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Scavenging effect of catechin on free radicals studied by molecular orbital calculation[1]

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ABSTRACT The eigenvectors, net charges, and electron population on catechin were calculated with molecular orbital method MNDO. It was found that the 2 hydroxyls on the benzene ring of the chromane were more active than the others on the other benzene ring when it reacted with a free radical.

KEY WORDS catechin; antioxidants; free radical scavengers; molecular structure

Catechins and green tea polyphenols scavenged O_2^- by about 74% and 72% respectively in irradiated riboflavin / EDTA system and scavenged almost all oxygen radicals produced from respiratory burst of polymorphonuclear leukocytes stimulated by phorbol myristate acetate, which indicated that they were effective scavengers of free radicals[1]. The water extracts of green tea and the antioxidants showed a strong inhibitory effect on the backward mutation induced by aflatoxin B_1 (AFB_1) and benzo(a)pyrene (BaP) in Salmonella typhimurium. The tea antioxidants inhibited gene forward mutation in V_{79} cells treated with AFB_1 and BaP, and also decreased the frequency of sister chromatid exchanges and chromsome aberration in V_{79} cells treated with AFB_1[2]. The main components of the water extracts of green tea are catechins. There has been no report about the molecular mechanism of scavenging free radicals by catechins in the literatures. In order to study the scavenging mechanism for oxygen radicals by catechins, the eigenvectors, net charges, electron mulliken population and other parameters of the atoms of the catechin molecules were calculated by MNDO method. The attack points on catechins by oxygen free radicals were suggested. The results were compared with that of molecular vitamin E, a well-known scavenger of free radicals *in vivo*. The results may be useful for selecting more effective scavengers of free radicals by theoretical method.

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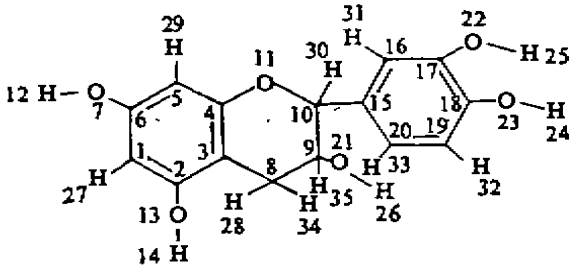
METHODS

The primary reasonable configurations of catechin and vitamin E were determined by configuration optimization with a molecular mechanical program MMP_2. Then the orbital parameters were calculated with MNDO method. Except some dissociation potentials of valance states, the primary atomic parameters were remained in the calculation and the potentials came from some special data means. The data were 13.6 ev for H_{1s}; 20.8 ev for C_{2s}; 11.3 ev for C_{2p}; 26.5 ev for N_{2s}; 13.6 ev for N_{2s}; 33.0 ev for O_{2s}; 16.2 ev for O_{2p}.

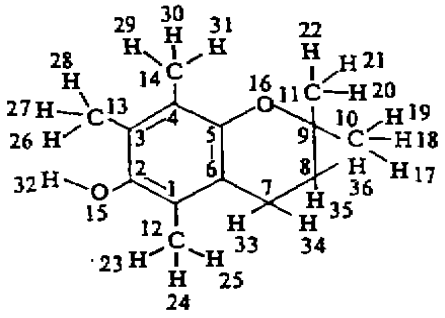
When the molecules of catechin and vitamin E were calculated, the parameters of 35 and 36 atoms were input respectively. The atom numbers of catechin and vitamin E were shown in Fig 1.

RESULTS

The optimal conformation of catechin in water solution The optimal conformation of catechin in water solution, the bond lengths and angles between the atoms from the calculation were shown in Fig 2 and Fig 3. The



Catechin



Vitamin E

Fig 1. Atom numbers of catechin and vitamin E.

chromane ring was located on the xy plane and the other benzene ring was perpendicular to this plane and located on z direction.

Structural characteristics of catechin deduced from eigenvectors of highest occupied orbitals The highest occupied orbital is the main taking part in the chemical reaction. The structural characteristic of a molecule can be found from the distributions of different eigenvectors. The electrons of S, Px, and Py orbital ussualy form σ bond and the electrons of Pz form π bond. The electron was mainly in Pz orbitals from the carbon atoms of benzene in the chromane plane and the oxygen atoms 7, 11, and 13 (Tab 1), meaning that the benzene ring, together with the 2 oxygen

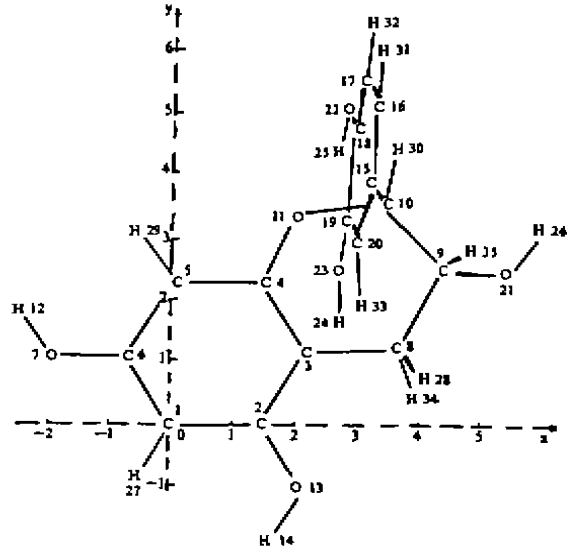


Fig 2. Optimal conformation of catechin.

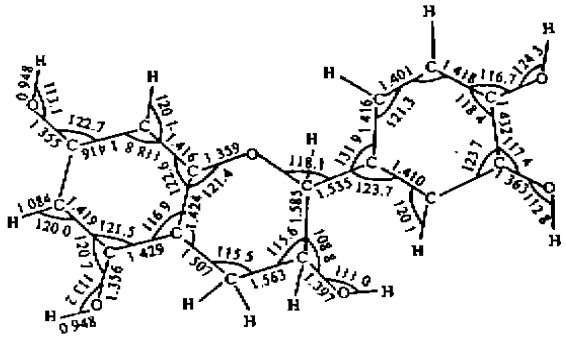


Fig 3. Bond lengths and angles between atoms of catechin.

atoms of hydroxyl groups connecting to it and the oxygen atom in pyrane ring formed a big conjugative system. The 2 hydroxyl groups were not a real hydroxyl but a middle form between a hydroxyl and a quinone. The oxygen atom in pyrane ring enlarged this big conjugative system and increased the contribution of quinone. The electrons were delocated on this big conjugative system. The other benzene ring was perpendicular to the

chromane plane, so the electron of Px on the carbon 15, 16, 17, 18, 19, 20 and oxygen atoms 22 and 23 participate to form a big bond (Tab 1) but smaller than that on chromane. These 2 hydroxyl groups also had some components of quinone. But the electrons on the oxygen atom of hydroxyl groups in pyrane ring (atom 21 in Tab 1) did not have this character.

Structural characteristics of vitamin E deduced from the same method The eigenvectors in carbon atoms and oxygen atoms of hydroxyl group of benzene ring of vitamin E calculated with the same method were shown in Tab 1. The eigenvectors of the highest occupied orbital of vitamin E had almost the same distribution as that of the catechin, eg. The distributions on Pz were much more than on other orbitals, meaning that vitamin E also existed in a middle form between hydroxyl benzen and quinone.

Electron mulliken population and net charge differences between oxygen and hydrogen atoms of hydroxyls on benzene ring of the chromane and the other benzene ring The electron mulliken populations between oxygen and hydrogen atoms of the hydroxyls on the other benzene ring were larger than those on the benzene of the chromane (Tab 2), suggesting that the bonds of O–H of the hydroxyls on the other benzene ring were stronger than that on the benzene of the chromane. Because this population indicated the bond strength between the 2 atoms. This also meant that the H atoms of the hydroxyls on the benzene ring of the chromane were easier to leave when it reacted with a oxygen free radical. The net charge differences between the oxygen and hydrogen atoms of hydroxyls on the other benzene ring were also larger than that of the benzene ring on the chromane plane (Tab 2), which supported the above conclusion.

Tab 1. Coefficients of molecular orbital of catechin and vitamin E.

No of atom	Symbol	S	Px	Py	Pz
Catechin					
1	C	−0.00029	0.00009	0.00039	0.12063
2	C	−0.00018	0.00009	0.00015	0.01421
3	C	−0.00102	−0.00284	0.00001	−0.10488
4	C	0.00221	0.00659	0.00053	−0.08198
5	C	−0.00368	−0.00883	−0.00240	−0.04582
6	C	0.00194	0.00217	0.00381	0.05449
7	O	0.00020	−0.00285	−0.00214	−0.03952
8	C	−0.00291	0.00129	−0.00883	0.01886
9	C	0.01119	−0.02382	0.03280	−0.02099
10	C	0.00860	0.01739	−0.01257	−0.00069
11	O	−0.00487	−0.03313	0.01434	0.10626
13	O	0.00002	−0.00009	−0.00012	−0.01072
15	C	0.00194	−0.42309	0.03703	0.04854
16	C	−0.00007	−0.33721	0.02488	0.02885
17	C	0.00082	0.15590	−0.01278	−0.01205
18	C	0.00068	0.44988	−0.04104	−0.03814
19	C	0.00159	0.45048	−0.04082	−0.03424
20	C	−0.00358	0.04572	−0.00625	−0.00692
21	O	0.00327	0.00749	−0.00814	0.00956
22	O	0.00103	−0.33750	0.02676	0.02757
23	O	0.00344	−0.30055	0.01730	0.02950
Vitamin E					
1	C	−0.00506	−0.00965	0.00475	−0.32464
2	C	0.00413	0.00769	−0.00803	−0.45681
3	C	0.00273	−0.01359	0.00515	−0.23705
4	C	−0.01054	0.02080	−0.00718	0.31951
5	C	0.01220	0.01057	−0.00004	0.24776
15	O	−0.00062	0.00032	0.00180	0.25095

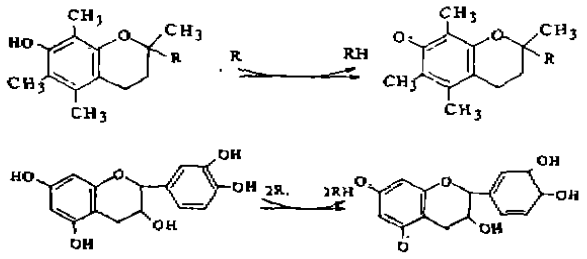
Tab 2. Eelectron mulliken populations (MP) and the net charge differences (ΔE) between oxygen and hydrogen atoms of hydroxyls on benzene ring of chromane and other bezene ring.

	Chromane		Benzene	
O	O7	O13	O22	O23
H	H12	H14	H25	H24
MP	0.870686	0.871558	0.874609	0.874587
E	0.4443	0.4471	0.4554	0.4684

DISCUSSION

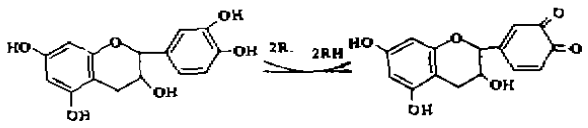
From above results it can be found that with regard to the benzene ring of the

chromane plane, both catechin and vitamin E are almost the same structure, eg. Both are in a middle forms between hydroxyl benzene and quinone, which is easy to react with free radicals to form quinone free radicals. When vitamin E reacts with a free radical it will form a quinone free radical[3]. So we suggest that, when catechin reacts with a free radical, it also forms a quinone free radical on its benzene ring of chromane plane and maybe on other benzene ring. So that one catechin molecular can react with more than one free radical molecular. This conclusion is consistent with the result of our experiment that the scavenging effect of catechin on O_2^- is larger than that of vitamin E[1].

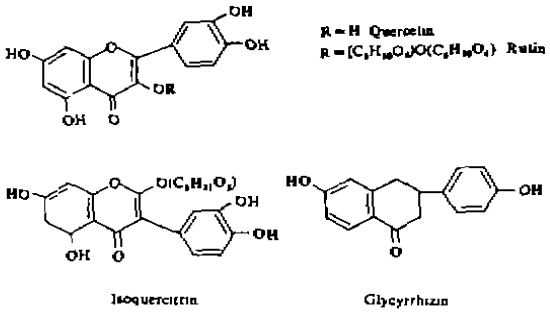


It has been regarded that, when catechin reacts with free radicals, only two hydroxyl groups on the other benzene ring are included without using the hydroxyls on the benzene of the chromane. Our results show that the electron mulliken populations and the net charge differences between the oxygen and hydrogen atoms of hydroxyl groups on the other benzene ring are larger than those on the benzene ring of the chromane plane and the conjugative system on the benzene ring of the chromane plane is bigger than that of other benzene ring. So when they react with oxygen free radicals, the first attacked points should be at the two hydroxyl groups on the benzene ring of chromane plane.

We have also studied rutin, querectin, isoquerectin, and glycyrrhizin, which structures similar to catechin. It was found that



the scavenging effects of these traditional chinese medicines on O_2^- were also very strong[4,5], confirming our above suggestion. The structures of these medicines are:



The method of DMNO used in this work is an approximate one. A more accurate method will be used for further calculation to prove our above result and to study more precisely on the nature of this kind of antioxidants.

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用分子轨道计算方法研究儿茶素对自由基的清除作用

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提要 本文用分子轨道法计算了儿茶素分子各原子的本征向量、净电荷和电子分布等。发现当儿茶素与自由基反应时，苯并吡喃平面苯环上两个羟基比另一个苯环上的两个羟基活泼。

关键词 儿茶素；抗氧化剂；自由基清除剂；分子结构

清除作用，

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Effect of cimetidine on isolated rat myocardial reperfusion injury[1]

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ABSTRACT The effects of cimetidine (Cim) on ventricular fibrillation threshold (VFT), diastolic excitation threshold (DET), effective refractory period (ERP), and vulnerable period (VP), were determined in both stable perfusion and postischemic reperfusion rat hearts. The results showed that reperfusion after 15 min global myocardial ischemia caused a significant decrease VFT and ERP, and an increase in VP and DET. Cim 1 mmol · L^{-1} prevented the lowering in VFT, shortening in ERP, and lengthening in VP from the postischemic reperfusion. Cim 0.1 mmol · L^{-1} attenuated the exacerbation of VFT and VP. Cim 0.01 mmol · L^{-1} did not exert any noticeable influence on the electrophysiological parameters. It was shown that Cim 1 mmol · L^{-1} protected myocardium against the aggravation of electrophysiological characteristics induced by postischemic reperfusion.

KEY WORDS ventricular fibrillation; electrophysiology; cimetidine; myocardial reperfusion injury

Serious arrhythmias even as ventricular fibrillation (VF) are frequently induced by postischemic reperfusion[1]. A mass of histamine release is probably an important factor in the appearance of arrhythmias[2]. It has been proved that cimetidine, an H_2–receptor antagonist, has an anti–arrhythmia action during ischemia[3]. Nevertheless, the effects of Cim on reperfusion arrhythmias and its related mechanism are still uncertain. Furthermore, so far very few reports[4] have been found about the action of Cim on myocardial electrophysiological characteristics during reperfusion, this study was to investigate in isolated rat hearts whether Cim could prevent myocardial reperfusion changes.

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MATERIALS AND METHODS

Cim was obtained from Shanghai First Pharmaceutical Factory.

Wistar rats were bred from the Experimental Animal Center of Zhejiang Medical University.

ZMUP–1 computerized measuring apparatus for electrophysiological parameters was made by the subsidiary factory of Zhejiang Medical University.

Preparation of isolated hearts Forty–six rats weighing 230 ± *s* 21 g were used.