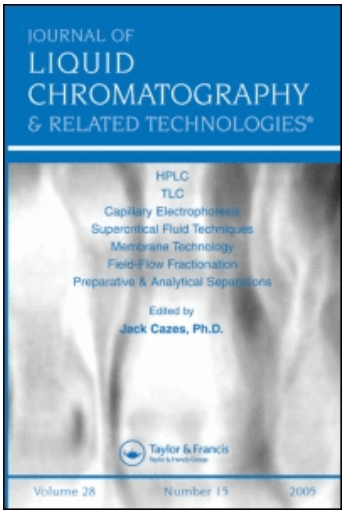

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Structure Retention Relationship for Steroid Hormones. Functional Groups as Structural Descriptors

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STRUCTURE RETENTION RELATIONSHIP FOR STEROID HORMONES. FUNCTIONAL GROUPS AS STRUCTURAL DESCRIPTORS

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ABSTRACT

Quantitative Structure Retention Relationship was investigated for a group of thirty steroid hormones. Structure of every compound was represented as a combination of the estrane skeleton and three to nine elements of a set of eighteen molecular fragments. Capacity factors of these compounds were represented as a sum of contributions due to the individual molecular fragments. From the temperature dependence of capacity factors the enthalpy and entropy of the retention process of these compounds were determined. It was shown that the enthalpy can also be considered as additive. Contributions to the capacity factor and to the enthalpy due to individual molecular fragments were calculated by the multiple regression method.

INTRODUCTION

Correlation of the molecular structure with physicochemical properties and biological activity seemed attractive since long ago [1, 2]. In chromatography, this correlation was named quantitative structure - retention relationship (QSRR) [3]. There are two ways of approaching this problem. In one of them the retention parameters are expressed as a function of the properties belonging to the molecule

as a whole, e. g. dipole moments, molecular refractivity, hydrophobicity, topological indexes and many others [2, 4-8]. In the second, contributions of the individual substituents to retention are considered. Additivity of substituent effects can be interpreted on the basis of linear Gibbs energy (or linear free energy relationship - LFER) [1, 3, 4, 9, 10] which was extensively used for explaining the effects of structural parameters on chemical rates and equilibria [11].

If the assumption on LFER is applicable, the following thermodynamic relationships for the capacity factor k':

$$k' = K \cdot \varphi \qquad (1)$$

where K is the partition coefficient of the solute molecules between stationary and mobile phases, φ is the volume phase ratio of the stationary to the mobile phase, and

$$\ln k' = -\frac{\Delta G}{RT} + \ln\varphi \qquad (2)$$

where ΔG - molar Gibbs energy, R - the molar gas constant, T - absolute temperature, lead to the expression

$$\ln k' = \sum_i \ln k_i' + \ln\varphi \qquad (3)$$

where k_i' denotes contribution from the i^{th} fragment of a given compound to k'. For a set of capacity factors of a number of compounds we can find contributions k_i' using the least squares method for solving the system of linear equations. The method was comprehensively outlined in the paper of Chen and Horváth [3]; details being available in textbooks of statistics, e.g. [12]. The results of such QSRR investigation for a large number of aromatic - aliphatic acids (experimental data from [13], paper chromatography) were presented in [3]. Papers on QSRR using RPLC data for catecholamine derivatives [3], cardiac glycosides and steroid hormones [14] as well as benzodiazepines [15] were also concerned with the estimation of contributions of various molecular fragments (atomic groups, substituents) to retention.

QSRR was also investigated in a somewhat simplified way, i. e. by comparison of retention parameters of two compounds differing in only one molecular feature,

present in one compound, and absent in another one. This method was used for steroid hormones in [16] (normal phase LC) and in [17] (RPLC).

Such a simplified approach does not provide simultaneous contributions of all substituents, whereas the method treating a group of compounds as composed of a set of "building blocks" can in principle give contributions from all the elements of the set.

The group of compounds selected in this study consisted of 30 steroid hormones. Every compound can be represented by combining three to nine elements of the set of eighteen characteristic atomic groups and the estrane skeleton. As a matter of fact, in the beginning the number of characteristic groups was 19; then it was reduced to 18: the reasons will be given later on. We anticipated that the LFER assumption holds in this case, i. e. for every compound:

$$\ln k' = \text{const.} + \sum_{i=1}^{18} a_i \ln k_i' \qquad (4)$$

where the constant is the contribution from the estrane skeleton and includes also $\ln\varphi$. All the coefficients a_i take only two values: 1 (when the corresponding atom group is present in a given molecule) or 0 (when it is missing).

Eqn. 2 can be also written as [10]:

$$\ln k' = -\frac{\Delta H}{RT} + \frac{\Delta S}{R} + \ln\varphi \qquad (5)$$

where ΔH, ΔS - denote enthalpy and entropy of the process of transfer of a solute molecule from mobile to stationary phase, respectively. Van't Hoff plots of ln k' vs 1/T offer the possibility of the estimation of ΔH and ΔS.

In this way ΔH and ΔS values were found. Next, it was checked if the values can also be treated as additive; if so, the following equations should be true:

$$\Delta H = \text{const.} + \sum_i a_i h_i \qquad (6)$$

$$\Delta S = \text{const.} + \sum_i a_i s_i \qquad (7)$$

where h_i and s_i - the increments to ΔH and ΔS of the characteristic atomic groups.

The main goal of this work was to verify the three relations 4, 6 and 7 experimentally.

EXPERIMENTAL

Materials

The names and CAS Registry Numbers of the steroids selected in this work are given in Table I. Solutions were prepared by diluting concentrated stock solutions (about 1 mg/ml, methanol) with mobile phase to the concentration about 25 μg/ml.

HPLC system

The HPLC system used was a Pye - Unicam PU 4100 (pump, oven and UV detector), Shimadzu RCA integrator, and a Varian fluorescence detector (for the measurement of void volume V_o). The chromatograms of steroid compounds were monitored at 210 nm (3, 9, 18, 22, 23, 25, 27, 28), 280 nm (10-12) and 240 nm (the rest). The column used were Partisil 10 ODS and Partisil 10 ODS-1, both 250 x 4.6 mm. Methanol-water was used as a mobile phase (55:45), flow rate was 1,5 ml/min. Capacity factors k' were measured on the column 1 for 30 steroids as a function of temperature at 35, 40, 45, 50, 55 and 60°C (only for lynestrenol at 40-65°). They were also measured on the column 2 at constant temperature, 40°C. Void volume V_o was determined as proposed by Neidhart et al [18], their method amounts to the doping of the mobile phase with a fluorophore (quinine sulphate), injection of undoped mobile phase, and measuring time of the decrease of fluorescence. Phase ratio φ was evaluated as the column inner volume minus V_o, divided by V_o. At 40°C φ was equal 0,48.

Statistics

Statistical analysis of data was performed with Stratgraphics Plus, version 6.0.

RESULTS AND DISCUSSION

Capacity factors k' were measured for 30 steroids as a function of temperature on the column 1. The measurements were also carried out on the column 2 at

TABLE 1
Names and CAS Registry Numbers of the Studied Set of Compounds

Compound		CAS Registry Number
1	Cortisone	[53-06-5]
2	Medroxyprogesterone acetate	[71-58-9]
3	Lynestrenol	[52-76-6]
4	Testosterone	[58-22-0]
5	Nandrolone	[434-22-0]
6	Progesterone	[57-83-0]
7	Prednisone	[53-03-2]
8	Prednisolone	[50-24-8]
9	Androsterone	[53-41-8]
10	Ethinyloestradiol	[57-63-6]
11	Estradiol	[50-28-2]
12	Estrone	[53-16-7]
13	Hydrocortisone	[50-23-7]
14	Methyltestosterone	[58-18-4]
15	Methylprednisolone	[83-43-2]
16	Ethisterone	[434-03-7]
17	Norethisterone	[68-22-4]
18	Methylandrostenediol	[521-10-8]
19	Methandienone	[72-63-9]
20	Methylprednisolone acetate	[53-36-1]
21	Prednisolone acetate	[52-21-1]
22	5α-androstane-3,17-dione	[846-48-8]
23	5α-androstane-17α-methyl-17β-ol-3-one	[521-11-9]
24	Cortexolone	[152-58-9]
25	Pregnenolone	[145-13-1]
26	1,4-androstadien-3,17-dione	[897-06-3]
27	5α-androstan-17β-ol-3-one	[521-18-6]
28	5α-androstan-3β-ol-17-one	[481-29-8]
29	Hydroxyprogesterone	[68-96-7]
30	Medroxyprogesterone	[520-85-4]

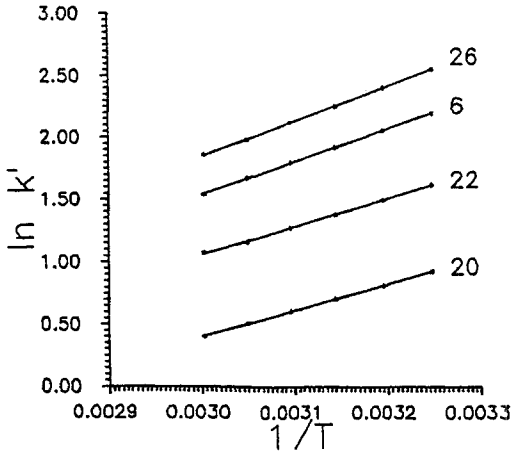


Figure 1 Plot of lnk' vs 1/T for several steroids. Column: Partisil ODS-1, mobile phase, methanol - water (55:45). See Table I for the names of compounds.

constant temperature of 40°C. Several plots of lnk' vs 1/T are shown in Figure 1. Correlation coefficients of the linear regression lnk' vs 1/T are very near to 1, so the assumption that the mechanism of the process is invariant over the temperature range investigated and the enthalpy is constant is justified. Values of ΔH and ΔS calculated from eqn. 5 are listed in Table II along with the measured values of k'.

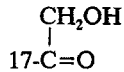
In order to verify if the LFER can be accepted in this investigation, the linear regression of lnk' on ΔH was calculated. The correlation coefficient of this regression was high, R = - 0.9774, so the relations given by eqns. 4,6 and 7 should be true. Hence the sets of lnk', ΔH and ΔS were used to calculate the increments $\mathbf{lnk_i'}$ to lnk', the increments h_i to ΔH and the increments s_i to ΔS by means of multiple linear regression.

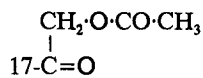
During this calculation a difficulty was encountered. In the matrix of coefficients a_i one or more columns appeared to be linear combinations of others. To overcome this problem it was necessary to delete the column corresponding to the C(20)=0 group. As a consequence the character of neighbouring groups was changed:

TABLE 2
Values of k', ΔH and ΔS of the Studied Steroids. Column: Partisil ODS-1, mobile phase: methanol-water (55:45), temp. 40°C (for k'). The enthalpy and entropy units are kcal·mol^{-1} and cal·mol^{-1}·deg^{-1}, respectively.

Compound	k'	ΔH	ΔS
1	0.9437	-3.13	-8.60
2	6.712	-5.17	-11.3
3	28.30	-6.52	-12.7
4	4.146	-4.50	-10.1
5	3.573	-4.40	-10.1
6	7.649	-5.29	-11.4
7	0.8779	-3.01	-8.36
8	1.033	-3.26	-8.84
9	6.810	-4.88	-10.4
10	3.204	-4.73	-11.3
11	3.462	-4.73	-11.2
12	2.749	-4.32	-10.3
13	1.094	-3.27	-8.78
14	5.413	-4.74	-10.3
15	1.509	-3.66	-9.37
16	3.495	-4.34	-9.91
17	3.033	-4.23	-9.85
18	6.803	-5.14	-11.1
19	3.802	-4.26	-9.47
20	2.189	-4.23	-10.5
21	1.519	-3.79	-9.77
22	4.353	-4.47	-9.89
23	7.675	-4.80	-9.89
24	1.935	-3.76	-9.22
25	10.86	-5.69	-12.0
26	4.774	-4.76	-10.6
27	5.834	-5.11	-11.3
28	5.844	-4.89	-10.6
29	2.175	-3.84	-9.21
30	3.430	-4.40	-10.1

instead of (21)CH_3 we have 

instead of (21)CH_2OH we have 

whereas 21($CH_2 \cdot O \cdot CO \cdot CH_3$) was converted to 

Taking the value of the coefficient of determination, R^2, as a criterion, one can see that the additivity of lnk′ is good (R^2 equals to 0.9693 and 0.9778 respectively for columns 1 and 2) and the additivity of ΔH is seen to be fairly good ($R^2 = 0.9328$). However, one can hardly agree that such an additive dependence for ΔS ($R^2 = 0.6672$) exists. Contributions h_i to ΔH are given in Table 3. The greatest positive partial enthalpies for C(3)=0 and 3-OH are measures of the heat consumption by these atomic groups during the transfer from the mobile to stationary phases. This heat is probably needed for the breaking of hydrogen bonds between these groups and the alcoholic - aqueous environment. Interestingly enough, the same groups in other positions (C(11)=0, C(17)=0, 11-OH and 17-OH) have far smaller partial enthalpies. Such functional groups as C = O and C-OH have essentially the same values when situated at the same site.

The contributions from double bonds between different atoms are also different. The large negative constant can be interpreted in such a way that the basic core of all these compounds, the estrane skeleton, has far greater affinity to hydrocarbon chains of the stationary phase than to the polar species of the mobile phase.

Apart from the relations based on the LFER assumption we have also tried to determine the increments to capacity factor according to:

$$k' = \text{constant} + \sum_{i}^{18} a_i \, k_i' \qquad (8)$$

Values of R^2 for such a multiple regression were equal to 0.9786 and 0.9855 for columns 1 and 2 respectively. Comparison with the values of R^2 for multiple linear regression using eqn. 4 shows that eqn. 8 describes the contributions of individual

TABLE 3

The Enthalpy Increments h_i and the Capacity Factor Increments k_i' due to the Individual Molecular Fragments.

	Molecular fragment	Enthalpy increments h_i	Capacity factor increments k_i'
1	1(2)C=C	0.01±0.1	-0.04±0.5
2	4(5)C=C	0.2±0.2	-1.8±0.6
3	C(3)=O	2.3±0.3	-26±1
4	C(11)=O	0.7±0.3	-1±1
5	C(17)=O	1.6±0.3	-6±1
6	3-OH	2.1±0.3	-25±1
7	11-OH	0.5±0.2	-1±0.9
8	17-OH	1.3±0.2	-5.8±0.9
9	(19)CH_3	-0.3±0.2	0.6±0.7
10	CH_3 17-C=O	0.8±0.2	-1.7±0.8
11	6-CH_3	-0.5±0.2	0.8±0.6
12	CH_2OH 17-C=O	0.9±0.2	-2.2±0.9
13	6π electrons	0.1±0.3	-2.7±1
14	17-C≡CH	0.2±0.2	-0.5±0.6
15	5(6)C=C	0.06±0.3	-0.2±1
16	17-CH_3	0.1±0.2	0.8±0.6
17	17-O·CO·CH_3	-0.8±0.3	3.6±1
18	CH_2·O·CO·CH_3 17-C=O	-0.5±0.2	0.5±0.7
	constant	-8.3±0.4	37±1

groups to retention better than eqn. 4. The F values point to the same conclusion. Contributions k_i' to k', according to eqn. 8, are given in Table 3. It can be seen that the same groups which have the largest positive increments to ΔH show the largest negative contributions to k'. Nevertheless, the correlation between k' and ΔH (R = -0.8188) is rather poor. It can also be seen in Table 3 that not all the contributions are statistically significant. According to the results of the Student's test one could exclude variables (contributions from fragments) 1, 4, 7, 14, 15, 18 by

taking significance level equal to 0.3. After elimination of these variables R^2 increased from 0.9786 to 0.9829.

It is known from the published data [19, 20] that in the class of steroid compounds the retention is not only determined by the kind of substituents but also depends on their stereochemical position. Thus the analysis of additive contributions should discern not only different substituents but also their stereochemistry, e. g. 3α-OH, 3β-OH and aromatic 3-OH ought to be treated separately. So we have tried to do so but an attempt to follow this idea resulted in the increase of linearly dependent columns and the calculations had to be abandoned.

In order to determine the actual predictive value of the procedure the method of cross - validation was applied [21]. The correlation coefficient of these cross - validated vs. observed values was 0.9038.

CONCLUSIONS

The capacity factor of a steroid hormone can be represented as a sum of increments due to individual molecular fragments (atomic groups). The linear representation given by eqn. 8 seems to be more reliable than the logarithmic relation of eqn. 4. The possibility of the additive representation for retention enthalpy and entropy was also checked and it was found that only the enthalpy can be considered as additive. Nevertheless the high value of the coefficient of determination in the multiple linear regression of lnk' indicates that LFER is a good empirical rule in this case. So correlation coefficients of regresion insufficiently descriminate between theoretical models [22, 23].

The knowledge of contributions from various atomic groups can be used to predict chromatographic behaviour of other compounds and to understand better interactions between solute and its environment. The investigation can be continued taking into account the discrimination of substituents with regard to their geometric isomerism.

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