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Relationship between structure and anti-oxidation of tocopherol with molecular orbit theory[1]

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KEY WORDS vitamin E; antioxidants; free radicals; lipid peroxides; molecular models; structure-activity relationship

AIM: To explore the relationship between different structures of tocopherol (Toc) and some phenol compounds and their anti-oxidative activities. **METHODS:** Use the *ab initio* calculation of molecular mechanics and quantum chemistry. **RESULTS:** The anti-oxidation of Toc was related to the ability to release active hydrogen, ie, related to the O-H electron populations, frontier orbital energy (au), and the decreased amount of energy at the reaction ending stage. The order of hydroxyl O-H electron populations in different Toc model molecules were $\alpha < \gamma \leqslant \beta < \delta$, which was consistent with their anti-oxidation reported. **CONCULSION:** The molecular orbit (MO) theory and the quantum chemical parameters can be used to analyze the anti-oxidation of phenol compounds with different structures.

There are two classes of vitamin E, ie, tocopherol and tocotrienol. The tocopherol (Toc) can be classified into α-, β-, γ-, and δ-tocopherol based on their structures. The α-tocopherol is the strongest in bioactivity and is commonly called vitamin E. Besides its action of promoting reproductive ability[1], Toc has the functions of anti-aging, preventing and inhibiting cancer[2,3]. Its important function of anti-oxidation, which can be used to prevent or decrease the injury induced by the lipid peroxidation, was discovered with the development of free radical biology and medicine[4-6]. So Toc has been widely used in the clinic and animal or cellular experiments as an antioxidant. It was the phenol structure to inhibit the lipid peroxidation, ie, it can release the active hydrogen in the hydroxyl group to combine the free radicals so that it can inhibit their attack on the lipid. The lipid peroxidation is a chain reaction induced by free radical, which can be stopped when the free radical was cleaned.

$$L_1H \xrightarrow[h\nu]{\text{Free radical}} L_1^{\cdot} + H^{\cdot} \xrightarrow{O_2} L_1OO^{\cdot} \xrightarrow{L_2H}$$

$$L_1OOH + L_2^{\cdot} \xrightarrow{O_2} \xrightarrow{L_3H} \cdots\cdots \rightarrow \sum_{1-\infty}^{n} LnOOH + Ln^{\cdot}$$

The production of free radical in small amount ($L^{\cdot}$) can lead to the continuous oxidation and consumption of lipid on the membrane, and result in a lot of lipid peroxides (LOOH). The membrane was seriously injured. If there is Toc interfering in, such chain reaction can be stopped.

$$LOO^{\cdot} \xrightarrow{\text{Tocopherol}} LOOH + T^{*}$$

T^{*} is a more stable tocopherol free radical, which makes the reaction carry on to the product. A part of Toc can be transferred into the quinone structure in the model of membrane lipid peroxidation[7], which may be through the process in which the bond between $C_{19} - O_{14}$ was broken in the chromanoxy ring and combined with the $\cdot OH$ to form the quinone structure.

To examine the above reaction process of Toc from the middle stage of tocopherol free radical T^{*} to the quinone structure and explore if different numbers of the methyl group on the ring near the hydroxyl group would affect the release of the active hydrogen and their anti-oxidation, related molecular models were designed and the accurate quantum chemical calculation was

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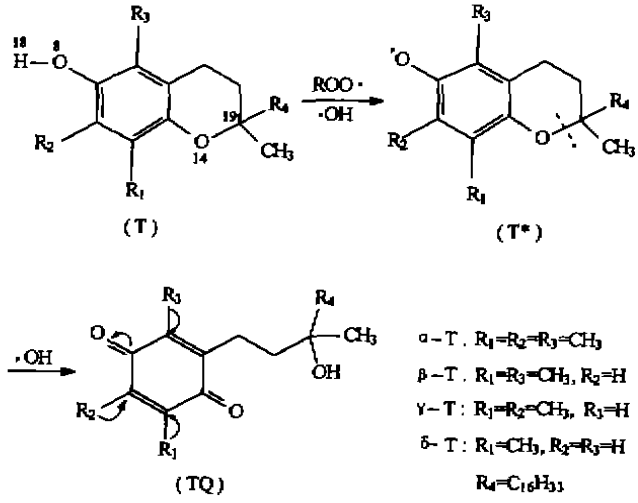

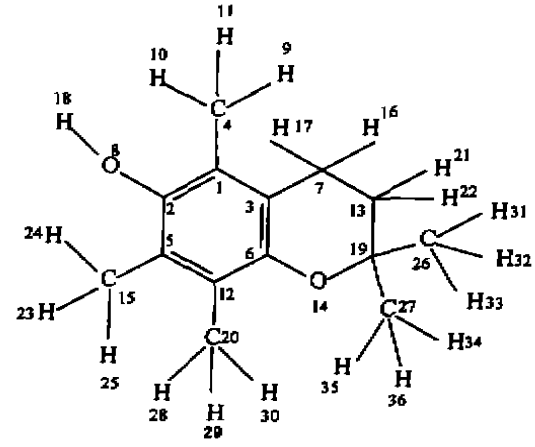

Fig 1. The atom number in Toc model molecules.

performed. The technique of electron spin resonance spectroscopy (ESR) has been used to detect the exist of Toc free radicals in the middle stage.

METHODS

Calculation proceeding The SYBYL/MAIMIN2 molecular mechanical proceeding was used to calculate the optimal structure[8]. If there was rotable bond, the SEARCH proceeding was used to search the related conformation, the search scope being 0 – 360° and the length of step being 10°. When the one with the lowest energy was found, it was calculated with the AM1 semi-experienced quantum chemical optimum calculation[9]. The molecular models were then calculated for the electron structure with the Gaussian 94 proceeding, *ab initio* STO-3G[10]. All the calculations were performed on the SGI computer working station in the State Key Laboratory of New Drug Research of Shanghai Institute of Materia Medica, Chinese Academy of Sciences.

Model molecules α-mT, β-mT, γ-mT, δ-mT: α, β, γ, δ- four different kinds of Toc model molecules with methyl group instead of the side chain on the original molecules. α-mT*, β-mT*, γ-mT*, δ-mT*: Toc free radical model molecules after dehydrogenation. α-mTQ, β-mTQ, γ-mTQ, δ-mTQ: Toc quinone model molecules. The atom number in Toc model molecules was shown in Fig 1.

ESR determination The determination was performed as the reference[11,12] with the improved reaction system and the determining condition for the Toc free radicals. Reaction system: α-Toc 0.1 mol·L^{-1}, $CuSO_4$ 0.5 mmol·L^{-1}, 1 % H_2O_2, 95 % alcohol (AR) as the solvent. Determining condition: microwave frequency (SF): 9.81 GHz; modulating frequency (MF): 100 kHz; modulating amplitude (MA): 0.1 mT; microwave power (SP): 20 mW, sweeping width (SW): 10 mT, room temperature: 25 ℃. Apparatus: Bruker ER200D-SRC electron spin resonance.

RESULTS

After the α, β, γ, and δ-Toc molecular models were calculated by the molecular mechanics and quantum chemistry to obtain the conformation with the lowest energy, the electron structure of each molecule was accurately calculated by the *ab initio* STO-3G. The results were shown in Tab 1.

The energy of frontier orbital HOMO in mT model molecules was decreased step by step from α-mT to δ-mT with *ab initio* STO-3G, while the energy difference with LUMO was increased gradually, consistent with the determining result of the T → T* reaction rate constant reported[13], in which the chemical activity of α-mT was the strongest. On the other side, the H_{18} net positive charges in the hydroxyl group were all the biggest in their Toc molecules, the order among them being α-mT > β-mT > γ-mT > δ-mT. Moreover, the PO data between O_8 – H_{18} were smaller, the order being $\alpha < \gamma \leqslant \beta < \delta$. The small PO data means the O-H bond was

Tab 1. Total energies (E), frontier orbital (HOMO, LUMO) energies (au), atomic net charge (O_8, H_{18}) and O_8-H_{18} electron populations (PO) of tocopherol.

	E	STO-3G HOMO	LUMO	O_8	H_{18}	$O_8 - H_{18}$(PO)
α-mT	-683.0867	-0.2106 (0.4789)*	0.2683	-0.2872	0.1945	0.2611
β-mT	-644.5088	-0.2117 (0.4806)*	0.2689	-0.2819	0.1927	0.2636
γ-mT	-644.5085	-0.2136 (0.4816)*	0.2680	-0.2812	0.1924	0.2635
δ-mT	-605.9263	-0.2158 (0.4843)*	0.2685	-0.2774	0.1923	0.2672

*$\triangle E_{HL} = E_{LUMO} - E_{HOMO}$

easily broken to release the hydrogen. The O-H bond in α-mT was the weakest, meaning that it was the easiest one to release the active hydrogen. When the free radical was combined with the active hydrogen, it would be cleaned (Tab 1). When Toc was turned into the mT* after dehydrogen ation, the system energy in mT* was higher than that in the original molecule, but the increased amount ΔE_{mT^*-mT}, including the energy in the active hydrogen (E_H = 0.466 hartree) was not very big, meaning that the system was still stable after dehydrogenation. The increased amount for α-mT was the smallest one, then the β-, γ-, and δ-mT (Tab 2).

Both of the mT* and mTQ, the main constitutes in the frontier orbital were the Pz orbit formed by the carbon atom on the benzene ring and two oxygen atoms (Tab 2). Their dihedral angle data (177° - 180°) showed that they were on the same plane basically and the electron in the Pz atomic orbit can produce conjugation by dispersion, beneficial to stable the system, ensuring the reaction to be carried on to the direction of forming the mT* and mTQ. Although the ΔE_{mT^*-mT} increased a little when the model Toc molecule was turned into the mT* after dehydrogenation, the energy in the ending stage would decrease greatly when they were turned into the mTQ. The order of their decreased amount was $\alpha > \gamma \geqslant \beta > \delta$. The molecular structure of mTQ has only one oxygen atom more than that of mT, in which the energy of the oxygen atom E_O was 73.8042 hartree after calculation. The general energy in mTQ, E_{mTQ} had only a little more than the addition of the energy in mT and the oxygen atom, the increassed amount of α-mTQ being the smallest one among them.

It was shown in Tab 2 that HOMO energy decreased from α-mT* to δ-mT* when Toc was turned into the mT* after dehydrogenation, meaning that chemical activity in α-mT* was the strongest one among them. The electron populations PO in $C_{19} - O_{14}$ of mT* were smaller than that in $C_6 - O_{14}$, showing that the $C_{19} - O_{14}$ bond being weaker comparatively. It would be possible that the $C_{19} - O_{14}$ bond was induced to break when the oxygen free radical in the system

Tab 2. Total energies (E), frontier orbital (HOMO, LUMO) energies (au), constitutes ($C_{benzene-ring}$, O_1), C-O electron populations and C_{19} net charges of tocopherol-radicals and quinone model with *ab initio* STO-3G.

	E	HOMO	LUMO	$\triangle E_{HL}$	Constitutes	$C_{19} - O_{14}$	$C_6 - O_{14}$	C_{19}
α-mT*	-682.5411 (0.0796)†	-0.2500	0.2748	0.5256	2Pz (C_B, O_8, O_{14})	0.2592	0.2791	0.1610
β-mT*	-643.9578 (0.0850)†	-0.2533	0.2730	0.5263	2Pz (C_B, O_8, O_{14})	0.2589	0.2790	0.1606
γ-mT*	-643.9575 (0.0850)†	-0.2545	0.2733	0.5278	2Pz (C_B, O_8, O_{14})	0.2591	0.2795	0.1609
δ-mT*	-605.3740 (0.0863)†	-0.2571	0.2712	0.5283	2Pz (C_B, O_8, O_{14})	0.2588	0.2794	0.1605
α-mTQ	-756.8451 (0.0458)#	-0.2777	0.1603	0.4381	2Pz (C_B, O_8, O_{14})			
β-mTQ	-718.2615 (0.0515)#	-0.2821	0.1583	0.4404	2Pz (C_B, O_8, O_{14})			
γ-mTQ	-718.2619 (0.0508)#	-0.2833	0.1575	0.4407	2Pz (C_B, O_8, O_{14})			
δ-mTQ	-679.6782 (0.0523)#	-0.2904	0.1555	0.4458	2Pz (C_B, O_8, O_{14})			

†$\triangle E_{MT^*-MT} = E_{MT^*} - (E_{MT} - E_H)$; #$\triangle E_{MTQ-MT} = E_{MTQ} - (E_{MT} + E_O)$

was accumulated to a certain concentration, and then it would combine with C_{19} to form the mTQ (Tab 3).

The main difference of the four Toc in their structures was the different numbers of the methyl on the ring. It was shown from Tab 3 that the electron charge on the methyl group would be changed with the increase of the methyl number, and it would produce $\delta - \pi$ super-conjugation effect by connecting with carbon atom in benzene ring due to the inducing effect of methyl group. When forming the mT* and mTQ structure, a part of the electron on the methyl would be transferred into inside of the ring, which made the electron cloud density in the conjugation system increased including that on the O_8. As the α-mT has three methyl groups which produce stronger inducing effect, it made the electron cloud density on the conjugation system and O_8 increased so much when forming the mTQ that the system became more stable with lower general energy. The δ-mT had only one methyl so that it produced weaker inducing effect and weaker conjugation effect than other three Toc.

It was found that there was particular absorption signal of free radical for α-Toc in the H_2O_2 and Cu^{2+} reaction system by ESR determination, which presented in hepto-splitted peak with binomial ratio (Fig 2).

This signal was declined to half in 10 min, showing that it was a stabler free radical. Based on the calculation for the α-mT*, the hepto-splitted peak was analyzed for the distribution of the electron spin densities (C_1 0.8931, C_2 −0.8664, C_3 −0.8365, C_5 0.8854, C_6 0.8018,

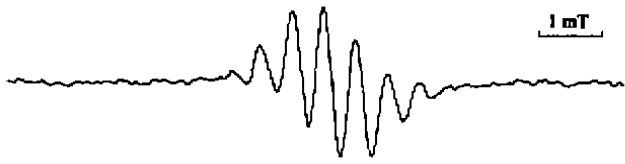

Fig 2. ESR spectra of α-tocopherol radical.

C_{12} −0.8473, O_8 0.9679), that in O_8 being the biggest (0.9679), then the C_1 (0.8931) and C_5 (0.8854). And the six protons in the R_2 and R_3 methyl groups were near the same because the R_2 and R_3 were on the both side of O_8 and the spin density on C_1 and C_5 were near the same. If determined by ESR, it should have seven lines on the spectrum according to the role of (2nI + 1), which was consistent with the result of practical determination. Although there were electron spin densities on the C_{12}, C_3, and C_6, there was no hydrogen atom nearby or with opposite spin direction for O_8, so that the ESR particular spectrum was not affected.

DISCUSSION

Tocs are phenol compounds, of which the anti-oxidation is related to the phenol hydroxyl number contained. Although Toc contains only one phenol hydroxyl group, the electron inducing effects by the methyl group on the hydroxyl group are not the same due to their different numbers and position, presenting on their different electron distribution, the different electron populations and the ability to release active hydrogen. After dehydrogenation, the Toc was turned into mT* and the electron might move to

Tab 3. The atom or group charge (O_8, H_{18}, R_1, R_2, R_3) and total offer electron of methyl ($\Sigma R = R_1 + R_2 + R_3$) in tocopherol and quinone model.

	O_8	H_{18}	R_1 (C_{20}, H_{28}, H_{29}, H_{30})	R_2 (C_{15}, H_{23}, H_{24}, H_{25})	R_3 (C_4, H_9, H_{10}, H_{11})	ΣR
α-mT	−0.2872	0.1945	0.0182	0.0186	−0.0004	0.0365
β-mT	−0.2819	0.1927	0.0223	−	0.0175	0.0399
γ-mT	−0.2813	0.1924	0.0199	0.0194	−	0.0393
δ-mT	−0.2774	0.1924	0.0252	−	−	0.0252
α-mTQ	−0.2197		0.0273	0.0279	0.0320	0.0872 (0.0507)*
β-mTQ	−0.2157		0.0355	−	0.0326	0.0681 (0.0283)*
γ-mTQ	−0.2154		0.0274	0.0287	−	0.0561 (0.0168)*
δ-mTQ	−0.2112		0.0356	−	−	0.0356 (0.0104)*

*$\triangle \Sigma R_{MTQ-MT}$

O_8, increasing the dispersing area due to the $\delta - \pi$ super conjugation by the methyl group, which was helpful to stabilize the system. α-Toc has three methyl groups, offering more electron cloud, so that the system became more stable, presenting that less active energy was needed to form the mT^* and the dehydrogenation was easily carried on. The mT^* was only present in the middle stage. It was more important that the $O_{14} - C_{19}$ bond on the chromanoxyl of the mT^* was weakened and easily broken due to the induction of methyl's. Moreover, the system energy had no obvious change after the bond broken by calculation, but the system energy was decreased greatly when forming mTQ on C_{19} with ·OH, offering the stable energy to the system. The stable energy represents the easiness to complete the anti-oxidation finally. The order of the stable energy for the four Toc molecules was $\alpha > \gamma \geqslant \beta > \delta$, consistent with their anti-oxidation reported in the reference[14]. This means that it was reasonable to use the calculating model with the methyl group instead of the side chain and the step by step comprehensive analysis. By the analysis of the quantum chemical parameters combined with the ESR determination, there were two possible mechanisms for the anti-oxidation of Toc: firstly, it can release the active hydrogen to combine the ROO· free radicals, then inhibit the chain reaction of the lipid peroxidation; secondly, the O-C bond of the chromanoxyl ring in mT^* may be broken to combine the ·OH and make them cleaned directly. The anti-oxidation of Toc was obviously related to the above two mechanisms or related to their molecular structures.

In order to explore the relationship between the antioxidation and the structure for other phenol compounds, three water soluble active ingredients extracted from *Salvia miltiorrhiza* Bunge were calculated with the quantum chemistry. It was proven by the experiments that the order of their antioxidation was salvianolic acid A > salvianolic acid B > rosmarinic acid, which have 7, 9, 5, phenol hydroxyl groups respectively. Their structures were shown in Fig 3.

It was difficult to explain why the activity of salvianolic acid A was stronger than salvianolic acid B if only according to the numbers of the

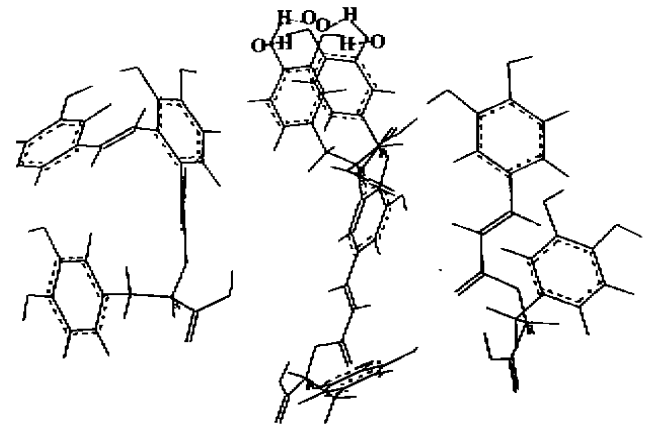

Fig 3. The space structures of salvianolic acid A, salvianolic acid B and rosmarinic acid.

phenol hydroxyl group for the former having seven while the later having nine. The quantum chemical calculation and the Conformation Search proceeding (the searched conformations for each molecule were more than 1.5×10^5) were used to find the one with the lowest energy, then calculated by the optimum accurate quantum chemistry. It was found that the phenol hydroxyl groups in these three molecules were the active groups with bigger positive charge on the H, but four groups in salvianolic acid B were very close in the space which may react each other to form the hydrogen bond, then the ability to release active hydrogen was decreased. So the antioxidation of salvianolic acid B was weaker than salvianolic acid A (it will be reported in another paper). It has been proven that the antioxidation of phenol compounds are not only related to the quantum chemical parameters such as: the electron distribution, electron populations, general energy, frontier orbital, etc, but related to their space structures as well.

The above results showed that the calculations with molecular orbital theory and electron structure are helpful to analyze the antioxidation of Toc with different structures and other phenol compounds, offering worthy information for designing or screening the effective antioxidants and medicine.

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应用分子轨道理论研究生育酚的结构与抗氧化活性的关系[1]

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关键词 维生素 E；抗氧化剂；自由基；脂质过氧化物；分子模型；结构-活性关系

目的：探索不同分子结构的生育酚和一些酚类化合物所具抗氧化作用的构效关系．**方法**：采用分子力学和量子化学从头计算方法，研究分析不同分子的电子结构与抗氧化活性关系．**结果**：生育酚的抗氧化活性与易释放活泼氢有关，活性大小与 O-H 间电子集居数、前线轨道能级及反应终态能量下降值有关，各种生育酚模型分子的羟基 O-H 间电子集居数排列顺序 $\alpha < \gamma \leqslant \beta < \delta$，与文献报道抗氧化活性的结论相一致．**结论**：应用分子轨道理论和量子化学指数可以帮助分析具有不同结构的酚类化合物的抗氧化活性．