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R_M VALUES OF STEROIDS AS AN EXPRESSION OF THEIR HYDROPHOBIC CHARACTER

II. EFFECT OF NEIGHBOURING GROUPS ON THE HYDROPHOBICITY OF SUBSTITUENTS

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SUMMARY

R_M values in reversed-phase thin-layer chromatography are suitable for the description of the hydrophobicity of compounds. In principle it is possible to calculate the R_M values of non-synthesized compounds with ΔR_M increments. However, the calculation of hydrophobicity by using an incremental system is often not exact, because the hydrophobicity of substituents depends on neighbouring groups and on the position of substitution. The influence of neighbouring groups on the hydrophobicity of substituents of steroids has been investigated and a feasible explanation is given for the dependence of hydrophobicity on neighbouring groups.

INTRODUCTION

In connection with quantitative structure–activity analyses, the R_M values of about 100 steroids were measured by means of reversed-phase thin-layer chromatography[1]. The non-polar stationary phase was silanized silica gel PF_{254} (Merck, Darmstadt, G.F.R.). Water–acetone mixtures were used as the polar mobile phase.

The exact measurement procedure was described in an earlier paper[1]. The R_M values were calculated by means of the equation

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

Analogous to the π values of Hansch *et al.*[2], ΔR_M values of a substituent X are defined as follows:

$$\Delta R_M(\mathrm{X}) = R_M(\mathrm{X}) - R_M(\mathrm{H}) \tag{2}$$

where $R_M(\mathrm{X})$ is the R_M value of the substituted compound, $R_M(\mathrm{H})$ the value of the

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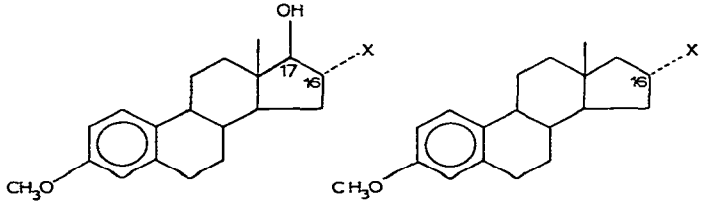
unsubstituted compound and $\Delta R_M(X)$ the hydrophobic increment of the substituent X. In principle it is possible to calculate the R_M value of a non-synthesized compound as

$$R_M(HX) = R_M(H) + \sum_i \Delta R_M(X_i). \quad (3)$$

Eqn. 3 is valid only if interactions of the substituents do not play a significant role.

RESULTS AND DISCUSSION

Among other steroids, compounds with a hetero substituent in the 16α-position and 17β-hydroxyl group (I) and steroids with a 16α-substituent only (II) have been investigated.



I II

The R_M values are dependent on the concentration of the organic component (acetone) in the mobile phase[3]. In an earlier paper it has already been shown that the R_M values relative to 45% acetone are suitable for the description of hydrophobicity[1]. These R_M values are summarized in Table I.

TABLE I

HYDROPHOBIC CONSTANTS

X	17β-H		17β-OH		π_{ar}	V_L
	R_M (0.45)	ΔR_M (0.45)	R_M (0.45)	ΔR_M (0.45)		
H	1.58	0	0.86	0	0	0.056
OH	0.80	−0.78	0.52	−0.34	−0.67	−0.403
Br	1.60	0.02	0.98	0.12	0.86	0.244
N_3	1.54	−0.04	0.97	0.11	0.46	0.281
SCN	1.27	−0.31	0.84	−0.02	0.41	0.120***
SeCN	1.34	−0.24	0.895	0.04	0.39*	—
SH	1.42	−0.16	0.88	0.02	0.39	0.160
$NHCOCH_3$	0.77	−0.81	0.50	−0.36	−0.97	−0.556
NCS	1.88	0.30	1.22	0.36	1.15	—
$SCH_2C_6H_5$	2.17	0.59	1.40	0.54	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. 1.

Table I shows that the ΔR_M values of the substituents X of the disubstituted series (17β-OH) are different from the ΔR_M values of the monosubstituted series (17β-H). This means that for the disubstituted compounds an intramolecular interaction takes place between the 17β-hydroxyl group and the substituent in the 16α-position. This results in a change in hydrophobicity of the substituent X and also a change in hydrophobicity of the 17β-hydroxyl group. This fact restricts the rule of additivity of hydrophobic increments. In the following only the change in hydrophobicity of the 16α-substituent will be considered.

The difference W (eqn. 4) between the ΔR_M values of X of the disubstituted compounds and the ΔR_M values of X of the monosubstituted steroids is used as a measure of the hydrophobic change for the 16α-substituent caused by intramolecular interaction with the hydroxyl group:

$$W = \Delta R_M\ (17\beta\text{-OH, } 16\alpha\text{-X}) - \Delta R_M\ (17\beta\text{-H, } 16\alpha\text{-X}) \tag{4}$$

In this connection $W > 0$ means that as a consequence of this interaction the hydrophobicity of the substituent is increased. On the other hand, $W < 0$ means that the hydrophobicity is decreased. The W values are given in Table II. As can be seen from Tables I and II, there is a relationship between this hydrophobic change and the hydrophobicity of the substituents themselves.

TABLE II

W VALUES OF SUBSTITUENTS (*cf.*, EQN. 4)

No.	*X*	*W*	*No.*	*X*	*W*
1	H	0	6	SeCN	0.28
2	OH	0.44	7	SH	0.18
3	Br	0.10	8	$NHCOCH_3$	0.45
4	N_3	0.15	9	NCS	0.06
5	SCN	0.29	10	$SCH_2C_6H_5$	−0.05

The W values have been correlated with the π values of Hansch *et al.*[2], the V_L ($= V_W - V_H$) values of Moriguchi *et al.*[4] (V_W is the Van der Waals volume and V_H a polar increment) and with the ΔR_M (0.45) values relative to the monosubstituted compounds:

$$W = 0.290\ (\pm 0.042) - 0.156\ (\pm 0.038)\ \pi_{ar} \tag{5}$$
$$n = 9;\ r = 0.943;\ t = 7.50;\ \alpha < 0.001$$

$$W = 0.262\ (\pm 0.039) - 0.371\ (\pm 0.087)\ V_L \tag{6}$$
$$n = 7;\ r = 0.969;\ t = 8.71;\ \alpha < 0.001$$

$$W = 0.153\ (\pm 0.019) - 0.367\ (\pm 0.041)\ \Delta R_M\ (0.45)_{17\beta\text{-H}} \tag{7}$$
$$n = 9;\ r = 0.987;\ t = 16.20;\ \alpha < 0.001$$

In these equations the W value of the unsubstituted compound (X = H) has

been neglected, because the hydrophobic increment for hydrogen is zero according to the definition of ΔR_M, and, therefore, the W value is also zero. However, this definition is modified by the fact that the hydrophobicity of hydrogen is influenced by the intramolecular interaction with neighbouring groups.

It is obvious from eqns. 5–7 that for hydrophilic substituents an increase in hydrophobicity takes place. On the other hand, if the hydrophobicity of the substituent is greater than a defined limit (here at about $\pi = 1.86$; $V_L = 0.71$ and $\Delta R_M = 0.42$), then the result of intramolecular interaction is a decrease in hydrophobicity. This may be explained as follows: a hydrate envelope around a substituent or, in general, around a molecule, is the greater and more regular the higher is the hydrophilicity of the substituent or of the molecule. In this case a "disturbance" by a neighbouring group becomes stronger because the formation of water contacts is hindered more drastically than in a hydrate envelope, which is not so regular.

For strong hydrophobic substituents one can assume that the energy of the transfer from water into the organic phase results from the disconnection of water contacts. If fewer of these water contacts are formed ("disturbance" by a neighbouring group), the energy of the phase transfer is decreased, which corresponds to a decrease in hydrophobicity. A decrease in the number of water contacts means an increase in hydrophobicity for polar groups and a decrease in hydrophobicity for non-polar groups.

Bush[5] reported the influence of steric interactions on hydrophobicity in adsorption chromatography and found qualitative analogous results.

The ratio R_M (16α-H; 17β-OH)/R_M (16α-H; 17β-H) = 0.54 describes the relative change in hydrophobicity caused by removal of the 17β-hydroxyl group. It is interesting that the ratio R_M (16α-X; 17β-OH)/R_M (16α-X; 17β-H) = 0.64 ± 0.02 is nearly constant (X ≠ H). Hence it follows that R_M (16α-X_1; 17β-OH) · R_M (16α-X_2; 17β-H) = R_M (16α-X_2; 17β-OH) · R_M (16α-X_1; 17β-H).

If the hydrophobicity of a substituent is influenced by neighbouring groups, then a dependence on the position of substitution should also exist, as intramolecular interactions change from position to position[3]. Methyl groups for which ΔR_M(0.45) were calculated are circled in Fig. 1.

There are considerable differences in the hydrophobicity of the methyl group depending on the position of substitution. The methyl group is the more hydrophobic the more water contacts can be formed. Hydrophobicity decreases, however, if the solvation is hindered.

A value ε was calculated by the following equation in order to obtain an approximate measure of the "shielding effect" of the neighbouring atoms:

$$\varepsilon = \sum_{i=1}^{n} V_{w,i}/r_i + 0.1 \cdot n \tag{8}$$

where n is the number of atoms (H and O) in close proximity to the methyl group, whose minimal distance is not greater than 2.5 Å, and r_i is the minimal distance ($r_i \leqslant 2.5$ Å). The values are taken from Dreiding models. V_w represents the Van der Waals volume of the corresponding neighbouring atom. The ΔR_M(0.45) and ε values are shown in Table III.

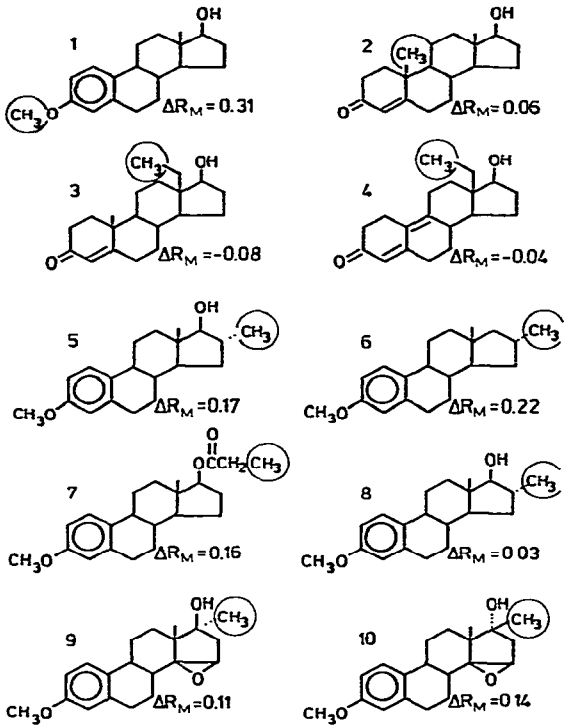


Fig. 1. ΔR_M values of the methyl group at different positions.

According to the definition of ε (eqn. 8) the "shielding effect" on the hydration of the methyl group (in other words, the steric hindrance of hydration) is proportional to ε. Therefore, the relationship $\Delta R_M = f(-\varepsilon)$ is confirmed by the following expression:

$$R_M = 0.330\ (\pm 0.091) - 0.320\ (\pm 0.132)\ \varepsilon \qquad (9)$$
$$n = 9;\ r = 0.865;\ t = 4.57;\ \alpha < 0.01$$

(without compound 3). An exponential dependence, however, appears to be more logical, because a totally shielded methyl group makes no contribution to hydrophobicity.

TABLE III

ΔR_M (0.45) AND ε VALUES

No.	ΔR_M	ε	*No.*	ΔR_M	ε
1	0.31	0.28	6	0.22	0.46
2	0.06	0.77	7	0.16	0.32
3	−0.08	1.54	8	0.03	0.70
4	−0.04	1.23	9	0.11	0.58
5	0.17	0.66	10	0.14	0.66

With $\Delta R'_M = \Delta R_M + 0.1$ the following equations are valid for all compounds:

$$\log \Delta R'_M = -0.097\ (\pm 0.149) - 0.967\ (\pm 0.184)\ \varepsilon \quad (10)$$

$$n = 10;\ r = 0.961;\ t = 9.83;\ \alpha < 0.001$$

and for ΔR_M

$$\Delta R_M = 0.800 \cdot e^{-\varepsilon/0.449} - 0.1 \quad (11)$$

The investigations have shown that the hydrophobicity of a substituent depends on the neighbouring group and, therefore, also on the position of substitution. A possible reason for the change in hydrophobicity is given by the decrease in the number of water contacts in the hydrate envelope around the substituent by intramolecular interaction.

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