Journal of Chromatography, 216 (1981) 69–78
Elsevier Scientific Publishing Company, Amsterdam — Printed in The Netherlands

CHROM. 14,017

R_M VALUES OF STEROIDS AS AN EXPRESSION OF THEIR HYDROPHOBIC CHARACTER

III. INTERPRETATION OF THE SLOPES OF THE R_M *VERSUS* ACETONE CONCENTRATION CURVES

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(First received January 21st, 1981; revised manuscript received May 20th, 1981)

SUMMARY

The use of R_M values in reversed-phase thin-layer chromatography as a parameter for the hydrophobicity of a compound is limited by the fact that the R_M values depend on the composition of the mobile phase. A possible explanation for this dependence is given and a hydrophobic parameter is suggested that is independent of the concentration of the organic component in the mobile phase.

INTRODUCTION

The hydrophobicity of drugs is important with regard to their biological activity, and a knowledge of the hydrophobicity is essential for establishing quantitative structure–activity relationships. The R_M values in reversed-phase thin-layer chromatography (RPTLC) are suitable for the description of the hydrophobicity of compounds because they can be determined very easily[1,2].

In RPTLC the stationary phase is non-polar [for instance, silica gel, impregnated with silicone oil or silanized silica gel PF_{254} (Merck, Darmstadt, G.F.R.)] and the mobile phase is polar (*e.g.*, water–acetone mixtures). The R_M values depend on the concentration of the organic component (acetone) in the mobile phase[1]. On plotting the R_M values *versus* the concentration of the organic component in the mobile phase, straight lines with different slopes for different compounds (i) result:

$$R_{m,i} = a_i - b_i z_{An} \quad (1)$$

with

$$z_{An} = V_{An}/(V_{An} + V_{H_2O}) \quad (2)$$

where V_{An} is the volume of acetone in the mobile phase and V_{H_2O} the volume of water.

0021-9673/81/0000–0000/$02.50

Taking R_M values estimated at different compositions of the mobile phase, different correlations with biological activities are possible. For this reason, a general problem arises when using R_M values as a hydrophobic parameter. Therefore, we looked for a hydrophobic parameter on the basis of R_M values but independent of the composition of the mobile phase.

In an earlier paper we discussed the influence of neighbouring groups on the hydrophobicity of substituents[3]. The change in hydrophobicity of such a substituent by a neighbouring group was interpreted by the disturbance of the hydrate envelope around the substituent. The results obtained in studying this intramolecular interaction have now been used to explain the different slopes of the straight lines between R_M values and the concentration of the organic component in the mobile phase. A second model for the interpretation is based on thermodynamic principles. Both models yield equivalent results.

RESULTS AND DISCUSSION

As a measure of hydrophobicity that is independent of the portion of the organic component, we propose a parameter that is obtained from the area below the straight line according to eqn. 1 (Fig. 1).

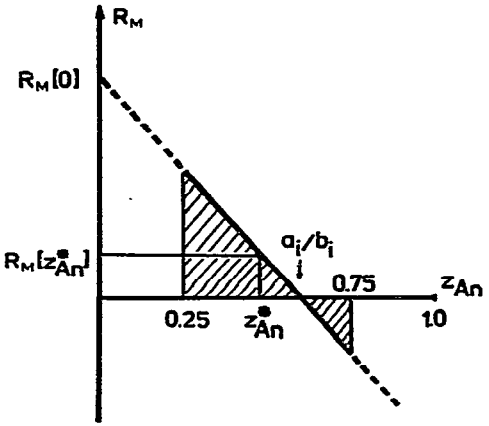

Fig. 1. Plot of R_M values *versus* concentration of organic component (acetone) in the mobile phase.

The difference between the areas above and below the z_{An} axis within the limits* from $z_{An} = 0.25$ to 0.75 is expressed by the integral

$$F_D = \int_{0.25}^{a_i/b_i} (a_i - b_i z_{An})\, dz_{An} + \int_{a_i/b_i}^{0.75} (a_i - b_i z_{An})\, dz_{An} \quad (3)$$

Solving this integral yields

$$F_D = (2a_i - b_i)/4 \quad (4)$$

* Integration within these limits to exclude deviations from linearity.

This expression should be considered as a measure of the hydrophobicity. Of course, another expression is conceivable: only the area over the z_{An} axis or the sum of the areas, for instance. Our intention is to obtain a parameter of hydrophobicity that is independent of partition of the mobile phase and possibly more reliable than a value that refers to any concentration, because the complete functional expression (eqn. 1) serves for the specification of the hydrophobicity.

A hydrophobic increment of substituents is defined analogous to the π values or the ΔR_M values:

$$\Delta F_D = F_D(X) - F_D(H). \tag{5}$$

In Table I, data for steroids with a hydroxyl group in the 17β-position and a 16α-hetero substituent and steroids with only a 16α-substituent are given.

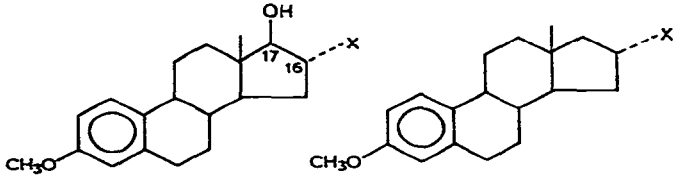

Table I includes the coefficients in eqn. 1, the correlation coefficient r (number of data points $n = 4$), the F_D and ΔF_D values, the π values of Hansch *et al.*[4] and the V_L ($= V_W - V_H$) values of Moriguchi *et al.*[5,6].

The ΔF_D values yield significant correlations with other hydrophobic parameters, *e.g.*, the π values of Hansch *et al.* and the V_L values of Moriguchi *et al.*:

17β-H:

$$\Delta F_D = -0.142\ (\pm\ 0.048) + 0.185\ (\pm\ 0.046)\ \pi_{ar} \tag{6}$$

$n = 10$; $r = 0.942$; $t = 7.51$; $\alpha < 0.001$;

17β-OH:

$$\Delta F_D = -0.024\ (\pm\ 0.021) + 0.116\ (\pm\ 0.020)\ \pi_{ar} \tag{7}$$

$n = 10$; $r = 0.963$; $t = 10.15$; $\alpha < 0.001$;

17β-H:

$$\Delta F_D = -0.119\ (\pm\ 0.019) + 0.451\ (\pm\ 0.045)\ V_L \tag{8}$$

$n = 10$; $r = 0.971$; $t = 9.99$; $\alpha < 0.001$;

17β-OH:

$$\Delta F_D = -0.013\ (\pm\ 0.011) + 0.276\ (\pm\ 0.026)\ V_L \tag{9}$$

$n = 10$; $r = 0.992$; $t = 18.86$; $\alpha < 0.001$.

These equations show that the F_D values are suitable as a hydrophobic parameter in reversed-phase thin-layer chromatography. The equations also show that the correlation is not improved over the correlation of $R_M(0)$ or $R_M(0.45)$ values[3]. The reason may be the correlation between b_i and a_i (see below) in the case of the steroids investigated; perhaps such a correlation does not exist for other substances class.

The change in hydrophobicity of the 16α-hetero substituent by intramolecular interaction with the 17β-hydroxyl group is attributed to a decrease in the number of

TABLE I

HYDROPHOBIC DATA FOR STEROIDS

X	17β-H					17β-OH					π_{ar}	V_L
	a_i	b_i	r	F_D	ΔF_D	a_i	b_i	r	F_D	ΔF_D		
H	3.81	4.97	0.99	0.66	0	2.31	3.28	0.99	0.34	0	0	0.056
OH	2.19	3.11	0.99	0.32	−0.34	1.54	2.26	0.90	0.20	−0.14	−0.67	−0.403
Br	3.78	4.84	0.97	0.68	0.02	2.38	3.11	0.98	0.42	0.08	0.86	0.244
N_3	3.62	4.63	0.96	0.65	−0.01	2.40	3.17	0.98	0.41	0.07	0.46	0.281
SCN	3.24	4.36	0.99	0.53	−0.13	2.39	3.43	0.98	0.34	0	0.41	0.120***
SeCN	3.56	4.93	0.99	0.55	−0.11	2.48	3.52	0.99	0.36	0.02	0.39*	0.138***
SH	3.56	4.75	0.99	0.59	−0.07	2.28	3.13	0.98	0.36	0.02	0.39	0.160
$NHCOCH_3$	2.13	3.02	0.99	0.31	−0.35	1.64	2.51	0.99	0.19	−0.15	−0.97	−0.556
NCS	4.39	5.58	0.99	0.80	0.14	3.15	4.31	0.99	0.50	0.16	1.15	0.438***
$SCH_2C_6H_5$	5.08	6.47	0.99	0.92	0.26	3.68	5.07	0.99	0.57	0.23	2.57**	0.887***

* $\pi(SeCN) = \pi(SeCH_3) - \pi(CH_3) + \pi(CN)$.
** $\pi(SCH_2C_6H_5) = \pi(SCH_3) + \pi(C_6H_5)$.
*** V_H value from ref. 5.

water contacts in the hydrate envelope around the substituent and the 17β-hydroxyl group[3]. This decrease in the number of water contacts has been estimated by a simple model, assuming the substituents and their hydrate envelopes to be spheres. The hatched "overlapping volume" (Fig. 2) has been calculated as a function of the substituent in the 16α-position.

For the calculation of the effective radius (r^e), a simplified model was used. Assuming that the hydrate envelope is a spherical shell with volume

$$V = \tfrac{4}{3}\pi\,[(r^e)^3 - (r^w)^3] \tag{10}$$

this volume should be proportional to the polar effect:

$$V = (1 + 10^3\ V_H)/V_W \tag{11}$$

A proportionality factor was neglected because only relative volume changes were included in the following calculations. In these equations, r^e is the effective radius, r^w the Van der Waals radius, V_H the hydrophilic increment of Moriguchi *et al.*[5,6] and V_W

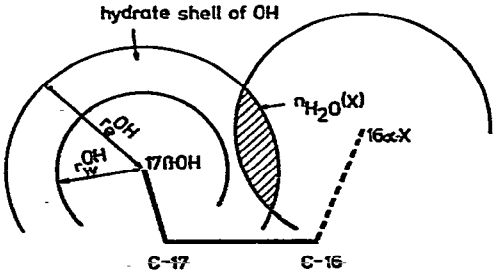

Fig. 2. Calculation model of hydrate envelopes.

the Van der Waals volume[5]. Division of eqn. 11 by the Van der Waals volume results in standardization to unit volume.

With

$$r^w = \sqrt[3]{3V_w/4\pi} \tag{12}$$

the following equation is obtained as an expression of the "effective radius":

$$r^e = \sqrt[3]{(3V_r/2\pi) + (r^w)^3}$$
$$= 0.620 \sqrt[3]{(1 + 10^3 V_H + V_w^2)/V_w} \tag{13}$$

The calculation of spherical areas with these "effective radii" gives values $F [= 4\pi (r^e)^2]$ correlating well with values of the "molecular surface area" (MSA) according to Hermann[7]:

$$\text{MSA} = -55.902 (\pm 15.662) + 3.499 (\pm 0.113) F \tag{14}$$
$$n = 16; r = 0.998; t = 55.36; \alpha < 0.001$$

(MSA of alcohols and hydrocarbons from ref. 8. Hence the "overlapping volume" follows:

$$V_g(X) = \tfrac{1}{3}\pi [h_{OH}^2(3r_{OH}^e - h_{OH}) + h_X^2(3r_X^e - h_X)] \tag{15}$$

where

$$h_{OH} = [(r_X^e)^2 - (r_{OH}^e - A_X)^2]/2A_X \tag{16}$$
$$h_X = [(r_{OH}^e)^2 - (r_X^e - A_X)^2]/2A_X \tag{17}$$

and

$$A_X = 1.204 \sqrt{3.970 + 0.119 r_X^k + 0.690 (r_X^k)^2} \tag{18}$$

(r^k is the covalent radius).

TABLE II

$n_{H_2O}(X)$ AND $h(X)$

X	$n_{H_2O}(X)$	$h(X)$
H	0	0
OH	0.39	0.26
Br	0.12	0.07
N_3	0.24	0.17
SCN	0.31	0.24
$NHCOCH_3$	0.46	0.32
SH	0.18	0.13
NCS	0.28	0.29
SCH_2Ph	0.59	0.73

The following equation yields the number of moles of water forced out of the hydrate envelope as to X = H:

$$n_{H_2O}(X) = \frac{[V_g(X) - V_g(H)] \cdot 6.023 \cdot 10^{23} \text{Å}^3 \text{ mole}}{18.05 \cdot 10^{24} \text{ Å}^3 \text{ mole}} \tag{19}$$

$$n_{H_2O}(X) = 0.033\,[V_g(X) - V_g(H)] \tag{20}$$

($18.05 \cdot 10^{24}$ is the molar volume of water in Å^3). These values are given in Table II.

The relative change in the hydrophobicity of X by the intramolecular interaction with the hydroxyl group was calculated by equation 21:

$$h(X) = 1 - \frac{R_M \left[\text{OH}, \text{X}\right] - R_M \left[\right]}{R_M \left[\text{OH}\right] + R_M \left[\text{X}\right] - 2R_M \left[\right]} \tag{21}$$

These values are also given in Table II.

A good correlation was found between the number of moles of water forced out of the hydrate envelope and the relative hydrophobic change (eqn. 22):

$$h(X) = -0.062\ (\pm\ 0.101) + 1.077\ (\pm\ 0.304)\ n_{H_2O}(X) \tag{22}$$
$$n = 10;\ r = 0.920;\ t = 6.61;\ \alpha < 0.001$$

With the estimated number of moles of water forming a hydrate envelope around the 17β-hydroxyl and 16α-X substituents without mutual influence [$n^{tot}_{H_2O}(X)$] and with the estimated number of moles of water not taking part in the formation of the hydrate envelope [$n_{H_2O}(X)$] as a result of the described intramolecular interaction, one can calculate the molar fraction of the latter:

$$x_{H_2O}(X) = \frac{n_{H_2O}(X)}{n^{tot}_{H_2O}(X)} \tag{23}$$

This molar fraction of water corresponds to a defined molar fraction of acetone in the mobile phase of the chromatographic system:

$$x_{An}(X) = 1 - x_{H_2O}(X) \tag{24}$$

This represents the correlation between the loss of water by intramolecular interaction and the same loss of water caused by an increase in the acetone concentration in

the mobile phase. As the acetone concentration was calculated as a volume fraction (z_{An}), a transformation was carried out:

$$z^*_{An}(X) = \frac{x_{An}(X)}{x_{An}(X) + \frac{M_{H_2O} \cdot \varrho_{An}}{M_{An} \cdot \varrho_{H_2O}} \cdot [1 - x_{An}(X)]} \tag{25}$$

where M_{H_2O} and M_{An} are the molecular weights of water and acetone, respectively, and ϱ_{H_2O} and ϱ_{An} are their densities.

The slopes of the straight lines according to eqn. 1 were calculated by eqn. 26 (see also Fig. 1):

$$\tan \alpha(X) = \frac{R_M(X)\,[z^*_{An}] - R_M(X)\,[0]}{z^*_{An}(X)} \tag{26}$$

The relative change in the hydrophobicity caused by varying the acetone concentration in the mobile phase from $z_{An} = 0$ to $z_{An} = z^*_{An}$ was calculated by means of the equation

$$q^*_{An}(X) = \frac{R_M(X)\,[z_{An}]}{R_M(X)\,[0]} \tag{27}$$

Solving eqn. 27 for $R_M(X)\,[z^*_{An}]$ and putting this expression into eqn. 26 gives eqn. 28 as an expression for the slopes of the straight lines:

$$\tan \alpha(X) = \frac{R_M(X)\,[0]\,[q^*(X) - 1]}{z^*_{An}(X)} \tag{28}$$

Assuming that the decrease in the amount of water in the mobile phase results in a relative change in hydrophobicity [$h^*(X)$] corresponding to the change in hydrophobicity caused by a decreasing in the amount of water [$h(X)$] as a result of the intramolecular interaction, one can develop the equation

$$\begin{aligned} h^*(X) &\approx h(X) \\ h^*(X) &= ch(X) \end{aligned} \tag{29}$$

where $h^*(X) = 1 - q^*(X)$ and c = constant, and for tan $\alpha(X)$:

$$\tan \alpha(X) = -cR_M(X)\,[0]\,h(X)/z^*_{An}(X) \tag{30}$$

A relationship exists between $h(X)$ and $z^*_{An}(X)$:

$$\begin{aligned} h(X) &= -0.061\ (\pm\ 0.089) + 3.400\ (\pm\ 0.847)\ z^*_{An}(X) \\ n &= 10;\ r = 0.935;\ t = 7.74;\ \alpha < 0.001 \end{aligned} \tag{31}$$

Hence eqn. 32 follows as a good approximation:

$$h(X) = 3.4\ z^*_{An}(X) \tag{32}$$

Eqn. 32 was substituted into eqn. 30 and the result was an expression of the slopes of the straight lines obtained by plotting the R_M values *versus* the concentration of the organic component in the mobile phase:

$$\tan \alpha(X) = -c' R_M(X)\,[0] \tag{33}$$

where $c' = 3.4\,c$.

A differentiation between the following three cases is possible:

(a) $R_M[0] > 0$: the straight line falls more steeply the more hydrophobic is the molecule;

(b) $R_M[0] < 0$: the straight line rises more steeply the more hydrophilic is the molecule;

(c) $R_M[0] = 0$: $\tan \alpha(X) = 0$.

The first case has been proved by us and the other two are hypothetical.

The experimentally ascertained slopes of the R_M *versus* acetone concentration curves of steroids[6] can be correlated with $R_M[0]_i$ values (eqns. 34 and 35):

$$b_i = 0.083\ (\pm\ 0.538) - 1.262\ (\pm\ 0.206)\ R_M[0]_i \tag{34}$$
$$n = 99;\ r = 0.973;\ t = 41.52;\ \alpha < 0.001$$

As the constant term in eqn. 34 is not significantly different from zero, it follows that

$$b_i = \tan \alpha(i) = -1.262\ R_M[0]_i \tag{35}$$

The difference in the slopes is reduced to the decrease in the number of water contacts in the mixed solvate envelope around the molecule caused by the decrease in the proportion of water in the mobile phase. The decrease in the number of water contacts is dependent on the hydrophobicity of the dissolved compound.

A simple thermodynamic approach yields an analogous result. In this model the standard enthalpy change of the chromatographic process is composed of the standard enthalpy change of the process with pure water as mobile phase and the standard enthalpy change of solvation of the molecules by water and acetone. The standard enthalpy change of the whole chromatographic process is given by

$$R_{M,i} = \Delta G^{\circ}_{i,1}/RT = a_i + b_i z_{An} \tag{36}$$

For the chromatographic process with pure water as mobile phase ($z_{An} = 0$) one obtains

$$a_i = -\Delta G^{\circ}_{i,2}/RT \tag{37}$$

If the mobile phase consists of water and an organic component (acetone), which is miscible with water, a solvation equilibrium is established:

$$P_i = c_i^{An}/c_i^{H_2O} \tag{38}$$

c_i^{An} is the concentration of the molecules of compound i which are solvated by the

organic component and $c_i^{H_2O}$ is the concentration of the molecules solvated by water. It should be mentioned that this is only a simplified model, as in practice a mixed solvate envelope exists.

As the concentration of the mobile phase is not constant, we must write

$$c_i^{An} = q_i/V_{An} \tag{39}$$

and

$$c_i^{H_2O} = p_i/V_{H_2O} \tag{40}$$

With $q_i + p_i = r_i$ and $q_i/r_i + p_i/r_i = 1$, one obtains

$$\begin{aligned} P_i &= (1/z_{An} - 1)\, q_i/p_i \\ &= p_i\,(1 - z_{An})/z_{An}(1 - q_i) \end{aligned} \tag{41}$$

and

$$\ln P_i = -\Delta G_{i,3}^e/RT \tag{42}$$

The proportion of molecules (p_i) solvated by water is the smaller the higher is the proportion of the organic component in the mobile phase and the higher is the hydrophobicity of the molecule (i).

One can assume that $\Delta G_{i,2}^e$ contributes the more to the standard enthalpy change of the chromatographic process the higher is the proportion of water solvated molecules. On the other hand, with an increase in the concentration of the organic component, the influence of $\Delta G_{i,3}^e$ increases. $\Delta G_{i,3}^e$ has the opposite sign to $\Delta G_{i,2}^e$, because the partition is defined as a partition between a non-polar stationary phase and water and not, for instance, between this phase and acetone. Hence we obtain

$$-\Delta G_{i,1}^e = -(1 - z_{An})\,\Delta G_{i,2}^e + z_{An}\Delta G_{i,3}^e \tag{43}$$

Eqns. 36, 37 and 42 were substituted in eqn. 43 and, after division by RT, we obtained

$$a_i + b_i z_{An} = (1 - z_{An})\, a_i - z_{An} \ln P_i \tag{44}$$

and for $b_i = \tan \alpha_i$:

$$b_i = -a_i - \ln P_i = R_M[0]_i - \ln P_i \tag{45}$$

Because in P_i depends on the hydrophobicity, one can write

$$\ln P_i = kR_M[0]_i \tag{46}$$

and

$$b_i = = k' R_M[0]_i \tag{47}$$

with $k' = k - 1$. As $k' > 0$ it follows that $\ln P_i > 0$ and $P_i > 1$. Hence from eqn. 41 it follows that $q_i > z_{An}$ and $p_i < 1 - z_{An}$. If $z_{An} < 0.5$ then $p_i > q_i$ and if $z_{An} > 0.5$ then $p_i < q_i$.

In other words, from this model it follows that up to a concentration of the organic component in the mobile phase of 50% by volume the proportion of the molecules that are solvated by water may be greater than the proportion of acetone-solvated molecules. If the proportion of the organic component is greater than 50% (v/v) the proportion of the molecules solvated by water must be smaller than the proportion of acetone-solvated molecules.

REFERENCES

1 G. L. Biagi, A. M. Barbaro, O. Gandolfi, M. C. Guerra and G. Cantelli-Forti, *J. Med. Chem.*, 18 (1975) 873.
2 E. Tomlinson, *J. Chromatogr.*, 113 (1975) 1.
3 J. Draffehn, B. Schönecker and K. Ponsold, *J. Chromatogr.*, 216 (1981)
4 C. Hansch, A. Leo, S. H. Unger, K. H. Kim, D. Nikaitani and E. J. Lien, *J. Med. Chem.*, 16 (1973) 1207.
5 I. Moriguchi, Y. Kanada and K. Kamatsu, *Chem. Pharm. Bull.*, 24 (1976) 1799.
6 J. Draffehn, B. Schönecker and K. Ponsold, *J. Chromatogr.*, 205 (1981) 113.
7 R. B. Hermann, *J. Phys. Chem.*, 76 (1972) 2754.
8 G. L. Amidon, S. H. Yalkowski and S. Leung, *J. Pharm. Sci.*, 63 (1974) 3225.