F	*s*	R_M	R_F	*s*	R_M
XX	2-OC_3H_7	7.85	0.524	0.027	−0.042	0.510	0.053	−0.017
XXI	2-OC_4H_9	7.76	0.541		−0.071	0.546		−0.080
XXII	2-OC_5H_{11}	7.86	0.556		−0.098	0.569		−0.121
XXIII	2-OC_6H_{13}	7.75	0.573		−0.128	0.594		−0.165
XXIV	2-OC_7H_{15}	7.55	0.586		−0.153	0.623		−0.218
XXV	2-OC_8H_{17}	7.60	0.601		−0.178	0.663		−0.294
XXVI	3-OC_3H_7	7.91	0.289	0.060	0.391	0.325	0.060	0.317
XXVII	3-OC_4H_9	7.91	0.308		0.352	0.360		0.250
XXVIII	3-OC_5H_{11}	7.90	0.316		0.335	0.387		0.200
XXIX	3-OC_6H_{13}	7.81	0.333		0.302	0.410		0.158
XXX	3-OC_7H_{15}	7.80	0.346		0.276	0.425		0.131
XXXI	3-OC_8H_{17}	7.91	0.362		0.246	0.471		0.050
XXXII	4-OC_3H_7	7.80	0.223	0.021	0.542	0.346	0.047	0.276
XXXIII	4-OC_4H_9	7.86	0.233		0.517	0.369		0.233
XXXIV	4-OC_5H_{11}	7.82	0.245		0.489	0.394		0.187
XXXV	4-OC_6H_{13}	7.75	0.253		0.470	0.398		0.180
XXXVI	4-OC_7H_{15}	7.80	0.264		0.445	0.403		0.171
XXXVII	4-OC_8H_{17}	7.86	0.283		0.404	0.427		0.128
XXXVIII	—	—	—		—	0.381		0.211

TABLE III

SLOPE VALUES (*a*) OF RELATIONS BETWEEN R_M AND NUMBER OF CARBONS IN ALKOXY SUBSTITUENT, AND $R_{M(CH_2)}$ VALUES

Compound	*Adsorption chromatography*		*Partition chromatography*	
	a	$\Delta R_{M(CH_2)}$	*a*	$\Delta R_{M(CH_2)}$
I–VI	−0.0585	0.057	−0.1102	0.112
VII–XII	−0.0495	0.050	−0.2841	0.277
XIII–XVIII	−0.0385	0.039	−0.3040	0.300
XX–XXV	−0.0273	0.027	−0.0527	0.055
XXVI–XXXI	−0.0282	0.029	−0.0495	0.053
XXXII–XXXVII	−0.0264	0.030	−0.0267	0.030

The progress in the use of quantitative relationships between structure and activity has shown the importance of the hydrophobic nature of substances. The hydrophobicity of substances is usually characterized by the partition coefficient *P*. For our series of substances, the experimental partition coefficients were measured by a classical method in a pair of hydrophilic and hydrophobic solvents. *n*-Octanol was used as a model of the lipid phase and the water layer was represented by the phosphate buffer (pH 7.0). In later studies is presented the relationship between



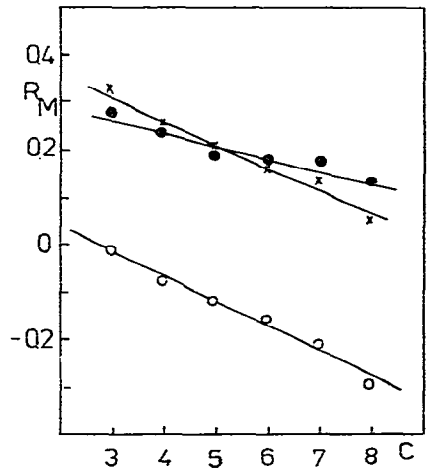
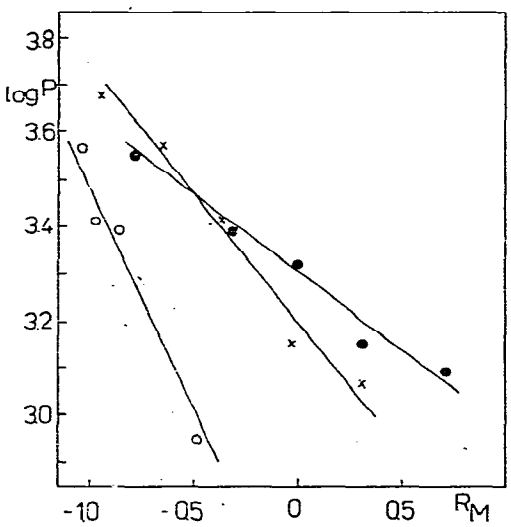


Fig. 3. Dependence of R_M values on the number of carbon atoms in the alkoxy substituent for morpholinoethyl esters of carbanilic acids, using partition chromatography. Derivatives as in Fig. 1.

Fig. 4. Dependence of R_M values on the number of carbon atoms in the alkoxy substituent for 2-(N,N-dipropylamino)ethyl esters of carbanilic acids, using partition chromatography. Derivatives as in Fig. 1.



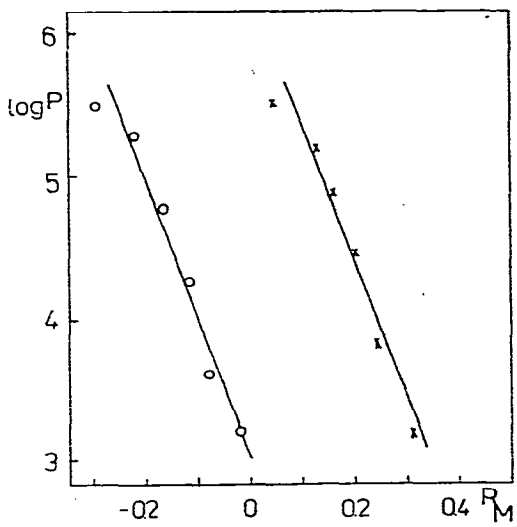


Fig. 5. Dependence of log P on R_M from partition chromatography for morpholinoethyl esters of carbanilic acids. Derivatives as in Fig. 1.

Fig. 6. Dependence of log P on R_M from partition chromatography for 2-(N,N-dipropylamino)ethyl esters of carbanilic acids. Derivatives ○ and × as in Fig. 1.

chromatographic parameters and other characteristics of hydrophobic properties, or the R_M values from partition chromatography are directly used to correlate the pharmacological characteristics and physico-chemical properties[13].

Before the chromatographic characteristic R_M could be correlated with the biological activity for a given group of substances, the validity of the relationship between log P and R_M (eqn. 2) had to be confirmed. This relationship is given for some groups of substances in Figs. 5 and 6. The individual points correspond to the members of the homologous series and they are linearly correlated. Mathematical expressions of these relationships are:

		r_k	s_r	
I–VI	$\log P = -1.0523\, R_M + 2.4481$	0.990	0.098	(3)
VII–XII	$\log P = -0.5265\, R_M + 3.1962$	0.990	0.083	(4)
XIII–XVIII	$\log P = -0.3187\, R_M + 3.2942$	0.988	0.088	(5)
XX–XXV	$\log P = -9.0550\, R_M + 3.0881$	0.977	0.107	(6)
XXVI–XXXI	$\log P = -9.2498\, R_M + 6.1937$	0.985	0.087	(7)

When other physico-chemical characteristics, *i.e.*, the pK_a values, were incorporated into the equations the correlation was not affected, eqns. 8–12, and the value of the correlation coefficient was not changed:

		r_k	s_r	
I–VI	$\log P = -1.1057\, R_M + 0.2035\, pK_a + 1.2875$	0.996	0.061	(8)
VII–XII	$\log P = -0.5520\, R_M + 0.3168\, pK_a + 1.3993$	0.990	0.080	(9)
XIII–XVIII	$\log P = -0.3212\, R_M + 0.0412\, pK_a + 3.0610$	0.988	0.087	(10)
XX–XXV	$\log P = -8.8162\, R_M - 0.2169\, pK_a + 4.7998$	0.977	0.107	(11)
XXVI–XXXI	$\log P = -8.7004\, R_M - 2.8276\, pK_a + 28.3598$	0.999	0.027	(12)

Introduction of Hansch's substitution constants into the correlations with ΔR_M gave the following equations:

		r_k	s_r	
I–VI	$\pi = -1.0441\, \Delta R_M - 0.3845$	0.964	0.188	(13)
VII–XII	$\pi = -0.5213\, \Delta R_M + 0.3617$	0.989	0.085	(14)
XIII–XVIII	$\pi = -0.3185\, \Delta R_M + 0.4732$	0.989	0.086	(15)

The relationship between the logarithm of the local-surface anaesthetic activity and the R_M values for morpholino-derivatives is shown in Fig. 7 and is described by:

		r_k	s_r	
I–VI	$\log U = -4.4364\, R_M - 1.9219$	0.955	0.172	(16)
VII–XII	$\log U = -1.4984\, R_M + 1.1001$	0.962	0.157	(17)
XIII–XVIII	$\log U = -1.1630\, R_M + 0.9831$	0.947	0.226	(18)

From the results of our work it is concluded that the partition coefficients can be replaced by the R_M values from partition chromatography of this series of substances having potential local anaesthetic activity. Since the R_M values are the chromatographic characteristics, all the advantages of chromatography can be exploited, *i.e.*, only small amounts of substance are needed and the determination of the substances is not necessary. Only one chromatogram is required to develop the complete

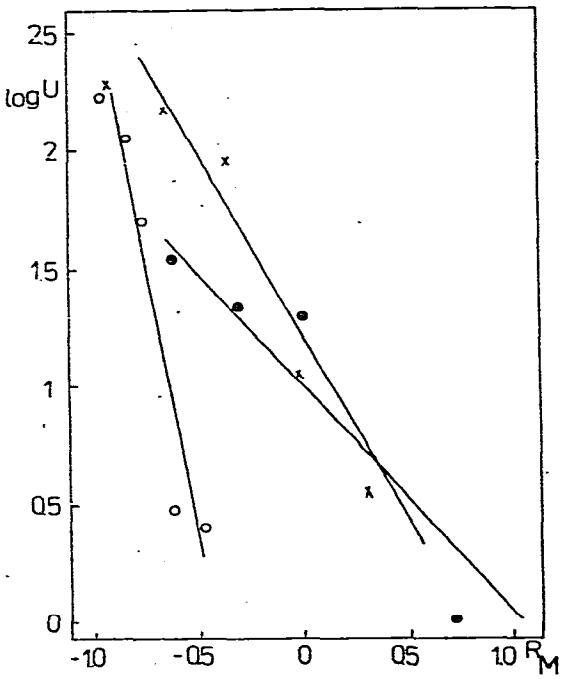


Fig. 7. Dependence of log U on R_M from partition chromatography for morpholinoethyl esters of carbanilic acids. Derivatives as in Fig. 1.

series of substances simultaneously and thus the required data are obtained under the same conditions.

ACKNOWLEDGEMENTS

The authors are very grateful to Dr. F. Kopecký, Department of Physical Chemistry, Pharmaceutical Faculty, Bratislava, for his assistance and for the computer program.

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