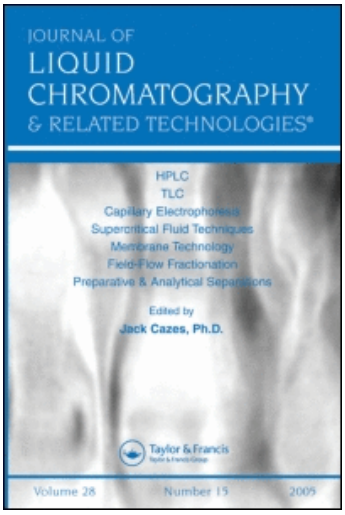

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TOPOLOGICAL INDEXES FOR EVALUATION OF THE SEPARATION OF *D* AND *L* AMINO ACIDS BY TLC>

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TOPOLOGICAL INDEXES FOR EVALUATION OF THE SEPARATION OF *D* AND *L* AMINO ACIDS BY TLC

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ABSTRACT

Selected amino acids have been separated into their enantiomers by thin layer chromatography on a chiral stationary phase. A new valence optical topological index (I^{v}_{opt}) and valence optical Gutman index (M^{v}_{2opt}) are proposed which enable distinction between isomers of *L* and *D* configuration.

INTRODUCTION

Thin layer, gas, and liquid chromatographic methods have been developed to separate amino acids enantiomers and their derivatives.[1-11] Analysis of amino acids and their derivatives have a great significance in medical diagnosis of hereditary metabolic diseases (phenylketonuria and other aminoaciduria).[12] My previous investigations have concerned a new optical topological index (I_{opt}) for predicting the separation of selected *D* and *L* optical isomers by TLC.[13]

The aim of the work reported herein was to propose new optical topological indexes and to evaluate the correlation between these and other topological indexes, and the R_F and R_M values of the optical isomers of *D* and *L* configuration of amino acids separated by TLC on a chiral stationary phase.

EXPERIMENTAL

Thin Layer Chromatography

The optical isomers of *D* and *L* amino acids: alanine, norvaline, norleucine, glutamic acid, phenylalanine, tyrosine, tryptophan, and proline were separated on Chir HPTLC plates (E. Merck, Darmstadt, Germany). Before use, plates were activated for 15 min at 100ºC. Amino acids were separated using methanol-water-acetonitrile (1:1:4) as the mobile phase.[13-16] The development distance was 11 cm. The chromatograms were visualized by dipping into a solution of ninhydrin (0.3%) in acetone and heating at 110ºC for 10 min.

Constant of the Pair Separation R_F^{α}

The constant of the pair separation (R_F^{α}) were calculated for the investigated pairs of optical isomers as the ratio of the R_F value of the *L* isomer to R_F value of the *D* isomer.[17]

Calculation of Topological Indexes Described in the Literature

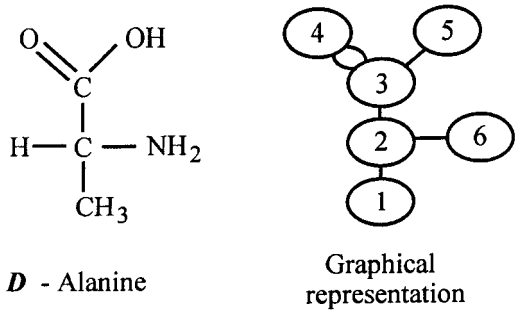
The Randić ($^0\chi^v$),[18] Wiener (W),[19,20] and optical topological (I_{opt})[13] indexes were calculated for the investigated amino acids.

Calculation of Topological Indexes Proposed in this Work

Valence optical topological index (I_{opt}^v)

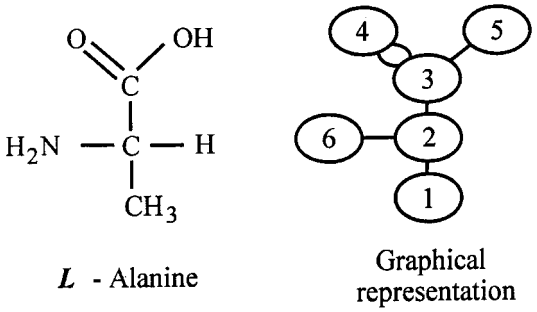
Definition of valence optical topological index

Let *G* be a molecular graph and $x_1,x_2,...,x_n$ its vertexes.[21,22] Let $Z^v = \left\| Z_i^v \right\|$ be an *n*-dimensional column-vector, such that its *i*-th component corresponds to the vertex of the molecular graph *G*. For all values of *i*, *i*=1, 2, ..., *n*, we choose



D - Alanine

Graphical representation



L - Alanine

Graphical representation

Figure 1. Graphical representation of the structures of *D* and *L* alanine.

$Z_i^v = \delta_i$, except if the *i*-th vertex of *G* represents an asymmetric (optically active) carbon atom. In this latter case, $Z_i^v = \delta_i$ if the respective carbon atom has configuration *D*, and $Z_i^v = -\delta_i$ if the configuration of that carbon atom is *L*.

The degree of the *i*-th vertex of the (hydrogen-suppressed) molecular graph is calculated as

$$\delta_i = \zeta_i - h_i \qquad (1)$$

where ζ_i is the number of valence electrons of the *i*-th atom in the molecule considered, and h_i is the number of hydrogen atoms attached to that atom.

Let $S'^{\nu} = \left\| S_i'^{\nu} \right\|$ be another column-vector calculated as

$$S'^{\nu} = [D] \bullet Z^{\nu} \quad (2)$$

where [D] is distance matrix of graph G,[18,20,22] and • indicates usual matrix multiplication.

Then, the valence optical topological index is given by

$$I_{opt}^{\nu} = \sum_{i=1}^{n} S_i'^{\nu} \quad (3)$$

Calculation of I_{opt}^{ν} for D- and L-alanine

Firstly, graphical representations of the structures of *D*- and *L*-alanine, with numbered vertexes, are established as in Fig. 1. The distance matrix, [D], is then constructed from these graphical representations, the elements being determined by means of the values given by Barysz et al.[20] (see Table 1).

The distance matrixes for the ***D* & *L*** amino acids are identical.

The distance matrix constructed above is multiplied by column vector Z_D^{ν} or Z_L^{ν} to give the column vector $\mathbf{S'^{\nu}}$ which consistis of elements $S_i'^{\nu}$.

[D] $\times$ Z_D^{ν} for *D*-alanine is given by:

$$\underset{\mathbf{[D]}}{\begin{bmatrix} 0 & 1 & 2 & 2{,}375 & 2{,}750 & 1{,}857 \\ 1 & 0 & 1 & 1{,}375 & 1{,}750 & 0{,}857 \\ 2 & 1 & 0 & 0{,}375 & 0{,}750 & 1{,}857 \\ 2{,}375 & 1{,}375 & 0{,}375 & 0{,}250 & 1{,}125 & 2{,}232 \\ 2{,}750 & 1{,}75 & 0{,}75 & 1{,}125 & 0{,}250 & 2{,}607 \\ 1{,}857 & 0{,}857 & 1{,}857 & 2{,}232 & 2{,}607 & 0{,}143 \end{bmatrix}} \cdot \underset{Z_D^{\nu}}{\begin{bmatrix} 1 \\ 3 \\ 4 \\ 6 \\ 5 \\ 3 \end{bmatrix}} = \underset{S_D'^{\nu}}{\begin{bmatrix} 44{,}571 \\ 24{,}571 \\ 16{,}571 \\ 21{,}821 \\ 26{,}821 \\ 38{,}712 \end{bmatrix}}$$

Therefore, I_{opt}^{ν} for *D*-alanine is calculated as follows:

$$I_{opt}^{\nu} = 44{,}571+24{,}571+16{,}571+21{,}821+26{,}821+38{,}712 = 173.067$$

Table 1

Distance Matrix [D] for *D*- and *L*-Alanine

Vertex Number	1	2	3	4	5	6
1	0	1,000	2,000	2,375	2,750	1,857
2	1,000	0	1,000	1,375	1,750	0,857
3	2,000	1,000	0	0,375	0,750	1,857
4	2,375	1,375	0,375	0,25	1,125	2,232
5	2,750	1,750	0,750	1,125	0,25	2,607
6	1,857	0,857	1,857	2,232	2,607	0,143

[D] $\times$ Z_L^v for *L*-alanine is given by:

$$\underset{\mathbf{[D]}}{\begin{bmatrix} 0 & 1 & 2 & 2{,}375 & 2{,}750 & 1{,}857 \\ 1 & 0 & 1 & 1{,}375 & 1{,}750 & 0{,}857 \\ 2 & 1 & 0 & 0{,}375 & 0{,}750 & 1{,}857 \\ 2{,}375 & 1{,}375 & 0{,}375 & 0{,}250 & 1{,}125 & 2{,}232 \\ 2{,}750 & 1{,}75 & 0{,}75 & 1{,}125 & 0{,}250 & 2{,}607 \\ 1{,}857 & 0{,}857 & 1{,}857 & 2{,}232 & 2{,}607 & 0{,}143 \end{bmatrix}} \cdot \underset{Z_D^v}{\begin{bmatrix} 1 \\ -3 \\ 4 \\ 6 \\ 5 \\ 3 \end{bmatrix}} = \underset{S_D'^v}{\begin{bmatrix} 38{,}571 \\ 24{,}571 \\ 10{,}571 \\ 13{,}571 \\ 16{,}321 \\ 33{,}570 \end{bmatrix}}$$

A calculation analogous to that for *D*-alanine shows that, for *L*-alanine, $I_{opt}^v = 137.175$

Valence optical Gutman index **(M_{2opt}^v)**

A valence optical Gutman index, M_{2opt}^v, similar to the Gutman index M_2, is given by

$$M^{\nu}_{2opt} = \sum_{\text{all edges}} (\delta_i \delta_j) \qquad (4)$$

where δ_i is calculated by Eq. (1); a value of "$+\delta_i$" is assigned to an asymmetric carbon atom bound to an amino group which gives the molecule the *D* configuration; a value of "$-\delta_i$" is assigned to an asymmetric carbon atom bound to an amino group which gives the molecule the *L* configuration.

Calculation of M^{ν}_{2opt} ***for D- and L-alanine***

For ***D***- alanine:

$$M^{\nu}_{2opt} = (1\times3) + (3\times4) + (3\times3) + 2(4\times6) + (4\times5) = 92$$

For ***L***- alanine:

$$M^{\nu}_{2opt} = [1\times(-3)] + [(-3)\times4] + [(-3)\times3] + 2(4\times6) + (4\times5) = 44$$

RESULTS AND DISCUSSION

R_F and R_M values; numerical values of the topological indexes W, $^0\chi^{\nu}$, and I_{opt}; and numerical values of the new topological indexes I^{ν}_{opt} and M^{ν}_{2opt} calculated using the procedures described above are listed in Table 2 for the *D* and *L* amino acids investigated.

Numerical values of the topological indexes W and $^0\chi^{\nu}$ are equal for the pair of isomers, i.e., these indexes do not differentiate *L* and *D* isomers. In this work, applied *optical topological index* (I_{opt}), *valence optical topological index* (I^{ν}_{opt}), and *valence optical Gutman index* (M^{ν}_{2opt}) enable the differentation of optical isomers of *L* and *D* configuration.

Constants of the pair separation, R^{α}_F, are listed in Table 3 for the *L* and *D* amino acids investigated.

R^{α}_F values characterizes the separation of isomers pairs of *L* and *D* configuration. A similar method for evaluation of pair separations was applied by J. Śliwiok.[17]

Table 2

Numerical Values of R_F and R_M, and the Topological Indexes W, $^0\chi^\nu$, I_{opt}, I^ν_{opt}, and M^ν_{2opt}

Amino Acid	Retention Data*		Topological Indexes				
	R_F	R_M	W	$^0\chi^\nu$	I_{opt}	I^ν_{opt}	M^ν_{2opt}
D-Alanine	0.47	0.052	24.553	3.510	48.463	173.067	92
L-Alanine	0.53	-0.052	24.553	3.510	36.499	137.175	44
D-Norvaline	0.50	0.000	63.517	4.924	126.391	398.137	101
L-Norvaline	0.56	-0.105	63.517	4.924	104.427	332.245	47
D-Norleucine	0.51	-0.017	94.499	5.631	188.355	571.672	105
L-Norleucine	0.60	-0.176	94.499	5.631	158.391	481.780	51
D-Glutamic acid	0.52	-0.035	120.106	5.280	239.069	1000.777	175
L-Glutamic acid	0.58	-0.140	120.106	5.280	202.855	892.135	121
D-Phenylalanine	0.45	0.087	175.472	6.604	350.301	1261.313	163
L-Phenylalanine	0.54	-0.070	175.472	6.604	302.331	1117.403	109
D-Tyrosine	0.55	-0.087	218.713	6.974	436.533	1677.363	189
L-Tyrosine	0.65	-0.269	218.713	6.974	379.061	1504.947	135
D-Tryptophan	0.50	0.000	309.209	8.104	617.632	2272.183	238
L-Tryptophan	0.58	-0.140	309.209	8.104	541.948	2045.131	184
D-Proline	0.38	0.213	54.373	4.554	108.103	379.950	114
L-Proline	0.46	0.070	54.373	4.554	88.771	321.774	54

* Average of 8 measurements

Numerical values of the indexes $I_{opt(L/D)}$ ($I_{opt(L/D)} = \frac{I_{opt(L)}}{I_{opt(D)}}$) and $I^\nu_{opt(L/D)}$ ($I^\nu_{opt(L/D)} = \frac{I^\nu_{opt(L)}}{I^\nu_{opt(D)}}$) for optical isomers of *L* and *D* configuration can used to differentiate these isomers on a chromatographic and optical basis. Numerical values of the indexes $I_{opt(L/D)}$ and $I^\nu_{opt(L/D)}$ are comparable with numerical values of constants of the pair separation R^α_F for *L* and *D* isomers. However, the ratio of the M^ν_{2opt} value of the *L* isomer to the M^ν_{2opt} value of the

Table 3

Numerical Values of R_F^{α}, $I_{opt(L/D)}$, $I^{\nu}_{opt(L/D)}$, and $M^{\nu}_{2opt(L/D)}$

Amino Acid	$R_F^{\alpha} = \frac{R_{F(L)}}{R_{F(D)}}$	$I_{opt(L/D)} = \frac{I_{opt(L)}}{I_{opt(D)}}$	$I^{\nu}_{opt(L/D)}$	$M^{\nu}_{2opt(L/D)} = \frac{M^{\nu}_{2opt(L)}}{M^{\nu}_{2opt(D)}}$
Alanine	1.128	0.753	0.793	0.478
Norvaline	1.120	0.826	0.834	0.465
Norleucine	1.176	0.841	0.843	0.486
Glutamic acid	1.115	0.849	0.891	0.691
Phenylalanine	1.200	0.863	0.886	0.669
Tyrosine	1.182	0.868	0.897	0.714
Tryptophan	1.160	0.877	0.900	0.773
Proline	1.210	0.821	0.847	0.474

D isomer have different numerical values for different isomer pairs. The obtained values for the *optical topological indexes* and *valence optical topological indexes* have high repeatability that range from 0.753 to 0.877 and from 0.793 to 0.900 for the indexes $I_{opt(L/D)}$ and $I^{\nu}_{opt(L/D)}$, respectively. This indicates an existing regularity between the chemical consitution that is expressed by the *optical coefficients of distinction* ($I_{opt(L/D)}$ and $I^{\nu}_{opt(L/D)}$) and the chromatographic data that is obtained as the ratio of the $R_{F(L)}$ and $R_{F(D)}$ values (the constants of the isomer pairs R_F^{α}) for the *L* and *D* amino acids investigated.

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