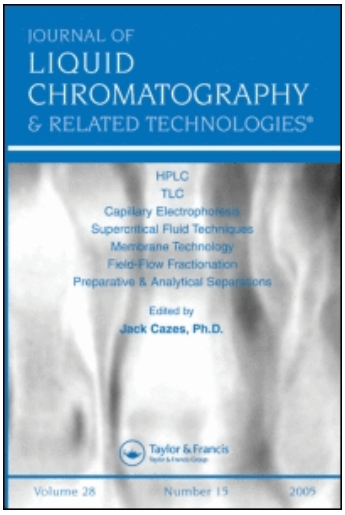

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Use of Selected Topological Indexes for Evaluation of Lipophilicity of Steroid Compounds Investigated by RP-HPTLC

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Use of Selected Topological Indexes for Evaluation of Lipophilicity of Steroid Compounds Investigated by RP-HPTLC

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Abstract: The selected steroid compounds (androsterone, epi-androsterone, dehydroepi-androsterone, testosterone, stigmasterol, β-sitosterol, estradiol, hydrocortisone, and cholesterol) were investigated with the use reversed phase high performance thin layer chromatography on RP18W plates (#1.14296, E. Merck), using methanol–water, acetonitrile–water in different volume compositions as a mobile phases. The chromatographic parameters of lipophilicity (R_{MW}) of the studied steroids were determined. Topological indexes based on the adjacency matrix: Gutman (M, M^{ν}), Randic ($^{o}\chi^{\nu}$, $^{1}\chi^{\nu}$, $^{o}\chi^{\nu}$, and $^{1}\chi^{\nu}$), based on distance matrix: Rouvray (R), Wiener (W), and Pyka (A, ^{o}B, and ^{1}B), and also based on information theory (I_{SA} and $\bar{I}_{SA}$) and theoretical partition coefficients (Alog Ps, IAlog P, Clog P, log P_{Kowwin}, xlog P, and miLog P) for investigated steroids were calculated. It was found that lipophilicity determined chromatographically (R_{MW}) correlated best with topological indices $^{o}\chi^{\nu}$, $^{1}\chi^{\nu}$, R, W, A, and ^{1}B. However, theoretical partition coefficient values (Alog Ps, IAlog P, Clog P, log P_{Kowwin}, xlog P, and miLog P) correlated best with topological index $\bar{I}_{SA}$.

Keywords: Lipophilicity parameter R_{MW}, QSAR, RP-HPTLC, Steroid drug, Theoretical partition coefficient, Topological index

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INTRODUCTION

Steroids are compounds having a four ringed carbon skeleton derived from 1,2-cyclopentanoperhydrophenanthrene with a cyclic structure. Many steroids are present in plants and animals. Cholesterol is the most important sterol representative of the animal origin. A series of cholesterol analogs have been isolated from many organisms. Steroids have definite physiological and pharmacological activity.[1–4] Some steroid compounds i.e., testosterone, estradiol, estriol, progesterone, hydrocortisone, β-sitosterol are used as drugs in modern therapy.

Nowadays, during the study of the activity of chemical compounds, much attention is paid to QSAR (quantitative structure activity relationships) derived to find the correlation between a given activity and the chemical structure of a compound. QSAR techniques play a crucial role in the design of substances for use as drugs. QSAR frequently utilize structural descriptors, i.e., quantities calculated from chemical formulae or by use of quantum mechanical methods.[5–7]

The scientific literature contains many examples of the use of structural descriptors for analysis of different classes of chemical compounds by use of QSAR.[6,7] The work discussed in this paper is a continuation of our previous investigations to determine the possibility of applying selected structural descriptors (topological indexes) in the analysis of QSAR (quantitative structure activity relationships), QSPR (quantitative structure property relationships), and QSRR (quantitative structure retention relationships) for different groups of organic compounds.[8,9] The objective of this work was the discovery of relationships between lipophilicity obtained by use of RP-HPTLC, theoretical partition coefficients, and selected topological indexes.

EXPERIMENTAL

Chemicals

The following components of the mobile phase: methanol (Merck, Germany; for liquid chromatography), acetonitrile (Merck, Germany; for liquid chromatography), and redistilated water were used for RP-HPTLC analysis. The commercial samples of androsterone (A), epi-androsterone (EP), dehydroepi-androsterone (DHEA), testosterone (T), stigmasterol (ST), β-sitosterol (S), estradiol (E), hydrocortisone (H), and cholesterol (CH) (E. Merck, Germany) were used as test

solutes. Methanol (POCh, Gliwice, Poland; pure p. a.), ethanol (ZPS Polmos, Kutno, Poland; pure p. a.), chloroform (POCh, Gliwice, Poland; pure p. a.), and acetone (Chempur, Piekary Slaskie, Poland; pure p. a.) were used to prepare the solutions of steroid compounds. Sulfuric acid, 95% (Chempur, Piekary Slaskie, Poland; pure p. a.) and methanol (POCh, Gliwice, Poland; pure p. a.) were used to prepare the visualizing reagent.

Sample Preparation

Standard solutions of steroid compounds (5 mg/1 mL) were prepared in methanol (for androsterone, epi-androsterone, and estradiol, cholesterol), chloroform (for dehydro-epi-androsterone, stigmasterol, and β-sitosterol), ethanol (for testosterone), and a mixture of chloroform and acetone (7 + 3, v/v; for hydrocortisone).

Reversed Phase Thin Layer Chromatography

Thin layer chromatography was done on RP-HPTLC RP18W (E. Merck, #1.14296) glass plates. Solutions of examined bile acids were spotted on chromatographic plates in quantities of 10 μg of each steroid in 2 μL of solution. The particular compounds were spotted separately on the plates. The chromatograms were developed by using the mixture of organic modifier water in the following volume compositions:[10]

- methanol–water, the content of methanol in mobile phase was gradually varied by 5% (%, v/v) from 50–100 (%, v/v);
- acetonitrile–water, the content of acetonitrile in mobile phase was gradually varied by 5% (%, v/v) from 30–80 (%, v/v).

Fifty mL of mobile phase was placed into a classical chromatographic chamber (Camag, Switzerland). The chamber was saturated with solvent for 20 min. The chromatograms were developed at room temperature, e.g., 20°C. The development distance was 8.5 cm. The plates were dried at room temperature, e.g., 20°C. The mixture of sulfuric acid and methanol (1:9, v/v) was used as the visualizing agent, and a 10 cm × 20 cm plate was sprayed with 5 mL of this visualizing agent. The plate was then heated at 120°C for 15 min. A Camag densitometer was used to obtain R_F values. The chromatograms were done in triplicate and mean R_F values were calculated.

Theoretical Partition Coefficients

The values of theoretical partition coefficients such as: Alog Ps, IAlog P, Clog P, $\log P_{Kowwin}$, xlog P, and miLog P were calculated with the use of the Internet databases.[11]

Application of Reversed Phase High Performance Thin Layer Chromatography for Determination of Lipophilicity of Examined Steroids

The parameter of lipophilicity determined by RP-HPTLC can be expressed by R_M value and can be calculated using the formula (1). The R_M values obtained for studied steroids on RP18 W plates, using the following mobile phases: methanol–water and acetonitrile–water, were extrapolated to zero concentration of organic modifier in eluent (R_{MW}), in accordance with Soczewinski-Wachtmeister equation:[5]

$$R_M = R_{MW} - S \times \varphi \quad (1)$$

where: R_M is the R_M value of the examined substance by content w of the volume fraction of the organic modifier in mobile phase; R_{MW} is the theoretical value of R_M of analyte extrapolated to zero concentration of organic modifier in mobile phase; S is the slope of the regression curve; φ is the volume fraction of organic modifier in the mobile phase.

Calculation of Topological Indices

Selected topological indexes based on adjacency matrix: Gutman (M, M^{ν}),[6,7,12,13] Randic ($^{o}\chi^{\nu}$, $^{1}\chi^{\nu}$, $^{o}\chi^{\nu}$, and $^{1}\chi^{\nu}$),[6,7,12,13] based on distance matrix: Rouvray (R),[6,12,13] Wiener (W),[6,12–14] and Pyka (A, ^{o}B, and ^{1}B),[15] and also based on information theory (I_{SA} and $\bar{I}_{SA}$)[6–8] were calculated. Pyka, Rouvray, and Wiener indexes were calculated by building a distance matrix and determining its elements by means of values given by Barysz et al.[16] The methods of calculation of topological indexes have been described elsewhere.[6,7,12,13]

Regression Analysis

Regression analysis by the least squares method was performed using the computer software Statistica 8.0.

RESULTS AND DISCUSSION

The selected steroid compounds (androsterone, epi-androsterone, dehydro-epiandrosterone, testosterone, stigmasterol, β-sitosterol, estradiol, hydrocortisone, and cholesterol) were studied. The above mentioned nine steroid compounds were investigated with the use of reversed phase high performance thin layer chromatography on RP18W plates (#1.14296, E. Merck), using methanol–water, acetonitrile–water in different volume compositions as a mobile phase. The R_M values obtained for the studied steroids were extrapolated to zero concentration of organic modifier in the mobile phase in accordance with Soczewinski-Wachtmeister equation (1), and R_{MW} were calculated. The chromatographic lipophilicity R_{MW} and theoretical partition coefficients calculated by use of different methods (Alog Ps, IAlog P, Clog P, log P_{Kowwin}, xlog P, and miLog P) for these compounds are presented in Table 1. The obtained values of $R_{MW(m)}$, $R_{MW(a)}$ lipophilicity parameters indicate that hydrocortisone shows the lowest lipophilic properties. Androsterone, epi-androsterone, dehydro-epi-androsterone, testosterone, and estradiol have intermediate lipophilic properties. However, stigmasterol, β-sitosterol, and cholesterol have the highest lipophilicities.

The values of the topological indexes based on distance and adjacency matrixes, and based on information theory are presented in Table 2.

Table 1. Lipophilicity values R_{MW} ($R_{MW(m)}$ and $R_{MW(a)}$) and values of theoretical partition coefficients calculated by use of different theoretical methods for the steroid compounds

Steroid symbol	Chromatographic lipophilicity parameter R_{MW}		Theoretical log P					
	$R_{MW(m)}$	$R_{MW(a)}$	Alog Ps	IAlog P	Clog P	log P_{Kowin}	xlog P	miLog P
A	2.693	2.107	3.71	3.46	3.55	3.07	4.30	3.742
EP	2.507	2.365	3.71	3.46	3.55	3.07	4.30	3.742
DHEA	2.392	2.447	3.53	3.04	3.07	2.98	3.04	3.765
T	2.240	2.277	2.99	3.24	3.22	3.27	3.60	3.765
E	2.388	2.071	3.57	3.50	3.78	3.94	4.23	4.482
ST	13.496	11.806	6.51	9.52	9.96	9.40	8.44	7.818
S	12.695	11.710	7.24	9.64	10.45	9.65	9.06	8.058
H	1.277	1.424	1.71	1.71	1.70	1.62	0.52	1.445
CH	7.659	12.451	7.00	8.89	9.52	8.74	8.20	7.469

$R_{MW(m)}$ – lipophilicity parameter obtained by use methanol–water mobile phase.
$R_{MW(a)}$ – lipophilicity parameter obtained by use acetonitrile–water mobile phase.

Table 2. Numerical values of selected topological indexes for investigated steroid compounds

Steroid symbol	Topological indexes based on												
	Distance matrix					Adjacency matrix						Information theory	
	R	W	A	^{o}B	^{1}B	$^{o}\chi^{\nu}$	$^{1}\chi^{\nu}$	M^{ν}	M	$^{o}\chi$	$^{1}\chi$	I_{SA}	$\bar{I}_{SA}$
A	1569.50	785.00	348.00	2.4579	0.3434	13.6062	9.1634	192	136	14.4578	9.6319	59.39	1.16
EP	1569.50	785.00	348.00	2.4579	0.3434	13.6062	9.1634	192	136	14.4578	9.6319	59.39	1.16
DHEA	1522.50	761.50	337.56	2.4956	0.3539	13.3990	8.8587	204	148	14.2507	9.3272	57.82	1.18
T	1517.50	759.00	335.86	2.4970	0.3541	13.3990	8.8696	204	148	14.2507	9.3382	57.82	1.18
E	1298.96	642.72	294.78	2.5087	0.3762	12.1785	8.0926	202	154	13.2840	8.6882	53.12	1.20
ST	5086.75	2544.00	967.34	2.3709	0.2196	20.2394	13.2620	222	198	20.7922	13.5812	81.32	1.04
S	4829.25	2415.00	903.18	2.4058	0.2232	20.9215	13.5785	204	180	21.4743	13.8976	82.69	1.03
H	2684.25	1342.75	535.510	2.5911	0.3032	15.7017	9.9570	322	186	17.9578	11.2423	74.17	1.32
CH	3972.25	1986.25	772.28	2.3970	0.2394	19.3442	12.6298	194	170	19.8970	12.9490	77.05	1.04

Table 3. Correlation coefficient (r) of linear dependence between lipophilic parameter and topological index ($P_L = a \times I_t + b$) for investigated steroid compounds

	$R_{MW(m)}$	$R_{MW(a)}$	Alog Ps	IAlog P	Clog P	Log P_{Kowin}	xlog P	miLog P
R	**0.935**	0.926	0.817	0.903	0.900	0.896	0.778	0.803
W	**0.935**	0.926	0.816	0.902	0.900	0.896	0.777	0.802
A	**0.935**	0.929	0.819	0.905	0.902	0.898	0.781	0.806
^{o}B	−0.826	−0.836	−0.919	−0.887	−0.880	−0.866	**−0.946**	−0.915
^{1}B	−0.901	−0.930	−0.819	−0.894	−0.891	−0.879	−0.769	−0.783
$^{o}\chi^{\nu}$	0.915	0.939	0.839	0.908	0.907	0.897	0.790	0.806
$^{1}\chi^{\nu}$	0.932	**0.953**	0.868	**0.930**	**0.928**	**0.917**	0.826	0.837
M^{ν}	−0.175	−0.211	−0.451	−0.306	−0.306	−0.301	−0.517	−0.468
M	0.690	0.651	0.445	0.595	0.593	0.610	0.395	0.469
$^{o}\chi$	0.862	0.885	0.754	0.840	0.840	0.830	0.695	0.717
$^{1}\chi$	0.894	0.914	0.800	0.878	0.877	0.865	0.748	0.763
I_{SA}	0.816	0.832	0.682	0.780	0.778	0.766	0.619	0.638
$\bar{I}_{SA}$	−0.855	−0.886	**−0.966**	**−0.930**	**−0.927**	**−0.918**	**−0.978**	**−0.963**

P_L – lipophilic parameter (R_{Mw} or theoretical partition coefficient).
I_t – topological index.

Different possibilities of application of the topological indexes in QSAR analysis to calculate certain lipophilicity data of examined steroid compounds, depending on examined chromatographic lipophilicity parameter and theoretical partition coefficient, were found (Table 3). Regression equations for respective lipophilicity parameters with the greatest correlation coefficients were found with the following structural descriptors:

- for lipophilicity parameter $R_{MW(m)}$, with topological indexes based on the distance matrix R, W and A;
- for lipophilicity parameter $R_{MW(a)}$, with topological indexes based on the distance matrix R, W, A, and ^{1}B, and with topological index based on adjacency matrix $^{1}\chi^{\nu}$;
- for Alog Ps and miLog P with topological index based on information theory $\bar{I}_{SA}$;
- for IAlog P, log P_{Kowin}, and Clog P with topological index based on adjacency matrix $^{1}\chi^{\nu}$ as well as with topological index based on information theory $\bar{I}_{SA}$;
- for xlog P with topological index based on distance matrix ^{o}B, and topological index based on information theory $\bar{I}_{SA}$.

The most statistically significant of the obtained regression equations are listed below:

$$R_{MW(m)} = -2.498(\pm 0.935) + 0.003(\pm 0.000) \times R$$
$$n = 9;\ r = 0.935;\ SD = 1.816;\ F = 48;\ p < 0.0005 \qquad (2)$$

$$R_{MW(a)} = -17.529(\pm 2.796) + 2.206(\pm 0.264) \times {}^{1}\chi^{\nu}$$
$$n = 9;\ r = 0.953;\ SD = 1.596;\ F = 69;\ p < 0.0005 \quad (3)$$

$$A\log Ps = 27.38(\pm 2.340) - 20.02(\pm 2.04) \times \bar{I}_{SA}.$$
$$n = 9;\ r = -0.966;\ SD = 0.54;\ F = 44;\ p < 0.0005 \quad (4)$$

$$IA\log P = 41.08(\pm 5.37) - 31.36(\pm 4.68) \times \bar{I}_{SA}$$
$$n = 9;\ r = -0.930;\ SD = 1.25;\ F = 44;\ p < 0.0005 \quad (5)$$

$$C\log P = 44.38(\pm 5.97) - 34.00(\pm 5.19) \times \bar{I}_{SA}$$
$$n = 9;\ r = -0.927;\ SD = 1.39;\ F = 42;\ p < 0.0005 \quad (6)$$

$$C\log P = -10.27(\pm 2.42) - 1.51(\pm 0.23) \times {}^{1}\chi^{\nu}$$
$$n = 9;\ r = 0.928;\ SD = 1.38;\ F = 43;\ p < 0.0005 \quad (7)$$

$$\log P_{Kowin} = 40.63(\pm 5.80) - 31.03(\pm 5.05) \times \bar{I}_{SA}$$
$$n = 9;\ r = -0.918;\ SD = 1.35;\ F = 37;\ p < 0.001 \quad (8)$$

$$x\log P = 39.04(\pm 2.72) - 29.65(\pm 2.37) \times \bar{I}_{SA}$$
$$n = 9;\ r = -0.978;\ SD = 0.63;\ F = 157;\ p < 0.0001 \quad (9)$$

$$mi\log P = 31.74(\pm 2.85) - 23.41(\pm 2.48) \times \bar{I}_{SA}$$
$$n = 9;\ r = -0.963;\ s = 0.66;\ F = 88;\ p < 0.0001 \quad (10)$$

Later, a test, which consisted of removing testosterone from the set of data was carried out. For example, Eq. (3) was recalculated and a new equation (11) was obtained in which one of the points, i.e., testosterone, was omitted:

$$R_{MW(a)} = -17.706(\pm 3.184) + 2.220(\pm 0.295) \times {}^{1}\chi^{\nu}$$
$$n = 8;\ r = 0.951;\ SD = 1.720;\ F = 56;\ p < 0.0005 \quad (11)$$

The $R_{MW(a)}$ value for testosterone was predicted from Eq. (11) and it equal 1.984, whereas the experimental value was 2.277.

The high correlation coefficients and significance levels of Eqs. (2)–(11) and correlation coefficients presented in Table 3 are indicative of the special physicochemical importance of the topological indexes ${}^{o}\chi^{\nu}$, ${}^{1}\chi^{\nu}$, R, W, A, ^{1}B, and $\bar{I}_{SA}$. From data presented in this work, it is apparent that the above mentioned topological indexes describe additional important elements of chemical structure of the investigated steroid compounds not given by the other topological indexes.

Theoretical determination of log *P* values and chromatographic lipophilicity on the basis of the topological indexes of organic compounds has special significance if standards are not available. The method presented is very simple and can be recommended for study of quantitative structure activity relationships for steroid compounds. These methods of determining lipophilicity on the basis of topological indexes and chromatographic method complement well established methods and applications, i.e., methods of normal measurement in the *n*-octanol–water system. Because of experimental difficulties including solubility limits, different stability, creation of emulsions, or impurity of the compound studied, evaluation of log P value by the method proposed in this paper is analytically well founded. The methods presented for determination of chromatographic lipophilicity and log *P* values might also be useful in the design of other organic compounds.

Further investigations are in progress and concern the investigation in the range of applications of topological indexes and chromatographic technique in QSAR analysis.

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