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A. Pyka^a

^a Faculty of Pharmacy, Academy of Medicine, Sosnowiec, Poland

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

A. Pyka

Faculty of Pharmacy
Academy of Medicine
4, Jagiellońska Street
PL-41-200 Sosnowiec, Poland

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 ($I_{\text{opt}}^{\text{v}}$) and valence optical Gutman index ($M_{2\text{opt}}^{\text{v}}$) 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 100°C. 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 110°C 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

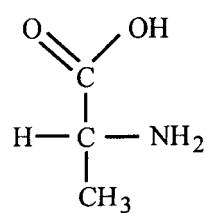
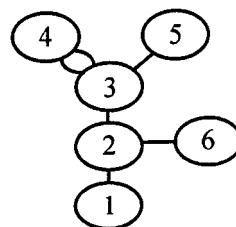
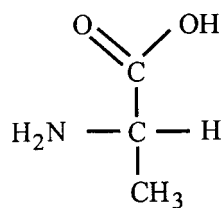
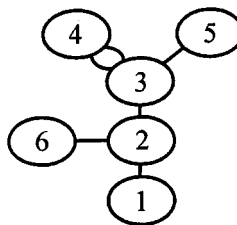
The Randić ($^0\chi^v$),¹⁸ Wiener (W),^{19,20} and optical topological (I_{opt})¹³ indexes were calculated for the investigated amino acids.

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, \dots, 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 $i, i=1, 2, \dots, n$, 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

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

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 $S^v = \|S_i^v\|$ be another column-vector calculated as

$$S^v = [D] \bullet Z^v \quad (2)$$

where $[D]$ is distance matrix of graph G ,^{18,20,22} and \bullet indicates usual matrix multiplication.

Then, the valence optical topological index is given by

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

Calculation of I_{opt}^v 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^v or Z_L^v to give the column vector S^v which consists of elements S_i^v .

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

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

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

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

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:

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

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

$$M_{2opt}^v = \sum_{\text{all edges}} (\delta_i \delta_j) \quad (4)$$

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_{2opt}^v for D- and L-alanine

For *D*- alanine:

$$M_{2opt}^v = (1 \times 3) + (3 \times 4) + (3 \times 3) + 2(4 \times 6) + (4 \times 5) = 92$$

For *L*- alanine:

$$M_{2opt}^v = [1 \times (-3)] + [(-3) \times 4] + [(-3) \times 3] + 2(4 \times 6) + (4 \times 5) = 44$$

RESULTS AND DISCUSSION

R_F and R_M values; numerical values of the topological indexes W , ${}^0\chi^v$, and I_{opt} ; and numerical values of the new topological indexes I_{opt}^v and M_{2opt}^v 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 ${}^0\chi^v$ 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_{opt}^v), and *valence optical Gutman index* (M_{2opt}^v) enable the differentiation of optical isomers of *L* and *D* configuration.

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

R_F^α 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 ,
 $^0\chi^v$, I_{opt} , I_{opt}^v , and M_{2opt}^v

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

$I_{opt(L/D)}^v$ ($I_{opt(L/D)}^v = \frac{I_{opt(L)}^v}{I_{opt(D)}^v}$) 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 $I_{opt(L/D)}^v$ are comparable with numerical values of constants of the pair separation R_F^α for *L* and *D* isomers. However, the ratio of the M_{2opt}^v value of the *L* isomer to the M_{2opt}^v value of the

Table 3

Numerical Values of R_F^α , $I_{\text{opt}}(L/D)$, $I_{\text{opt}}^v(L/D)$, and $M_{2\text{opt}}^v(L/D)$

| Amino Acid | R_F^α $= \frac{R_{F(L)}}{R_{F(D)}}$ | $I_{\text{opt}}(L/D)$ $= \frac{I_{\text{opt}}(L)}{I_{\text{opt}}(D)}$ | $I_{\text{opt}}^v(L/D)$ | $M_{2\text{opt}}^v(L/D)$ $= \frac{M_{2\text{opt}}^v(L)}{M_{2\text{opt}}^v(D)}$ |
|---------------|---|--|-------------------------|---|
| 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 $I_{\text{opt}}(L/D)$ and $I_{\text{opt}}^v(L/D)$, respectively. This indicates an existing regularity between the chemical constitution that is expressed by the *optical coefficients of distinction* ($I_{\text{opt}}(L/D)$ and $I_{\text{opt}}^v(L/D)$) 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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