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Chromatographic separation of tocopherols

A. Pyka^a, J. Sliwiok^{b,*}

^aFaculty of Pharmacy, Silesian Academy of Medicine, 4 Jagiellonska Street, PL41-200 Sosnowiec, Poland

^bInstitute of Chemistry, Silesian University, 9 Szkolna Street, PL40-006 Katowice, Poland

Abstract

α -, β -, γ -, and δ -Tocopherols were separated by reversed-phase high-performance thin-layer chromatography (C_{18} RP-HPTLC), normal-phase high-performance liquid chromatography (NP-HPLC), reversed-phase high-performance liquid chromatography (C_{18} RP-HPLC), and gas chromatography (GC). The selected topological indices based on connectivity (M , χ^v), on distance matrix (W , $^{\circ}B$, MTI) and on information theory (I_{AC} , \bar{I}_{AC}) were calculated for these tocopherols. The observed chromatographic separations of investigated tocopherols were compared. This comparison indicated that the C_{18} RP-HPTLC, NP-HPLC, and GC are the best techniques for the separation of these tocopherols. Topological index $^{\circ}B$ was the most significant. We obtained definite dependence between the numerical values of topological index $^{\circ}B$ and the chromatographic separation of the investigated tocopherols. © 2001 Elsevier Science B.V. All rights reserved.

Keywords: Topological indices; Tocopherols; Vitamins

1. Introduction

Vitamin E has been an enigma in nutrition research for over 60 years. In 1937, Emerson et al. [1] described the existence of various vitamin E homologues that had different abilities to prevent vitamin E deficiency. In nature, vitamin E occurs in eight different forms (α -, β -, γ - and δ -tocopherols and α -, β -, γ - and δ -tocotrienols) with varying biologic activities. Tocopherols are being intensively studied owing to their medical, biological, and physico-chemical significance [2–4]; the biological properties of α -tocopherol are of particular importance [5,6]. Of these eight compounds, α -tocopherol is reported to have the highest biological activity [7]. Tocopherol possesses three asymmetric carbon atoms. Because eight stereoisomeric tocopherols can be received. Natural α -tocopherol occurs as the enantiomer about

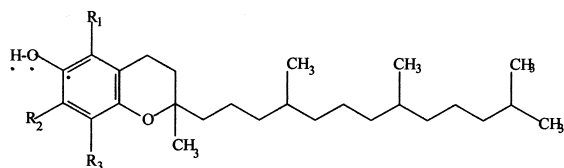
the configuration 2R, 4'R, 8'R. Semi-synthetic α -tocopherol is the mixture of the diastereoisomers about configuration 2R/S, 4'R and 8'R. Although γ -tocopherol is a more effective free radical scavenger than α -tocopherol in vitro [8], the reverse is true in vivo [9]. The biological activity of vitamin E has generally been associated with its well-defined antioxidant property, specifically against lipid peroxidation in biological membranes. The antioxidative effect of the different tocopherols may not be identical. It has been shown repeatedly in antioxidative tests with feedstuffs and fat that the tocopherols have an antioxidative activity of the following order: γ -, δ -, β -, and α -tocopherols [10,11]. Vitamin E occurs principally in wheat germ, vegetable oil, and vegetables. α -Tocopherol and γ -tocopherol are the most common of these eight naturally occurring vitamin E homologues in the human diet. Tocopherol is nearly insoluble in water, but is soluble in ethanol, ether, chloroform, acetone, and vegetable oils.

The problem of the separation of α -, β -, γ - and

*Corresponding author. Tel.: +48-322-599-978; fax: +48-322-599-978.

Table 1

Structures, molar masses (M_r), partition coefficient by Rekker ($\log P$), relative affinity of the tocopherols investigated, and the net electron charge (ΣNEC) on the compounds' $-\text{C}-\text{O}-\text{H}$ groups



Compound	R_1	R_2	R_3	M_r (g/mol)	$\log P$ [4]	ΣNEC [4]	Relative affinity (%) [7]
DL- α -Tocopherol	$-\text{CH}_3$	$-\text{CH}_3$	$-\text{CH}_3$	430.71	12.37	0.0039	100
DL- β -Tocopherol	$-\text{CH}_3$	$-\text{H}$	$-\text{CH}_3$	416.68	11.86	0.0223	38.1 ± 9.3
DL- γ -Tocopherol	$-\text{H}$	$-\text{CH}_3$	$-\text{CH}_3$	416.68	11.86	0.0327	8.9 ± 0.6
DL- δ -Tocopherol	$-\text{H}$	$-\text{H}$	$-\text{CH}_3$	402.65	11.36	0.0432	1.6 ± 0.3

δ -tocopherols has been the subject of numerous reviews [12,13].

The problem of the relationship between the structure of a molecule and its physical, chemical and biological properties is one of the most fundamental in chemistry. Therefore, the aim of these studies was to follow solutes of α -, β -, γ - and δ -tocopherols, which were separated by different chromatographic techniques and were characterized by selected topological indices. Table 1 lists general physicochemical data of investigated tocopherols.

2. Methods

2.1. Chemicals

If not otherwise specified, all used chemicals (analytical grade), reference tocopherols and HPTLC and HPLC solvents were from Merck, Darmstadt, Germany. Commercial samples of α -, β -, γ -, and δ -tocopherol (No. 15496, Merck) were used as test solutes.

2.2. Separation of α -, β -, γ -, and δ -tocopherols by RP-HPTLC

RP-HPTLC was performed on 10 cm \times 10 cm C_{18} reversed-phase UV_{254} HPTLC plates (No. 1.13724, Merck). Solution of a mixture of tocopherols was spotted on a chromatographic plate in quantities of 10 μg of each tocopherol in 2 μl of chloroform.

Mixtures of ethanol and water were used as mobile phases: mixtures containing ethanol and water in the volume proportions 10:0, 9.5:0.5, and 9:1 are designated S1, S2, and S3, respectively. Chromatograms were visualized with a mixture of equal volumes of solutions of dipyriddy in methanol (0.5%) and ferric chloride in methanol (0.2%).

2.3. Separation of α -, β -, γ -, and δ -tocopherols by NP-HPLC

Normal-phase HPLC conditions were as follows: HPLC system Knauer (Berlin, Germany); column: Hypersil Silica 5 μm (250 mm \times 4.6 mm) (Termo-Quest, Egelsbach, Germany); mobile phase: hexane–amyl alcohol (99.5:0.5, v/v); eluent flow: 2 ml/min; Knauer pump (Berlin, Germany), detection: UV at 290 nm (Knauer); injection volume: 20 μl of a standard mixture of α -, β -, γ -, and δ -tocopherols in ethanol. Experiments were performed at 293 K.

2.4. Separation of α -, β -, γ -, and δ -tocopherols by RP-HPLC

Reversed-phase HPLC conditions were as follows: HPLC system Knauer; column: RP- C_{18} , 5 μm (150 mm \times 4 mm) (Separon, Czech Republic); mobile phase: methanol–water (9:1, v/v); eluent flow: 1.5 ml/min; Knauer pump, detection: UV at 290 nm (Knauer); injection volume: 20 μl of a standard mixture of α -, β -, γ -, and δ -tocopherols in ethanol. Experiments were performed at 293 K.

2.5. Separation of α -, β -, γ -, and δ -tocopherols by GC

Trimethylsilyl (TMS) derivatives of α -, β -, γ -, and δ -tocopherols were separated. TMS derivatives were made by heating the mixture of tocopherols with 100 μ l *N*-methyl-*N*-trimethylsilyltrifluoroacetamide (MSTFA) (Aldrich Chemical Company, USA) for 25 min at 60°C. GC conditions were as follows: Carlo Erba Mega 5300 instrument (Italy); column: DB-5, 30 m \times 0.32 mm (J&W, USA); film thickness 0.25 μ m; oven: 200°C, 5°C/min to 310°C; He flow: 4 ml³/min; injection on colon; injection volume: 1.5 μ l of a standard mixture of α -, β -, γ -, and δ -tocopherols as TMS derivatives.

2.6. Calculation of topological indices

The topological indices based on connectivity: *Gutman* (*M*) [14–16], and *Randić* ($^1\chi^v$) [14–17], on distance matrix: *Wiener* (*W*) [14–16,18], *Pyka* ($^{\circ}B$) [16,19], and *Schultz* (MTI – Molecular Topological Index) [14,20], and on information theory: atomic composition index (I_{AC}) and mean of atomic composition index (\bar{I}_{AC}) [14,15] were calculated for the investigated compounds. The *Wiener*, *Pyka*, and *Schultz* indices were calculated by building the distance matrix and determining its elements by means of values given by *Barysz* et al. [21].

The methods of calculation of the topological indexes were presented in numerous review works [14–16].

3. Results and discussion

The separations of α -, β -, γ -, and δ -tocopherols on RP-C₁₈ plates with HPTLC by use of ethanol and water in different volume ratios as mobile phases are introduced in Table 2. The best separations were achieved with mobile phases S1 and S2 (Table 2).

In preceding investigations the separation of the above-mentioned substances was achieved by partition TLC technique, obtaining similar separation [22].

The following investigations referred to separation

Table 2

R_F values of the tocopherols investigated by C₁₈ RP-HPTLC

Compound	R_F^a		
	S1 ^b	S2	S3
α -Tocopherol	0.49	0.31	0.18
β -Tocopherol	0.53	0.37	0.20
γ -Tocopherol	0.57	0.41	0.22
δ -Tocopherol	0.63	0.47	0.24

^a Mean values, $n=5$.

^b S1, S2, S3 contained ethanol and water in the proportions 10:0, 9.5:0.5, and 9:1, v/v, respectively.

of these tocopherols using NP-HPLC. Fig. 1 shows the separation of the examined tocopherols.

The next separation of investigated tocopherols was done using the C₁₈-RP-HPLC technique and the

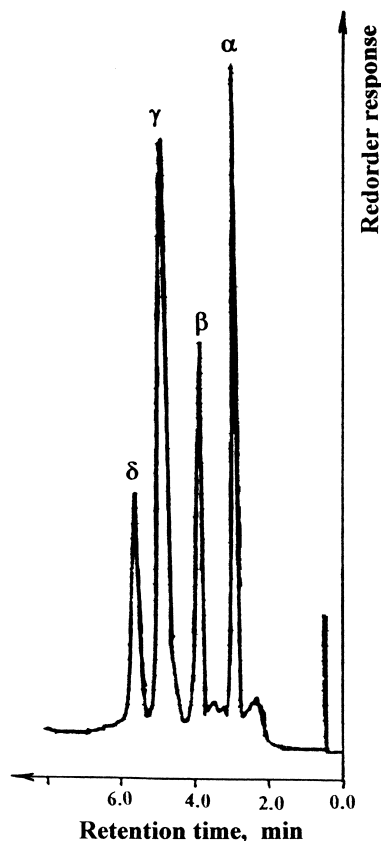


Fig. 1. NP-HPLC chromatogram of a standard mixture of tocopherols: 15 ng δ -tocopherol (5.60 min), 20 ng γ -tocopherol (4.88 min), 15 ng β -tocopherol (3.86 min), and 25 ng α -tocopherol (2.80 min); injected sample volume 20 μ l.

mixture of methanol and water as the mobile phase. Under these conditions there was no separation of β - and γ -tocopherols. Data are shown in Fig. 2.

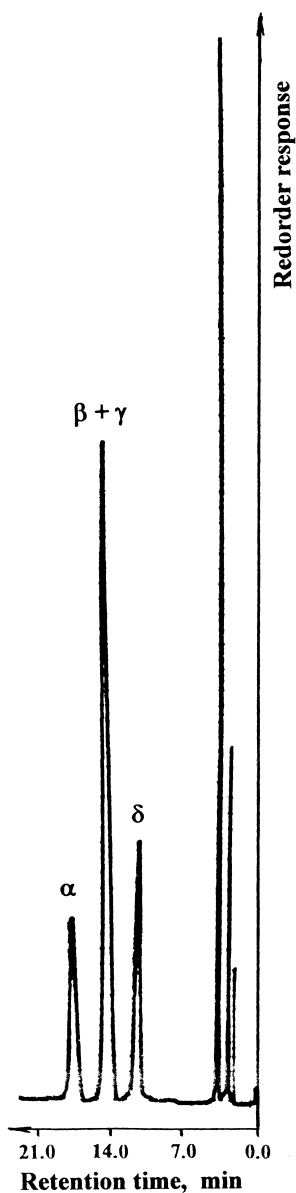


Fig. 2. C_{18} RP-HPLC chromatogram of a standard-mixture of tocopherols: 10 ng α -tocopherol (17.66 min), 35 ng β - and γ -tocopherol (13.98 min), and 15 ng δ -tocopherol (10.80 min); injected sample volume 20 μ l.

Also GC was used for the separation of the above-mentioned TMS-tocopherols. The data obtained are shown in Fig. 3.

One of the current tendencies in chemical investigations is the prediction of physicochemical and biological properties of chemical compounds from their structural parameters. The fundamental finding of these investigations is the fact that the structure of

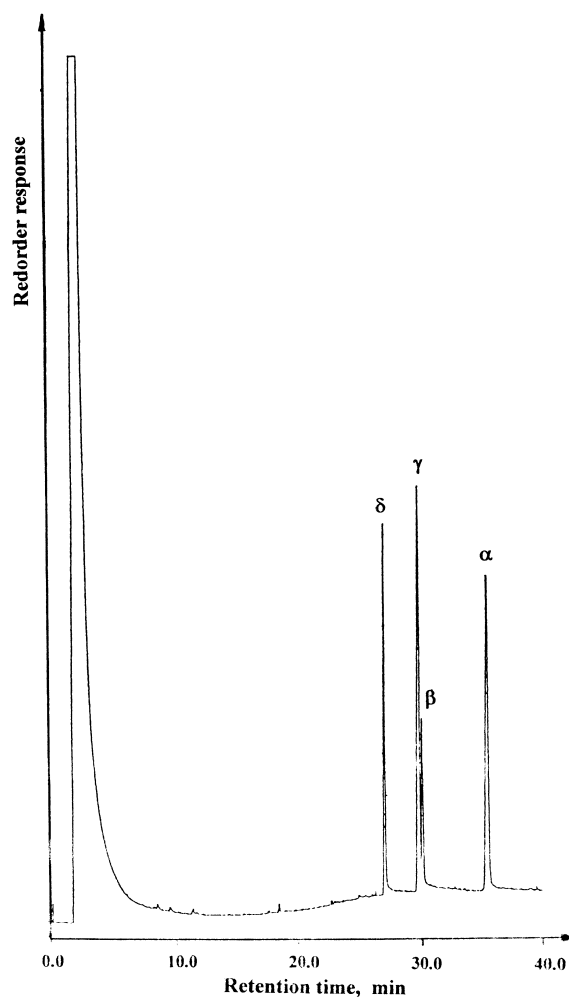


Fig. 3. GC chromatogram of a standard mixture of α , β , γ and δ -tocopherols as TMS derivatives: 20 ng TMS- δ -tocopherol (27.11 min), 20 ng TMS- γ -tocopherol (29.86 min), 15 ng TMS- β -tocopherol (30.11 min), and 20 ng TMS- α -tocopherol (35.40 min); injected sample volume 1.5 μ l.

a molecule determines its properties [23–26]. Only the quantum mechanics completely describes the structure of a molecule, characterizing its geometrical and electron structure. The topological indices, which descend from a graph theory, are the simplest way of a structural description of a molecule. The topological indices encode the structural information of a molecule. The topological index characterizes a molecule by a simple number [24,25]. In this connection, the selected topological indices were used to show the dependence among the numerical values of the topological indices and the chromatographic separation of the investigated tocopherols.

Presented in Table 3, the topological indexes clearly show that β - and γ -tocopherols as positional isomers possess the same numerical values of topological indices ${}^1\chi^v$, M , I_{AC} and \bar{I}_{AC} . However, the numerical values of the topological indices W and MTI for α -, γ -, β -, and δ -tocopherols, in the given sequence, grow smaller (decrease). The numerical values of the topological indices oB grow smaller (decrease) in following order: α -, β -, γ -, and δ -tocopherol. But, the difference between the numerical values of the topological index oB is very little for β - and γ -tocopherol. The definite dependence between the numerical values of topological index oB and the chromatographic separation of the investigated tocopherols was obtained. In the given sequence, retention times of tocopherols investigated by normal-phase HPLC grow; additionally, their retention times in GC grow smaller.

Simultaneously, attention was paid to the sum of the net electron charge on the compounds' $-C-O-H$ groups and to the steric effects which exist around the $-OH$ groups of the examined tocopherols [4].

Also the foregoing data correlate with the numerical values of topological index oB .

4. Conclusion

The chromatographic conditions which were applied in the NP-HPLC, C_{18} RP-HPTLC and GC can be used to separate α -, β -, γ -, and δ -tocopherols in various biological samples. Additionally, the structures of the particular investigated tocopherols are the best described by the topological index oB .

Further investigations concerning the physico-chemical significance of the topological index oB in connection with the chromatographic data are being continued. Future investigations will also be concentrated on research of a new structural parameter (topological index) which will better differentiate between the isomeric β - and γ -tocopherols.

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Table 3
Numerical values of topological indices for the tocopherols investigated

Compound	Topological indices						
	${}^1\chi^v$	M	W	oB	MTI	I_{AC} (bits)	\bar{I}_{AC} (bits)
α -Tocopherol	13.0432	252	3165.14	2.2070	15 433.13	88.45	1.092
β -Tocopherol	12.6205	244	2944.79	2.1769	14 554.13	85.58	1.097
γ -Tocopherol	12.6205	244	2945.96	2.1764	14 560.14	85.58	1.097
δ -Tocopherol	12.1979	236	2728.94	2.1479	13 692.47	82.70	1.103

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