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Antioxidant and photo-antioxidant abilities of catechins

Yoshiyuki Dobashi *, Takashi Hirano, Morio Hirano, Yasukazu Ohkatsu

Department of Applied Chemistry, Faculty of Engineering, Kogakuin University, 1-24-2 Nishishinjuku, Shinjuku-ku, Tokyo 163-8677, Japan

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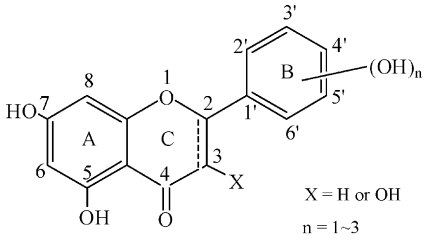
Abstract

Catechins are studied vigorously, in particular, concerning the antioxidant ability as polyphenol, which is generally considered responsible for photo-antioxidant natures of catechins. In this study, the antioxidant and photo-antioxidant abilities of (+)-catechin, (−)-epicatechin, and epigallocatechin have been investigated kinetically and discussed in detail. As a result, this study clarifies that a strong antioxidant is not always a good photo-antioxidant as well as that catechins show a high antioxidant ability in an initial oxidation process and acquire a photo-antioxidant ability with the progress of photo-oxidation. In particular, (+)-catechin and (−)-epicatechin convert into quercetin to show a remarkable photo-antioxidant ability almost comparable to quercetin. In this case, (−)-epicatechin has a higher photo-antioxidant ability than (+)-catechin, although they differ only in the configurations. These facts are explained by the direct conversion of the catechins into quercetin.

Keywords: Flavonoid; Catechin; UV; Photo-antioxidant; UVA

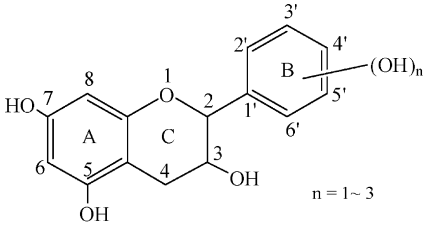
1. Introduction

Plants do photosynthesis using a part of sunlight, but suffer little damage from UV rays included in the sunlight. For this purpose, plants produce various substrates with antioxidant abilities, such as flavonoids generally known as polyphenols [1]. Almost all flavonoids consist of A–C rings as shown above, and their structures and functions are examined and discussed in detail [2]. For example, the B ring contributes to the antioxidant ability and the hydroxyl groups of the A ring do not function as an antioxidant. On the contrary, the 5-OH group shows a photo-antioxidant ability together with the carbonyl group on 4-position, because such a structure, namely 5-hydroxylbenzoyl structure, resembles a commercial UV absorber of 2-hydroxybenzophenone.



Basic structure of flavonoids

Catechins are kinds of flavonoids with a basic structure shown right. The extraction method [3,4] of catechins or the isolation and separation methods from many plants or fruits [5–8] are investigated energetically, and the effects of catechins on a skin are also studied concerning UV shielding [9,10]. Recently, many researches have been reported on the antioxidant abilities of catechins belonging to polyphenols [11–16].



Basic structure of catechins

* Corresponding author. Tel.: +81 3 3340 2703; fax: +81 3 3340 0147.
E-mail address: dobashi-y@u00.itscom.net (Y. Dobashi).

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Among these papers, the authors have paid attention to the photo-antioxidant ability shown by catechins, because they do not have the above-mentioned 5-hydroxybenzoyl structure as well as they hardly absorb UV rays above 290 nm which is the minimum wavelength of UV reaching the earth. Many studies explain such a photo-antioxidant ability of catechins by the antioxidant ability of scavenging oxygen-centered radicals, but this explanation seems insufficient.

This study will clarify why catechins have the photo-antioxidant ability, and, based on the result, discuss the possibility to develop new light stabilizers as unknown up to date.

2. Experimental

2.1. Reagents

(+)-Catechin was purchased from Tokyo Kasei Kogyo Co. Ltd. (−)-Epicatechin, epigallocatechin, (±)-α-tocopherol, and quercetin are purchased from Wako Pure Chemical Industries Ltd. (±)-Taxifolin was purchased from Sigma–Aldrich Corp. These reagents were used without purification.

2,2′-Azo-bis-isobutyronitrile (AIBN) and ethyl linoleate were purchased from Tokyo Kasei Kogyo Co. Ltd. Benzonitrile was purchased from Wako Pure Chemical Industries Ltd. These reagents were purified by general procedures (AIBN: recrystalization from methanol; and ethyl linoleate and benzonitrile: distillation).

2.2. Measurement of antioxidant activity

The antioxidant ability of a catechin was measured using an air-tight reaction system. After a flavonoid or α-tocopherol, ethyl linoleate (substrate), and AIBN (initiator) were dissolved in benzonitrile under O_2, the autoxidation was carried out at 50 °C with irradiating under UV rays or diffused light.

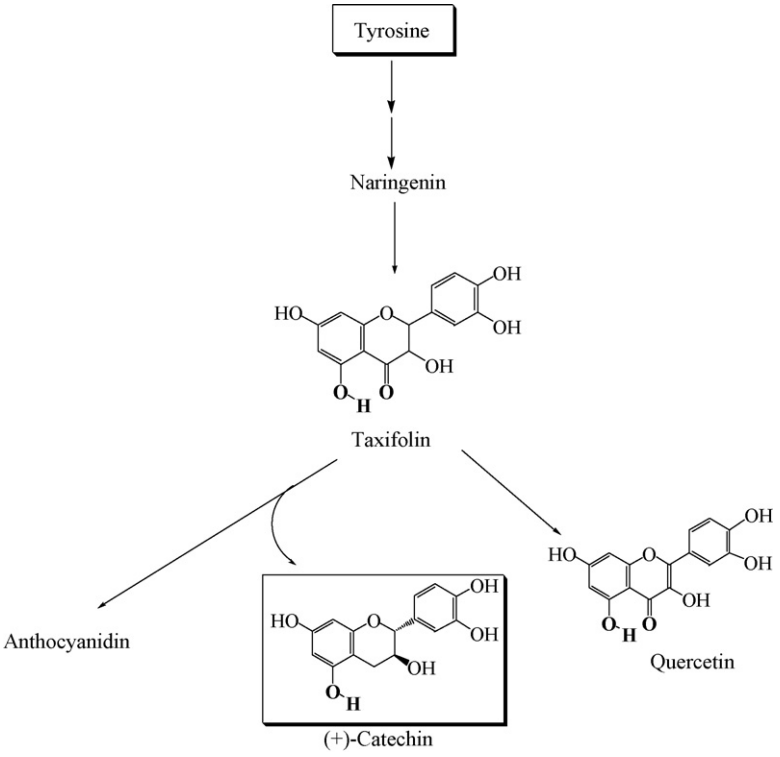
Antioxidant ability and photo-antioxidant ability are reported as a relative oxidation rate, namely, a rate of oxidation velocities in the presence to the absence of flavonoid or α-tocopherol. The equation is shown below

$$\text{Relative oxidation rate}\,(\%) = \frac{\text{velocity in presence of flavonoid}}{\text{velocity in absence of flavonoid}} \times 100$$

The lower the relative oxidation rate (%) is, the higher the antioxidant ability.

2.3. Instrumental analysis

UV absorption spectra were measured by a UV-2450 spectrometer (SHIMADZU). The light source of photo-oxidation was an ultra high-pressure mercury vapor lamp, the house of which was SW-U1501HQ (Ushio Co. Ltd.). As UV cut filters, UV-33 (ASAHI TECHNO GLASS Co.) was used. The oxidation velocity was measured using a DD200 (Toyoda Machine Works Ltd.) as transducer.



Scheme 1. Metabolic pathway of tyrosine to catechin.

3. Results and discussion

3.1. Relationship between metabolic pathway of flavonoids and antioxidant or photo-antioxidant abilities

Shown in Scheme 1 is the metabolic pathway of flavonoids used in this study. The starting substrate of flavonoids, naringenin, first converts into taxifolin, a part of which further converts into quercetin with a double bond in the C ring. The remains enter into another pathway to colored anthocyanidin compounds. Catechins form on this pathway. Generally, metabolites should have some meaning or aim for plant's maintaining the life, and no aimless metabolites must form. However, catechins are final metabolites, the peculiar meaning of which is unknown in a metabolic system. The authors are also interested in this point.

Shown in Fig. 1 is the oxidation reaction of ethyl linoleate in the presence of an above-mentioned flavonoid or α-tocopherol with a very strong antioxidant ability in a living body [17,18]. The ethyl gallate of gallo-ester was also used and shown in the figure, because many catechins exist as a gallate in plants. Ethyl gallate and (+)-catechin control the oxidation less in the initial oxidation process compared with α-tocopherol, but continue to inhibit the oxidation over a longer period. Therefore, such catechins seem good antioxidants overall. On the other hand, (−)-epicatechin behaves like the aforesaid catechin, but shows better antioxidant effect than (+)-catechin over all the period. The difference between (−)-epicatechin and (+)-catechin was also reported by Villano et al. [19], who did not discuss the reason why they show different antioxidant abilities, although they differ only in the configurations. This difference of stereo-structures, however, is important to clarify the photo-antioxidant ability of catechins as discussed later.

Shown in Table 1 are the antioxidant and photo-antioxidant abilities of flavonoids with their photo-chemical properties. All flavonoids show lower antioxidant activities under diffused light

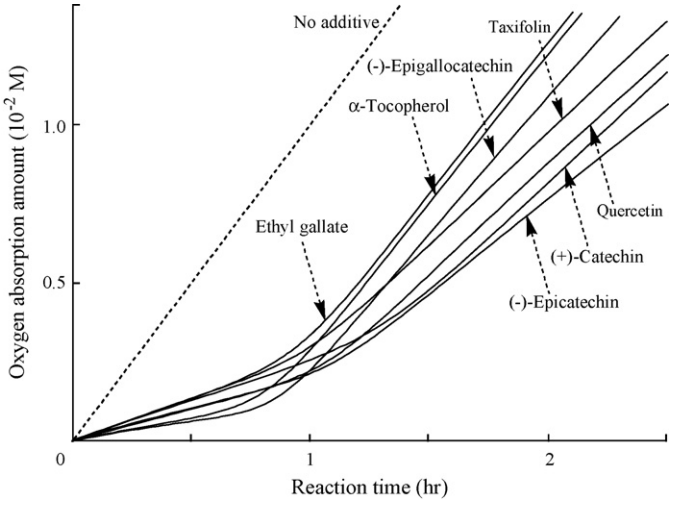


Fig. 1. Oxidation of ethyl linoleate in presence of tocopherol or flavonoid. Oxidation condition: [flavonoid, α-tocopherol, or ethyl gallate] = 5×10^{-5} M, [ethyl linoleate] = 0.6 M and [AIBN] = 10^{-2} M in benzonitrile at 50 °C under diffused light.

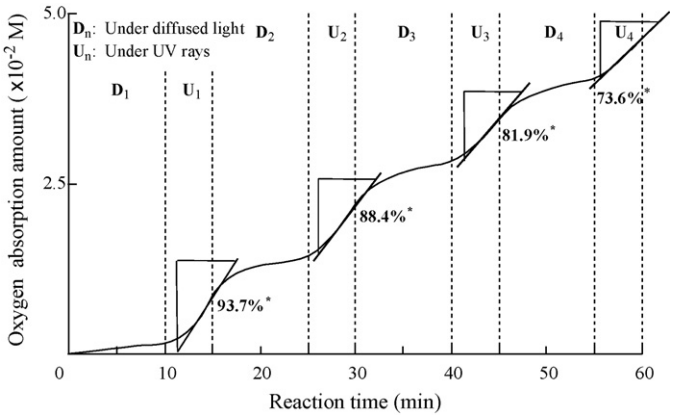


Fig. 2. Oxygen absorption amount in presence of (+)-catechin. Oxidation condition: [(+)-catechin] = 10^{-4}, [AIBN] = 10^{-2} M and [ethyl linolate] = 0.6 M in benzonitrile at 50 °C. *Relative oxidation rate.

than α-tocopherol except epigallocatechin. On the other hand, a photo-antioxidant activity must depend on the UV-absorbing ability of a substrate. All catechins absorb little harmful UV rays, while quercetin and taxifolin with 5-hydroxybenzoyl structure considerably absorb such UV rays. However, the catechins show higher photo-antioxidant abilities compared with taxifolin. In addition, α-tocopherol promotes the photo-oxidation at 5×10^{-4} M concentration, instead of inhibiting it, and ethyl gallate does not show any photo-antioxidant ability regardless of the concentration. This result shows that a radical-scavenging ability is not related directly to the photo-oxidation inhibition. There still remains a problem why a catechin functions as photo-antioxidant, although it does not absorb UV rays effectively.

3.2. Photo-antioxidant ability of catechin

Shown in Fig. 2 is an interesting experimental result. This shows the oxygen absorption amount in the oxidation reaction, in the presence of (+)-catechin, performed alternately under UV irradiation and under diffused light. Of course, the oxidation velocity is faster under UV due to the photo-initiation. The percentage in figure shows the rate of oxygen absorption velocities based on the velocity in the absence of (+)-catechin. The photo-oxidation velocity decreases step by step with repeated UV irradiation. This fact means the formation of a compound with a photo-antioxidant ability during the progress of oxidation.

As one of possibilities, (+)-catechin is assumed to go back to its precursor, taxifolin, to acquire a photo-antioxidant ability. That is, a catechin might get a carbonyl group by photo-oxidation of the 4-position of C ring. In order to confirm whether (+)-catechin actually converts into the precursor taxifolin, the oxidation solution was subjected to thin layer chromatography (TLC). Fig. 3 shows the chromatograms of flavonoids alone and of oxidation solutions. The big spots (clearly colored with iodine and dimly colored by UV radiation of 365 nm) at the top on the chromatogram of (+)-catechin are based on AIBN, and include an overlapped small spot (R_f = 0.85) absorbing UV ray (365 nm). This spot is considered to be that of oxidation products

Table 1
Antioxidant and photo-antioxidant activities of flavonoid

Compound	λ_{max} (nm)[a]	ε[a]	Relative oxidation rate (%)[b]		
			Diffused light (5×10^{-5} M)[c]	UV rays	
				(10^{-4} M)[c]	(5×10^{-4} M)[c]
α-Tocopherol			14.0	100	110
Ethyl gallate			26.5	100	100
(+)-Catechin	293	4,400	25.5	77.2	50.0
(−)-Epicatechin	293	4,400	21.8	77.0	31.0
(−)-Epigallocatechin	298	4,500	12.5	93.0	40.4
Quercetin	378	19,600	21.4	70.2	2.70
Taxifolin	330 292	27,300 26,500	26.8	88.5	62.1

[a] Measurement condition of UV spectra: [flavonoid, α-tocopherol, or ethyl gallate] = 10^{-5} M in ethanol at room temperature.
[b] Oxidation condition: [ethyl linoleate] = 0.6 M and [AIBN] = 10^{-2} M in benzonitrile at 50 °C.
[c] Concentration of flavonoid.

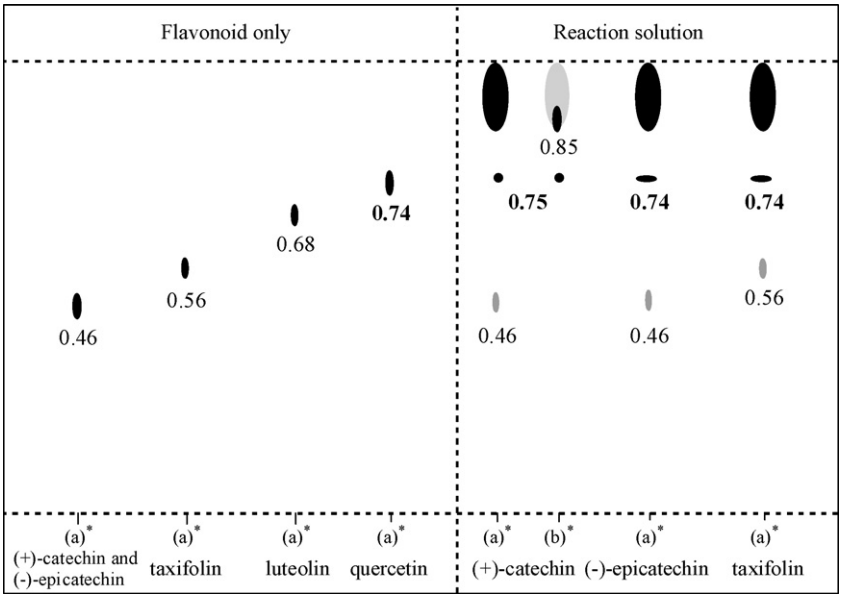


Fig. 3. R_f values of flavonoid before and after oxidation. Developing solvent: MeOH/CH_2Cl_2 = 1/4; thin layer: silica gel. Oxidation condition: [flavonoid] = 10^{-4} M and [AIBN] = 10^{-2} M in benzonitrile at 50 °C under diffused light for 120 min. *Spots detected (a) with iodine and (b) by UV (365 nm) radiation.

derived from (+)-catechin, because AIBN and its decomposition products do not absorb the UV ray, while the product from the catechin is less polar product from the standpoint of R_f values, compared with quercetin, and can absorb UV rays of higher wavelength range. Of course, the spot of (+)-catechin ($R_f = 0.46$) was not observed by the UV ray (365 nm). The small spots at the bottom on the chromatogram are based on the unreacted starting flavonoids. On comparing the other new spots ($R_f = 0.74$–0.75), (+)-catechin and (−)-epicatechin showed the spot with the same R_f value as quercetin. Contrary to the expectation, taxifolin could not be detected at all. The fact, found in this study, that taxifolin becomes quercetin by photo-oxidation, however, is very interesting on considering the metabolic pathway of flavonoids. Thus, the spots from oxidation solutions suggest the direct conversion of catechin into quercetin, because taxifolin is not oxidized so fast as it forms as an intermediate.

In general, only TLC is insufficient to identify a compound. Therefore, the formation of quercetin was confirmed using a UV spectroscopic method, which is often utilized for the identification of flavonoids. The characteristic λ_{max} and ε of UV spectra of flavonoids well reflect the difference in their fundamental skeleton and the number and position of hydroxyl groups [20]. For example, flavones or flavonols have two λ_{max}'s of Band I (300–380 nm, mainly based on a cinnamoyl group including Δ^2 and 4-keto in B and C rings) and Band II (240–280 nm, mainly based on a benzoyl group including 4-keto in A and C rings). On the other hand, isoflavones or flavanones and dihydroflavonols having no double bond in C ring show a strong absorption in the Band II, but a rather weak absorption in a lower wavelength range of Band I [21]. That is to say, a flavonoid with such a double bond shows an absorption maximum in a longer wavelength range because of on enlarged conjugation system. Shown in Fig. 4 are UV spectra of general flavonoids. It is seen that the absorption spectra are very different between quercetin and luteorin with double bond in C ring and (+)-catechin or taxifolin with no double bond. Therefore, a new absorption maximum should appear clearly in the range of 300–380 nm, if (+)-catechin gets 4-keto group and the double bond like quercetin.

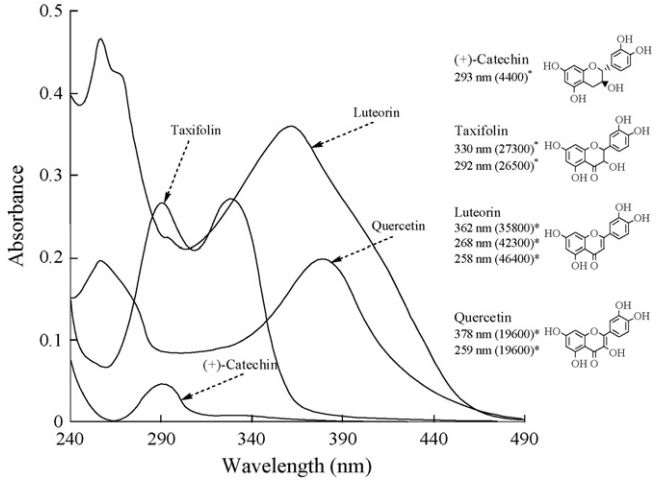


Fig. 4. UV spectra of flavonoids. Measurement condition of UV spectra: [flavonoid] = 10^{-5} M in ethanol at room temperature. *λ_{max} (ε).

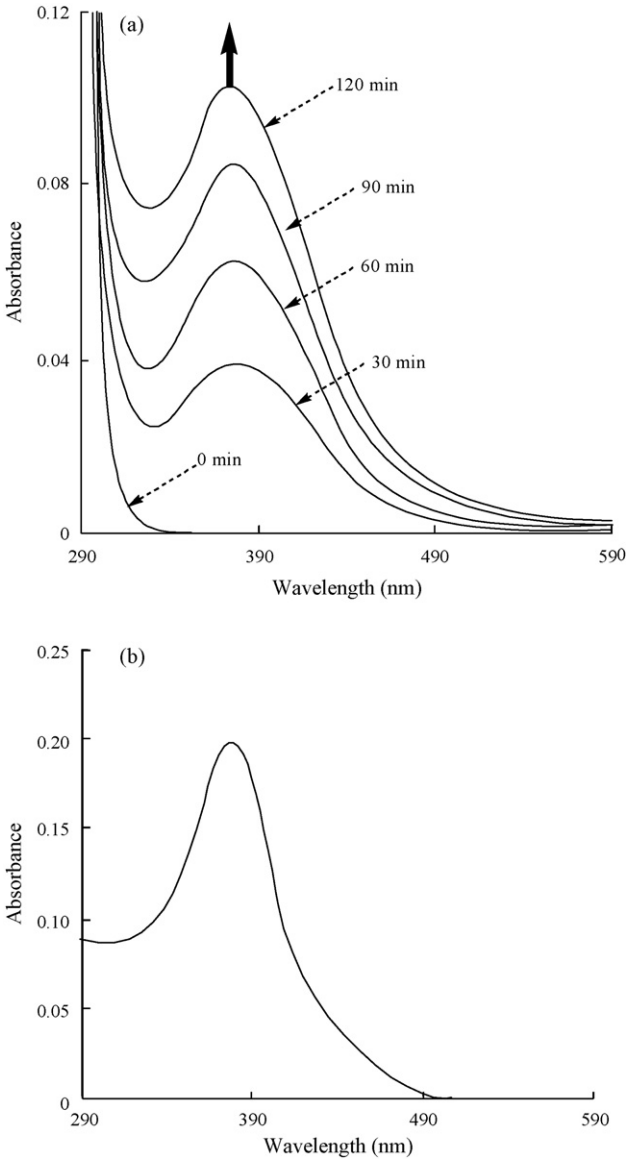


Fig. 5. (a) Differential spectra of (+)-catechin after oxidation. Oxidation condition: [(+)-catechin] = 10^{-4} M and [AIBN] = 10^{-2} M in benzonitrile at 50 °C under diffused light. Measurement condition of UV spectra: at room temperature. (b) UV spectrum of quercetin. Measurement condition of UV spectra: [quercetin] = 10^{-5} M in ethanol at room temperature.

(+)-Catechin alone was photo-oxidized in the presence of AIBN while taking UV spectra with time. The use of AIBN as initiator is a general technique in an oxidation reaction [22]. AIBN is cleaved by light and heat to form a carbon-centered radical, which promptly becomes the peroxy radical in oxygen-containing atmosphere. Therefore, the peroxy radical is a main radical in this study. The oxidation of (+)-catechin was carried out under diffused light, because the irradiation of UV rays obstructs the UV spectroscopic measurement due to the fast change of UV spectra with time. Fig. 5a shows differential spectra of the reaction solution obtained by subtracting the AIBN spectrum. The decrease in the absorption of (+)-catechin cannot be confirmed due to its rather high original concentration, but a new absorption appears and increases around 380 nm with

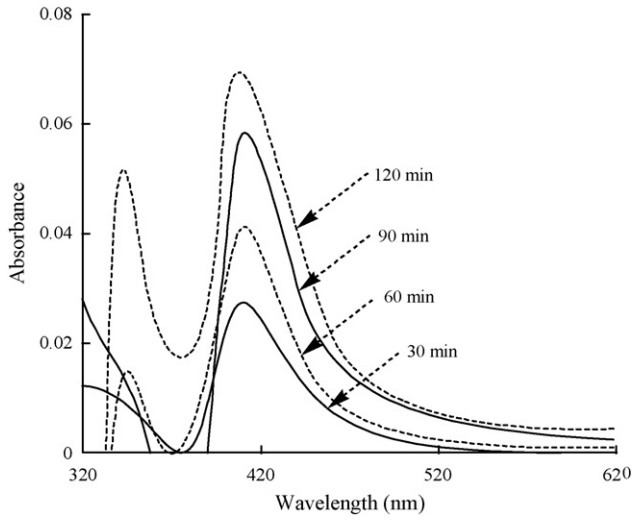


Fig. 6. Differential spectra of (+)-catechin during oxidation. Oxidation condition: [(+)-catechin] = 10^{-4} M and [AIBN] = 10^{-2} M in benzonitrile at 50 °C under diffused light. Measurement condition of UV spectra: at room temperature.

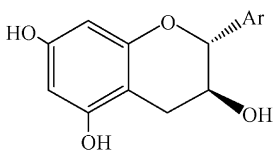
time. This absorption spectrum well resembles that of quercetin shown in Fig. 5b and indicates the direct formation of quercetin from (+)-catechin. The same phenomena are observed for (−)-epicatechin and taxifolin as well.

When Fig. 5a and b were compared, the absorption band around 380 nm of Fig. 5a looks a little broad compared with that of quercetin, suggesting the overlap of another spectrum. Thus, the spectra shown in Fig. 6 are obtained as differential spectra by subtracting Fig. 5b from Fig. 5a. As expected, a compound absorbing the light around about 400 nm forms or exists. This product is assumed as a product with *o*-benzoquinone structure forming after B ring scavenges peroxy radicals in comparison with the result of epigallocatechin as discussed in Section 3.4. In general, an *o*-benzoquinone structure has two absorption bands at about 370 and 570 nm [23], but the latter band is not observed clearly because of the very low extinction coefficient of ε = about 20. On the contrary, the former band can be clearly seen even in the case of a little existence because of the rather higher ε. The chemical structure giving the absorption bands of Fig. 6 must correspond surely to *o*-benzoquinone-like structure.

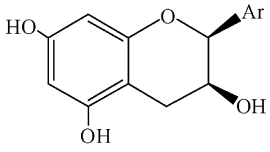
Summarized in Table 2 are the relative rates of oxygen absorption velocities for flavonoids in the oxidation performed under the alternative irradiation of diffused light and UV light: the oxidation conditions as well as the symbols, U_1–U_4, are the same as those in Fig. 2. Quercetin does not change the relative rate with time, but the other substrates decrease the relative rates, corresponding to the increase in the photo-antioxidant abilities. That is to say, the table well supports the formation of quercetin from (+)-catechin, (−)-epicatechin, and taxifolin with the progress of oxidation. Referring to Table 2 again, the degree of decrease in the relative rate toward quercetin is more remarkable for (+)-catechin and (−)-epicatechin than for taxifolin. This result also supports the direct conversion of the catechins to quercetin without going through taxifolin. The relative rate during the U_4 period for (+)-catechin and (−)-epicatechin approaches that of quercetin. This shows the conversion of the greater part of such a catechin into quercetin.

3.3. Conversion of catechin into quercetin

On watching a catechin as polyphenol, it is very interesting which hydroxyl group works as a radical scavenger. If it is a hydroxyl group in B ring, the quinone structure of B ring forms and does not give any quercetin. Therefore, quercetin will form reasonably only when a hydroxyl group on A ring scavenges a peroxy radical. The A ring has two hydroxyl groups, the one of which, on 5-position, will take part preferentially, in the reaction because the resulting phenoxy radical is more stable. The phenoxy radical can abstract a hydrogen on 4-position to subsequently form the benzyl radical as shown in Scheme 2: this process has been found by Ohkatsu and Nishiyama [24] and supported by MOPAC calculation [25]. The resulting benzyl radical becomes more stable by coupling with a peroxy radical. If the product simply decomposes by light, a carbonyl group would form on 4-position to give taxifolin. Therefore, another carbonyl formation process must be considered. If such a peroxy radical-coupled intermediate forms a hydrogen bond with a hydrogen on 2-position of C ring and reacts with oxygen, both 4-oxo group and 2,3-C=C bond necessary for quercetin form at the same time. This process is no more than estimation, but seems well to explain the difference in the conversion of (+)-catechin and (−)-epicatechin into quercetin. These catechins have the following configurations:



(+)-catechin



(-)-epicatechin

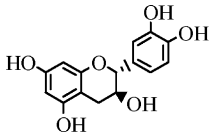
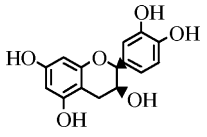
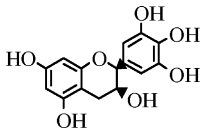
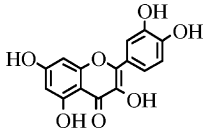
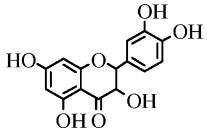
(+)-Catechin has a *trans*-conformation on 2,3-positions, while (−)-epicatechin, *cis*-conformation. Therefore, the above-mentioned intermediate is easier to form for (−)-epicatechin due to less steric hindrance of the hydroxyl group on 3-position. According to this idea, the difference in photo-antioxidant abilities of (+)-catechin and (−)-epicatechin shown in Table 2 can be understood well.

On the other hand, the hydroxyl groups on catechin B ring can also scavenge peroxy radicals. In this case, a catechin will change into a compound with *o*-quinone structure. The above-mentioned result of quercetin's forming as one of oxidation products of (+)-catechin (Fig. 3), however, may suggest that the 5-hydroxyl on A ring scavenges a peroxy radical in parallel with hydroxyls on B ring.

3.4. Antioxidant activities of epicatechin and epigallocatechin

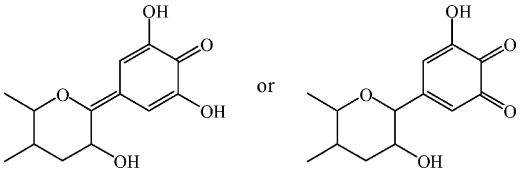
Epicatechin and epigallocatechin have different chemical structures, although the configuration is same. Therefore, it is estimated that both exhibit almost similar photo-antioxidant abilities. However, the relative oxidation rate of epigallocatechin is better under diffused light as shown in Table 1, and its photo-antioxidant activity is worse compared with that of epicatechin. This fact indicates that the gallo group scavenges

Table 2
Effect of on–off of UV rays on relative oxidation rate

Compound	Relative oxidation rate under UV (%)			
	U_1	U_2	U_3	U_4
 (+)-Catechin	93.7	88.4	81.9	73.6
 (−)-Epicatechin	89.0	73.5	72.5	68.6
 (−)-Epigallocatechin	100	98.0	87.2	89.5
 Quercetin	–	68.1	68.1	68.1
 Taxifolin	95.9	93.1	91.1	79.5

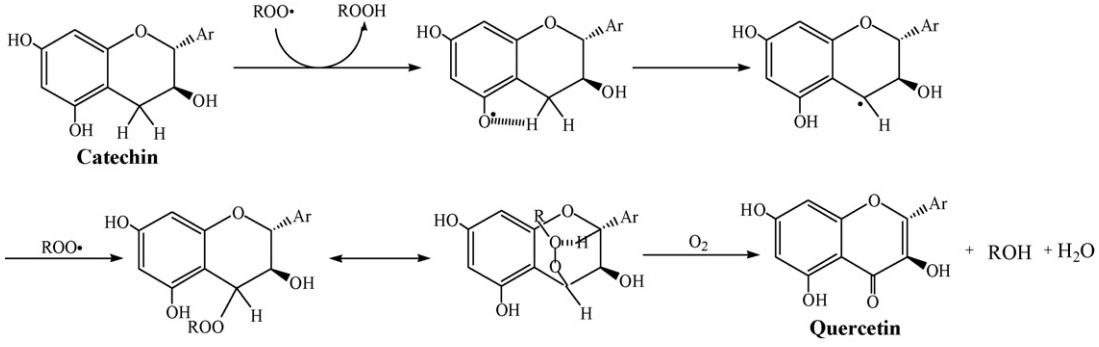
Oxidation condition: [ethyl linoleate] = 0.6 M and [AIBN] = 10^{-2} M in benzonitrile at 50 °C.

peroxy radicals rather faster. Shown in Fig. 7 are UV–vis spectra of the reaction solution obtained using epigallocatechin instead of (+)-catechin under the same conditions as in Fig. 5a. In this case, the clear absorption band is not observed at 380 nm as the case of (+)-catechin, but an absorbance seems to increase with time near 380 nm. This may indicate that the similar reaction also proceeds in the case of epigallocatechin. However, Table 2 indicates that the reaction proceeds more slowly for the case of epigallocatechin than for (+)-catechin and (−)-epicatechin. Interestingly as shown in Fig. 7, however, epigallocatechin shows a weak absorption band around 459 nm, which grows up with time. This fact also supports the preferential attack of a peroxy radical on a hydroxyl group of B ring. That is to say, in the case of epigallocatechin, the gallo group converts into the quinoid structure as shown below.



In this case, the *p*-quinoid structure is estimated as a possible structure, because *p*-benzoquinone with a similar structure to *p*-quinoid shows a weak absorption at about 470 nm [23].

As the above-mentioned, epigallocatechin is not expected to show a good photo-antioxidant ability compared with epicatechin. This may be ascribable to the fact that the *p*-quinoid structure is difficult to form the enone structure in C ring. How-



Scheme 2. Formation mechanism of quercetin from catechin.

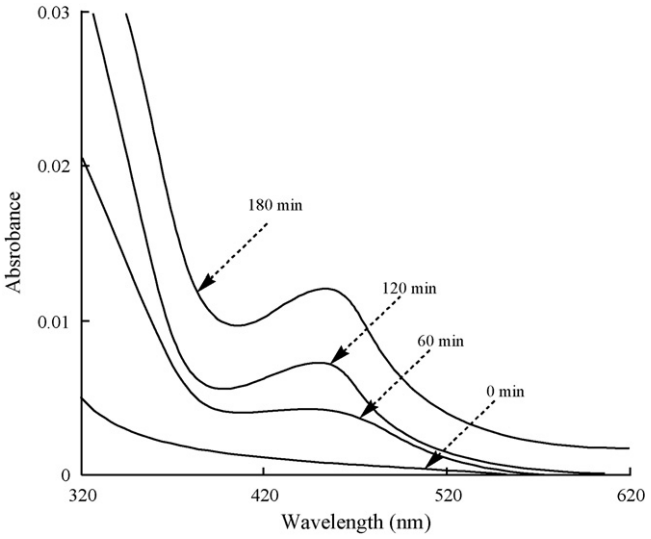


Fig. 7. Differential spectra of (−)-epigallocatechin during oxidation. Oxidation condition: [(−)-epigallocatechin] = 10^{-4} M and [AIBN] = 10^{-2} M in benzonitrile at 50 °C under diffused light. Measurement condition of UV spectra: at room temperature.

ever, epigallocatechin enhances the photo-antioxidant activity from 93.0% at the concentration of 10^{-4} M to 40.4% at the five times concentration (see Table 1). This fact may suggest the easier formation of the *o*-benzoquinone structure, at the higher concentration of epigallocatechin, which is effective to photo-antioxidant ability.

4. Conclusion

The eight substrates of catechin, gallocatechin, epicatechin, and epigallocatechin, and their esters of the hydroxyl group on 3-position with gallaic acid are mainly contained in green tea. Epigallocatechin gallate exist in the largest amount, followed by epigallocatechin, epicatechin, and epicatechin gallate. (+)-Catechin is only in a little amount. This study has first measured the antioxidant and photo-antioxidant abilities of these compounds to clarify a part of their roles in a living body. Gallates belonging to catechins have phenolic hydroxyl groups, coming from gallic acid, which generally do not show a high peroxy radical-scavenging ability due to the electron-withdrawing carboxyl group [26]. Nevertheless, the gallate group of catechins shows some antioxidant ability, but no photo-antioxidant ability as mentioned before: the antioxidant ability of the gallate being almost the same as that of (+)-catechin. Therefore, the study on epigallocatechin, epicatechin, and catechin will be sufficient to understand, especially, photo-antioxidant abilities of whole catechins.

As a result, epigallocatechin excels in antioxidant ability, while epicatechin and catechin, in photo-antioxidant ability. Sunlight liking tea plants are cultivated under strong sunlight. Such conditions are most suitable for damaging the plants, for example, by a photo-oxidation. Therefore, they contain more flavonoids compared with those of other plants. This reason may be explained as follows. Quercetin, kaempherol, myricetin, etc. exhibit high antioxidant and photo-antioxidant abilities, but are slightly soluble in an aqueous system and do not form quickly in plants. These flavonoids well protect tea plants from the photo-oxidation, but sometimes they will be insufficient to inhibit a suddenly occurring photo-damage such as photo-oxidation. In such an emergency case, some catechins, which are well soluble in water and acquire photo-antioxidant abilities immediately by sunbathing, will be able to function as precursors or stocks of photo-antioxidant flavonoids.

The other result obtained in this study is to provide a possibility to develop a new hydrogen bond type UVA. Such UVA can work as antioxidant in the dark and as photo-antioxidant which forms by photo-oxidation in the case of need. These compounds have not been proposed yet, but must be attractive depending on application fields.

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