Journal of Chromatography, 254 (1983) 277–281
Elsevier Scientific Publishing Company, Amsterdam — Printed in The Netherlands

CHROM. 15,342

Note

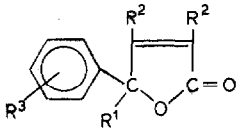
Relationship between R_M values and hydrophobicity

Chromatographic behaviour of crotonolactones

KVĚTOSLAVA DADÁKOVÁ*, JIŘÍ HARTL and KAREL WAISSER
Faculty of Pharmacy, Charles University, 501 65 Hradec Králové (Czechoslovakia)
(Received August 31st, 1982)

Recent studies on the relationships between physico-chemical properties and biological activities of drugs have shown the importance of the hydrophobic (or lipophilic) behaviour of drugs. The hydrophobicity of a drug is usually characterized by the partition coefficient obtained from studies of the distribution of the drug between immiscible polar and non-polar solvents, usually octanol and water. However, because of practical difficulties in the determination of the partition coefficient, chromatographic R_M values are often used as an expression of the hydrophobic chracter of molecules[1,2]. However, Gasparič[3] has warned that in partition thin-layer chromatography (TLC) also absorption on silica gel or cellulose can occur. The calculation of partition coefficients from hydrophobic fragmental constants appears to be another simple method for the estimation of the hydrophobicity of molecules[4,5]. Fragmental constants have a statistical character and calculated values of the partition coefficients are not so precise as the experimental ones.

This study aims to estimate the hydrophobicity of 4-aryl-2,3-dihalogeno-1,4-crotonolactones with antituberculotic activity. Experimental values obtained from partition TLC were compared with the calculated ones. In order to confirm that chromatography was based on partition principles, several concentrations of impregnating solutions were used to vary the thickness of the stationary phase.



EXPERIMENTAL

Chemicals

The compounds chromatographed were prepared using procedures described in the literature[6,7]. Their identities and purities were checked by the usual methods. All liquids used as stationary and mobile phases were of reagent-grade purity.

Thin-layer chromatography

All experiments were carried out on Lucefol (cellulose) sheets, 20 × 20 cm

0021-9673/83/0000–0000/$03.00

TABLE I

HYDROPHOBIC PARAMETERS OF 4-ARYL-2,3-DIHALOGENO-1,4-CROTONOLACTONES

Thin-layers were impregnated with solutions of various concentrations of formamide in ethanol.

Compounds				*20% Formamide*			*30% Formamide*			*50% Formamide*			*Δ log P**
No.	R^1	R^2	R^3	R_F	R_M	ΔR_M	R_F	R_M	ΔR_M	R_F	R_M	ΔR_M	
I	H	Cl	H	0.68	−0.33	0	0.51	−0.02	0	0.34	0.28	0	0
II	H	Cl	2′,4′,6′-$(CH_3)_3$	0.92	−1.04	−0.71	0.90	−0.96	−0.94	0.89	−0.91	−1.20	2.67
III	H	Cl	3′-NO_2	0.11	0.89	1.22	0.18	0.65	0.67	0	–	–	−0.03
IV	H	Br	H	0.57	−0.12	0.21	0.45	0.09	0.11	0.37	0.23	−0.06	0.28
V	H	Br	4′-F	0.46	0.07	0.40	0.34	0.28	0.31	0.23	0.53	0.24	0.65
VI	H	Br	4′-Cl	0.64	−0.25	0.08	0.49	0.02	0.04	0.43	0.12	0.17	1.22
VII	H	Br	3′,4′-Cl_2	0.77	−0.53	−0.53	0.68	−0.33	−0.31	0.61	−0.19	−0.48	2.26
VIII	H	Br	2′,5′-$(CH_3)_2$	0.85	−0.75	−0.42	0.76	−0.50	−0.48	0.74	−0.45	−0.74	2.06
IX	H	Br	2′,4′,6′-$(CH_3)_3$	0.89	−0.91	−0.58	0.86	−0.79	−0.78	0.84	−0.72	−1.01	2.95
X	4″-$CH_3C_6H_4$	Br	4′-CH_3	0.95	−1.26	−0.93	0.93	−1.12	−1.10	0.92	−1.06	−1.35	3.50

* Calculated by the method of ref. 5.

(Kavalier, Votice, Czechoslovakia). Impregnation of the layers was effected by the dipping technique. 20%, 30% and 50% solutions of formamide in ethanol were used for impregnation. The development was carried out by cyclohexane in glass tanks. The compounds were observed in UV light using a Camag Universallampe.

Calculations

R_M values were calculated from the obtained R_F values by the usual method[1]. ΔR_M values were determined with reference to 4-phenyl-2,3-dichloro-1,4-crotonolactone as the standard. Δ log P values were calculated according to Hansch and Leo[5] for the system octanol–water and they are also referred to 4-phenyl-2,3-dichloro-1,4-crotonolactone as the standard. This standardization of data was necessary as some structural effects on the value of the logarithm of the partition coefficient corresponding to the reference structure cannot be expressed by means of empirical rules. Regression equations were searched with the use of programme W-6 on a pocket computer SHARP PC 1211.

RESULTS AND DISCUSSION

The results are summarized in Table I. On increasing the thickness of the stationary phase, *i.e.*, by the use of more concentrated impregnating solutions, a decrease in the R_F values and thus an increase in the R_M values occurred. It can therefore be assumed that absorption was negligible and only partition effects occurred, the only exception being 4-(3′-nitrophenyl)-2,3-dichlorocrotonolactone (III). The correlation between ΔR_M and Δ log P values is expressed in eqns. 1–3.

At 20% formamide (Fig. 1):

$$\Delta \log P = -1.778\ \Delta R_M + 1.332 \quad (1)$$

$$r = 0.877,\ s = 0.66,\ F = 26.74,\ n = 10$$

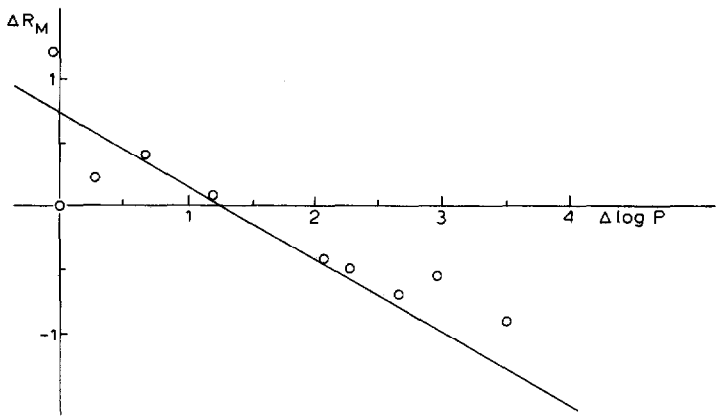


Fig. 1. Relationship between ΔR_M values (measured after impregnation of cellulose with 20% solutions of formamide in ethanol) and calculated Δ log P values.

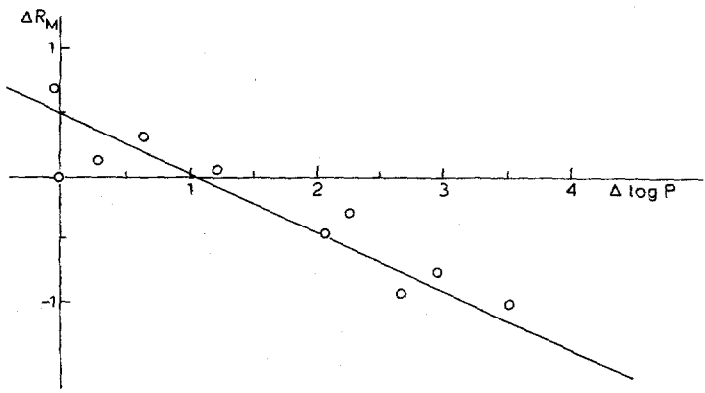


Fig. 2. Relationship between ΔR_M values (measured after impregnation of cellulose with 30% solutions of formamide in ethanol) and calculated Δ log P values.

At 30% formamide (Fig. 2):

$$\Delta \log P = -2.096\, \Delta R_M + 1.036 \qquad (2)$$
$$r = 0.927,\ s = 0.52,\ F = 48.57,\ n = 10$$

At 50% formamide:

$$\Delta \log P = -1.682\, \Delta R_M + 1.017 \qquad (3)$$
$$r = 0.919,\ s = 0.48,\ F = 32.57,\ n = 9$$

R_M values obtained at different thicknesses of the stationary phase were correlated with each other. Their correlation matrix according to eqn. 4

$$(R_M)_y = a\,(R_M)_x + b \qquad (4)$$

is presented in Table II.

The correlation of hydrophobic parameters with antituberculotic activity evaluated *in vitro* on Sauton's medium does not seem convincing. For example, an equation[8] is presented for MIC (minimum inhibitory concentration) as a function of the R_M data obtained at 20% formamide:

$$\log \text{MIC} = 0.745\, R_M + 0.434\, R_M^2 + 1.700 \qquad (5)$$
$$r = 0.737,\ s = 0.33,\ F = 4.16,\ n = 10$$

The present study succeeded in finding a suitable chromatographic system which could measure the hydrophobic properties of 4-aryl-2,3-dihalogenocrotonolactones. This is all the more interesting since it shows the similarity of the partition behaviour of this group of compounds in the octanol–water and cyclohexane–formamide systems.

However, the antituberculotic activity of these agents is connected with a greater number of molecular parameters and cannot be described by equations ex-

TABLE II

MATRICES OF REGRESSION COEFFICIENTS AND STATISTICAL CRITERIA FOR MUTUAL CORRELATION OF R_M VALUES OF 4-ARYL-2,3-DIHALOGENO-1,4-CROTONOLACTONES AT VARIOUS THICKNESSES OF THE STATIONARY PHASE

$(R_M)_y$	$(R_M)_x$		
	20%	*30%*	*50%*
20%		$a = 1.056$ $b = -0.144$ $r = 0.968$ $s = 0.17$ $F = 119.32$ $n = 10$	$a = 0.776$ $b = -0.381$ $r = 0.986$ $s = 0.08$ $F = 249.629$ $n = 9$*
30%	$a = 1.169$ $b = -0.019$ $r = 0.957$ $s = 0.20$ $F = 86.89$ $n = 10$		$a = 0.868$ $b = -0.159$ $r = 0.941$ $s = 0.06$ $F = 583.81$ $n = 9$*
50%	$a = 1.253$ $b = 0.472$ $r = 0.986$ $s = 0.10$ $F = 249.63$ $n = 9$*	$a = 1.132$ $b = 0.177$ $r = 0.994$ $s = 0.07$ $F = 591.83$ $n = 9$*	

* Compound III has been left out.

pressed in terms only of the hydrophobic properties of the molecules. Thus, the present paper forms an introduction to the correlation of antituberculotic properties with further parameters.

CONCLUSIONS

Experimental R_M values for a series of 4-aryl-2,3-dihalogeno-1,4-crotonolactones were determined using partition thin-layer chromatography to assess the hydrophobicity of these potential drugs. Statistically significant linear relationships were found between ΔR_M values and calculated Δ log P values. However, the hydrophobicities of these compounds do not give satisfactory correlation with the antibacterial activity against *Mycobacterium tuberculosis*.

REFERENCES

1 E. Tomlinson, *J. Chromatogr.*, 113 (1975) 1.
2 R. Kaliszan, *J. Chromatogr.*, 220 (1981) 71.
3 J. Gasparič, *J. Chromatogr.*, 196 (1980) 391.
4 R. F. Rekker, *The Hydrophobic Fragmental Constant*, Elsevier, Amsterdam, 1977.
5 C. Hansch and A. J. Leo, *Substituent Constants for Correlation Analysis in Chemistry and Biology*, Wiley, New York, 1979.
6 V. Ettel, M. Semonský and V. Zikán, *Chem. Listy*, 46 (1952) 232.
7 M. Semonský, J. Hartl, J. Křepelka, M. Beran, B. Kakáč, H. Veselá and V. Rejholec, *Collect. Czech. Chem. Commun.*, 40 (1975) 2869.
8 J. Hartl, K. Dadáková, Ž. Odlerová and K. Waisser, *Česk. Farm.*, in press.