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# Synthesis of the dl-ξ-Thiotocopherol: Newest Derivative of the dl-α-Tocopherol

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**Abstract:** Tocopherol is the most active vitamin and natural antioxidant existing in the nature known as vitamin E. Lacking of this vitamin makes drastic exchanges on the health of the living organisms. Their active chemical form is l-α-tocopherol substance. In this article, α-thiotocopherol a tocopherol derivative was synthesized via a precursor like dl-α-tocopherol, which has better antioxidant than natural α-tocopherol. And the last compound after separation and purification via TLC and PC procedures was analyzed by FTIR, GC-MS and elemental analysis, oxidative stability is tested with TGA method in air showing roughly antioxidant effect. Another approach is measurment of redox potential against a reference electrode under inert nitrogen atmosphere.

**Key words:** Tocopherol, phenols, substitute phenols, thiotocopherol, redox potential, thermal and oxidative stability.

## 1. Introduction

Vitamin E is a natural substance which has the cell protection function against biological toxic free radicals produced by body. This vitamin's chemical name is tocopherol, and observed tocopherols are four kind: α, β, γ, δ-tocopherols [1-4], main vitamin is l-α-tocopherol. Tocopherols are methyl substitued tocols. There are events for direct relation of vitamin activity with antioxidant effects [5-6]. This is one antidepressant buster's species and has protecting effect against cancer [7-8]. The free radicals cause the oxidative stress on living organisms. In literature it was seen that all tocopherols are derived from tocol, tocomonoenol, tocodienol and tocotrienols by substitiutions of the aromatic phenolic ringes with methyl groups. Antioxidant activity is a potential for scavenging of the free radicals. This fact can be measured experimentally or can be calculated theoretically or by semi theoretical approach [5, 9-11].

Redox potential is indicator for antioxidant activity, when it is diminishing to the more negative values, then reduction effect is increasing. Resonance formula of tocopherol explains the energetic stability of obtained tocopheryl radical after reaction of the tocopherol with free radicals.

ξ-thiotocopherol denotes that the true configuration of dl-thiotocopherol is yet unknown, instead of the symbol α the ζ(ksee) is useful for formulations.

## 2. Experiment

Sulfurisation reaction of the dl-α-tocopherol (Fig. 1a) was performed with Merck grade chemicals. Solvents for this synthesis are toluol or xylol. Pure red phosphorus was blended in a ceramic mortar with sulfur weighed on stochiometric ratio, after was heated

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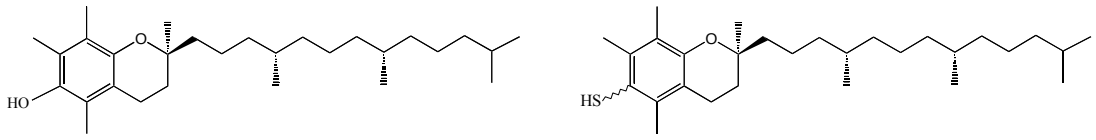



(a) (b)

**Fig. 1 (a) dl-α-tocopherol structural formula and (b) supposed formula for dl-ζ-thiotocopherol.**

in airless atmosphere, resulting reactive is $P_4S_{10}$, but here are some nonstochiometric phosphorous sulphides impurities. After fine milling, sulfurisation reactive is ready for utilisation.

1 mmol dl-α-tocopherol (0.4307 g, Merck) was solvated in xylene and mixed with 2 mmol $P_4S_{10}$ fine powder, during 48 h and continues mixing with a mixer, resulting product at room temperature is solvated from the dl-ξ-thiotocopherol [12-16] (Fig. 1b). Solution is filtering quickly with a fine filter paper, in the dark. The produced solution must be holding in dark closed bottle at room temperature for preventing autooxidations [9].

Ready standard TLC 20 × 20 cm glass plates with 0.20 mm silicagel film thickness are essayed, but plates preparated from us were more effective. Hydrated Silicagel $F_{254}$ (Camag) was spreaded on 20 × 20 cm glass plate with Camag applicator, obtained film is 0.5 mm thick. After drying of the plate at room temperature for 30 minutes, activation was made in a furnace at 120 $^oC$ for 60 minutes. Developing solutions for chromatography are: benzen (Bz)/MeOH (98/2 v/v); Bz/MeOH (100/4) and the best result is with Bz/MeOH (120/4 v/v) eluent. If there are trace water in pure solvents, cloudy like appearance of eluent has to remove with a part of anhydrous $Na_2SO_4$ salt.

With preparative TLC were obtained five bands, $R_1$-1.000, $R_2$-0.817(dl-ξ-thiotocopherol), $R_3$-0.692(dl-α-tocopherol), $R_4$-0.250 and $R_5$-0.000(impurities). Analysis of each TLC fractions was performed by GC-MS apparatus.

PC chromatography was performed with Whatman paper. This procedure is easy applicable, but with lower resolution. Solvent is $CH_2Cl_2$/n-hexane (100/100), bottom phase is $R_1$-0.878 (thiotocopherol) and upper is $R_2$-0.939 (dl-α-tocopherol), respectively [17].

Bands from TLC were extracted with diethyl ether, after evaporation of ether these fractions were acetylated directly with AcCl/pyridine reactive for 5 minutes. Resulted acetates were applied to the column [2, 18-21].

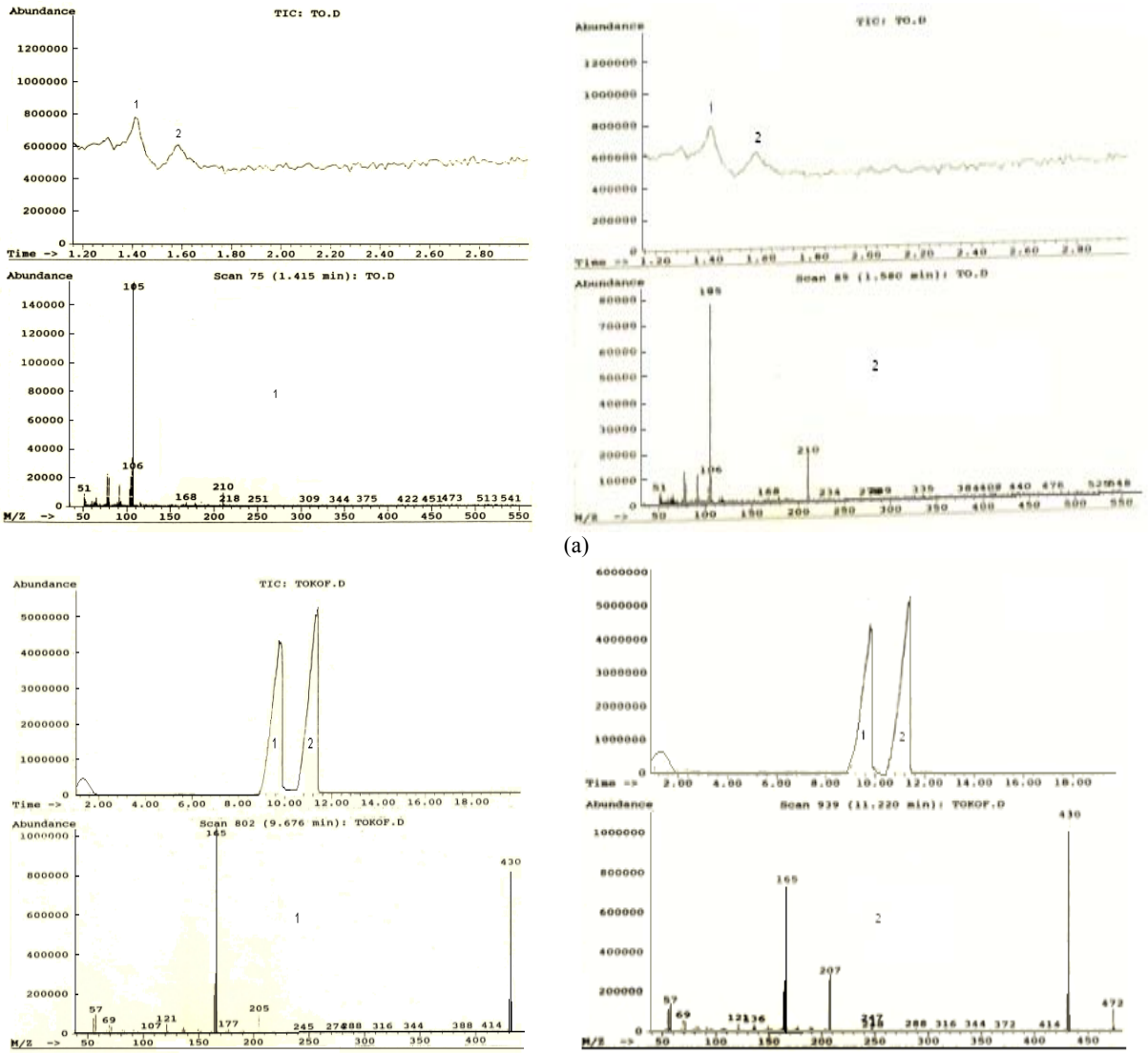
GC Chromatography analysis was set up with GC-MS 5890 Series II Hewlet Packard model apparatus. Working conditions are: Detector MS 5971 (280 $^oC$), column is cappilary 25 m long tubing, 1.9 mm ID and 0.33 μ film coatings with polymethyl silicone; injection block is at 240 $^oC$, carrier gas was nitrogen 1 mL/min. Essays were conducted in two conditions: thermal gradient of the column between 190-240 $^oC$ and isothermal at 240 $^oC$ temperature [2,18-22]. Data was analyzed via Chrom PC software program (Fig. 2).

FT-IR spectrums of the tocopherols were taken with Perkin Elmer BX50 spectrophotometer. Spectrums of α-tocopherol and ξ-thiotocopherol are given down in Fig. 3.

The elements analysis was completted by Leco CHNS-932 device connected to the VTF-900 pyrolysis furnace. Here is analysis of the thiotocopherol merely, because this is yet unknown structure. Measurment results are depicted in Table 1. Thiotocopherol is oil like liquid matter with sharp, pungent unpleasant odour and pale yellow color. Molecular weights are 446.779 g/mol for thiotocopherol and 430.71 g/mol for tocopherol.

TGA analysis of thiotocopherol and thiotocopherol acetate (some times are known with name TG) was carried out with Perkin Elmer Pyris 1 TGA & Spectrum 1 FT-IR apparatus. Scannings are between 20-500 $^oC$ with the speed of 20 $^oC$/min. Thiotocopherol and their acetate are insighted on the air atmosphere for some properties and the oxidative stabilities.

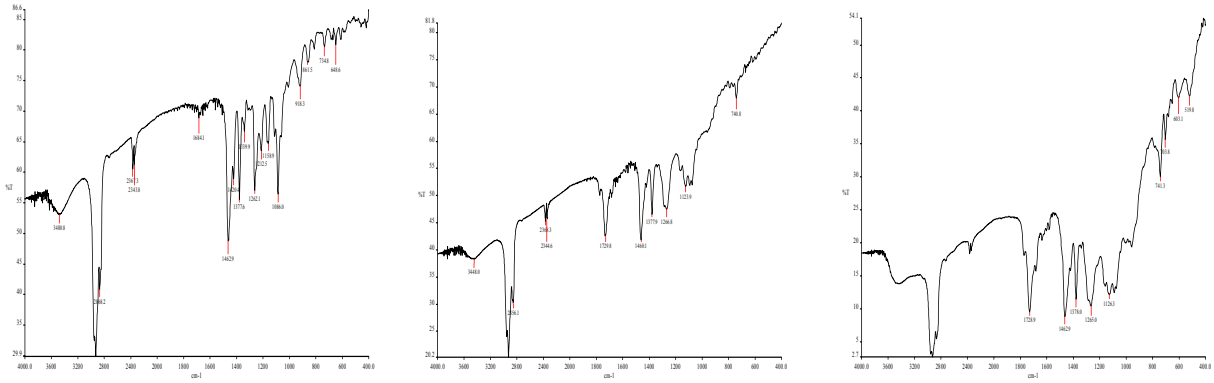
Melting points, thermal and oxidative degradation are understandable from thermograms and TGA is practical




(a)

(b)

**Fig. 2 GC-MS chromatographic separation and identification of some vitaminic fractions: (a) α-tocopherol (1) and thiotocopherol (2) acetates derivatives isothermal GC; (b) α-tocopherol (1) and thiotocopherol (2) acetates thermal gradients GC.**



(a) (b) (c)

**Fig. 3 FTIR spectra of (a) α-tocopherol, (b) ξ-thiotocopherol and (c) ξ-thiotocopherol acetate.**

**Table 1 Instrumental elements analysis for thiotocopherol, percentages are per weight.**

| Element | % C | % H | % N | % S | % O | Total |
|---|---|---|---|---|---|---|
| Measured | 77.29 | 10.84 | 0.00 | 7.91 | 3.95 | 99.99 |
| Theoretycal | 77.962 | 11.279 | 0.000 | 7.177 | 3.581 | 99.999 |

way for the antioxidants essays. Two thermograms are given in Fig. 4. TGA assisted by DTA and DSC is useful for structure determinations [22-24]. TGA and DSC simultaneous are applicable for the oil, the fat and the foods ingradients stability against deterioration. There are two mass variables: %W and derivative weight %ΔW.

Redox potential of thiotocopherol was measured in a beaker with a reference $Cu^{+2}$/Cu electrode and after adding of the 20 mL NaCl saturated absolute ethanol. Following solvatation of a few houndred mg of the thiotocopherol in alcohol solution, minutment it have to coat this beaker with stretch PE film and give nitrogen gas to the beaker for preventing aoutooxidations. All equipments were dipped in a thermostat bath with constant 25 $^{o}$C temperature.

Measurement was carried out after 20 minutes for reach the temperature steady-state case. Read out of the potential is with calibrated and precise Brymen 807 multimeter, the electrocell result is ΔE = 204.8 mV [25-26]. Constructed cell formula is $CuSO_4$ sat.aq./Cu//NaCl, EtOH//Cu,HS-T($N_2$). Predicted reactions for this cell are formulated down.

2 $Cu^{2+}$ + 2$e^-$ → $Cu_2^{2+}$ Reduction Half Cell Reaction (1)

2 HS-T – 2$e^-$ → 2 T-S Oxidation Half Cell Reaction (2)

## 3. Results and Discussion

Synthesized thiotocopherol structure was enlighted by spectroscopic methods; the elements percent is supported for the results. GC-MS chromatogram shows that substance is appropriate to the proposed formula. Synthesis of this substance must be over nucleophilic substitution mechanism, there is a replacment of the phenolic -OH group with HS- one.

Sample 1 and sample 2 were identified to be α-tocopherol and ξ-tocopherol. m/q = 205 is M+ radical cation and radical S. and m/q = 165 is mean peak for S+. [2, 16, 20-22], for the tocopherol basic peak is m/q = 165. In thiotocopherol GC-MS mass spectrum, the peak m/q = 64 is by the sulfure from the thiophenol structure (Fig. 2).

FTIR peak at 2962 cm-1 (sharp) is assymetric and symmetric -CH3 and C-H bond strenght. Between 900-600 cm-1 here is meaningful difference of both spectrums of the tocopherol and thiotocopherol, again in this region here can see the typical -SH bond peaks. The shapes of the hydrogen bond vibrations for two species are different.

Among 1600-2000 $cm^{-1}$ and 950-650 (fingerpring)

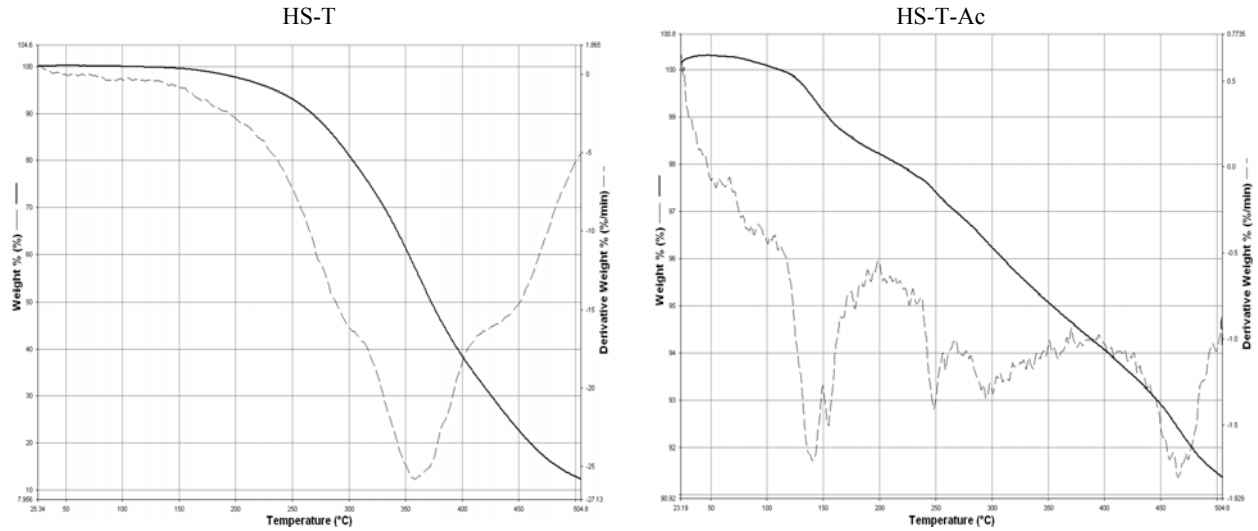



**Fig. 4 Thermograms of the thiotocopherol (HS-T) and thiotocopherol acetate (HS-T-Ac).**

$cm^{-1}$ regionprofile here is the same of the hexasubstitued benzene ring, this is the single aromatic ring of the tocopherol molecule. The peak at 1462 $cm^{-1}$ (sharp) and at 3040 $cm^{-1}$ (sharp) show aromatic C-H bending. Peak at 1460.1 $cm^{-1}$ is the cause of the aromaticity.

From the thermograms the melting point of HS-T can't distinct, because this is a liquid compound; for the deviation at $\%\Delta W = 360\ ^oC$ we can say there is an intense oxidation. It is clear that HS-Toc is stable in air maximum at 150 $^oC$, after here is rapid decrease in weight. The thermogram of HS-T-Ac is more complicated. This substance is solid, their melting point is aproximatelly 120-130 $^oC$, following the melting point here is the first deviation in $\%\Delta W$, this mean here is a thermal degradation. Acetate derivative is seen to be less stable than thiotocopherol.

## 4. Conclusions

The thiotocopherol's vitaminic activity is an open area for research. However, due to α-tocopherol's microsomes redox activation and direct antioxidant protection effect for cell wall, it is expecting similar effect for the thiotocopherol.

Redox potential isn't single fact, but cytological and tissue culture studies about this substance will give us more knowledge.

Antioxidant activity and vitaminic activity have to be compared with in vivo and in vitro studies. Again there is need for detailed destruction products and the fractions analysis, after chemical oxidations of thiotocopherol and thiotocopherol acetates.

Our research group expects the possible anticancerogen effectivity from this chemical, but before it have to research toxicity and tissue tolerance for this potential "vitamin".

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