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Reactivity of green tea catechins with formaldehyde

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Abstract In the reaction of green tea catechins with formaldehyde at room temperature (25°C), tea catechins were found to have reactivity. In particular, (−)-epicatechin gallate and (−)-epigallocatechin gallate, which have a galloyl moiety at the C-3 position, showed higher reactivity than (+)-catechin, (−)-epicatechin, or (−)-epigallocatechin. Reactivity of various kinds of simple phenolic compounds and flavonoids with formaldehyde was also examined. Among these compounds, only phloroglucinol showed reactivity to the same degree as that of nongalloylated catechins. These results suggest that factors for reactivity with formaldehyde at room temperature may be the presence of a phloroglucinolic A-ring structure and the absence of the electron-attractive group such as a carbonyl group in C-ring. The comparison of the reactivity of 3-*O*-acylated catechins with that of 3-*O*-galloylated catechins indicated that only a galloyl group effectively enhanced reactivity with formaldehyde.

Key words Tea catechins · Formaldehyde · Flavonoid · Volatile organic compounds (VOC) · Formaldehyde scavenger

Introduction

Pollution of indoor air by volatile organic compounds (VOCs) has become a problem owing to the advancements in building construction that improved indoor air-tightness and insulation in housing. Formaldehyde, which is contained in wood adhesive, has in recent years been the target of attention as one of the main indoor pollutants.[1–3] This is because formaldehyde is gradually released to the indoor air from wood products, and it is considered to have detrimental effects on the human body. Therefore, a major concern has been to remove free formaldehyde from indoor air.

There have been a number of proposals for utilizing condensed tannins from wood or bark sources as plywood adhesives.[4,5] In such studies, because of the use of tannins as phenol replacement in phenol-formaldehyde resins, attention has focused on the reaction of condensed tannins with formaldehyde. Condensed tannins are oligomeric or polymeric molecules based on several kinds of flavan-3-ol units; hence the reaction of formaldehyde with tannin model compounds, especially (+)-catechin [(+)-C], has been extensively investigated.[6–8] In these studies, it was found that electrophilic substitution by formaldehyde easily occurred at the C-8 and C-6 positions of (+)-C by the nucleophilicity of the A-ring.[9,10] On the other hand, green tea contains a large amount of flavan-3-ols such as (−)-epicatechin [(−)-EC], (−)-epigallocatechin [(−)-EGC], (−)-epicatechin gallate [(−)-ECg], and (−)-epigallocatechin gallate [(−)-EGCg]. We have conducted comprehensive research on the physiological functions of tea catechins.[11–15] However, little is known of the reactivity of formaldehyde with flavan-3-ols other than (+)-C. In view of the considerable significance of removing formaldehyde as a main indoor pollutant, this study was conducted to examine the reactivity of green tea catechins with formaldehyde, particularly at room temperature (25°C).

Materials and methods

Green tea catechins [(+)-C, (−)-EC, (−)-EGC, (−)-ECg, (−)-EGCg] were obtained from Sigma Chemical Co. (USA). Their structural formulas are shown in Fig. 1. (−)-Catechin [(−)-C] and (−)-Catechin gallate [(−)-Cg] prepared according to our method, previously reported.[13] Acyl derivatives of (−)-C and (−)-EGC (Fig. 2) were synthesized by the method previously described.[16]

The reactivity of green tea catechins and acylated catechins with formaldehyde was examined according to the

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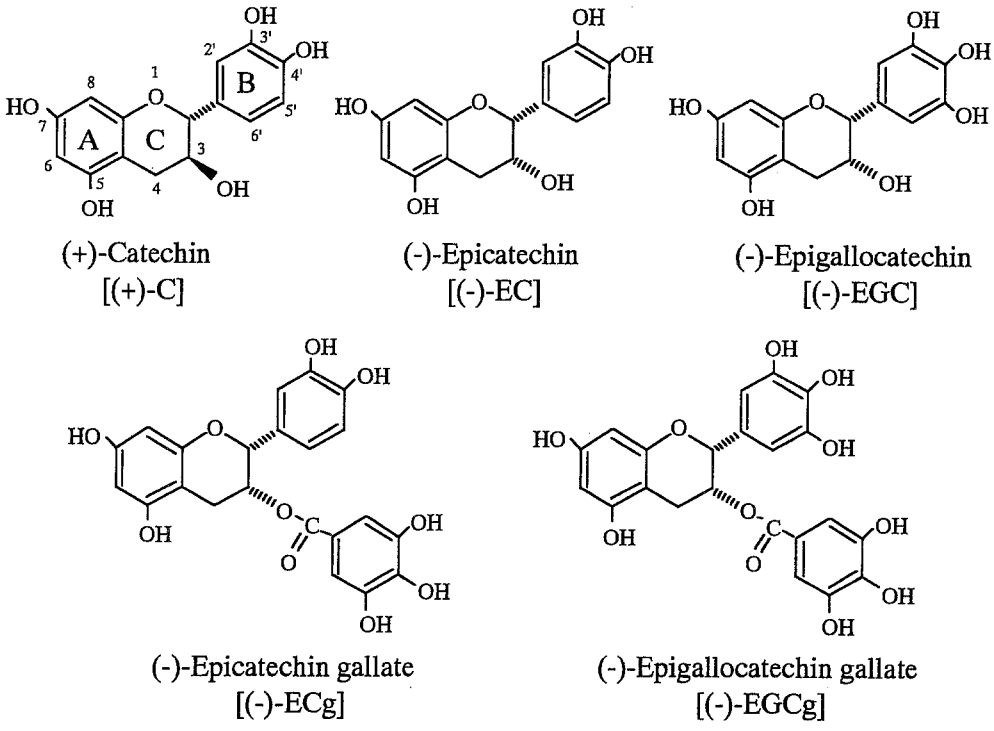
Fig. 1. Structures of green tea catechins



following method. Each sample was dissolved in distilled water to a concentration of 10 mM. The sample solution (0.17 ml) and 0.1% formaldehyde aqueous solution (0.05 ml) were added to a vial, and the total volume of the solution was adjusted to 10 ml with 100 mM phosphate-citrate buffer (pH 6.0). The reaction mixture (molar ratio of sample/formaldehyde 1:1) was incubated in an unsealed vial for 15 min at room temperature (25°C) with gentle stirring. To this mixture, 1 ml dinitrophenylhydrazine (DNPH) solution (2.4 mg DNPH/ml 30% perchloric acid) was added to convert the remaining formaldehyde to DNPH derivative.[17] After filtration with a membrane filter, the DNPH derivative was determined by high-performance liquid chromatography (HPLC). The reduction rate of formaldehyde was evaluated by comparing the value of the peak area of the DNPH derivative with that of the control. The HPLC analysis was conducted on a JASCO 800 series system [column 4.6 × 250 mm, Capcellpack ODS AG-120 (Shiseido Co., Tokyo); solvent 50% acetonitrile; detector ultraviolet (UV) 355 nm; flow rate 1 ml/min; oven temperature 40°C]. The reaction of these samples with formaldehyde in the case where the sample/formaldehyde molar ratio was 2:1 was examined under the same conditions as described above, except that 20 mM sample solution was used.

Simple phenolic compounds (Table 1) were purchased from Wako Pure Chemical Industries (Osaka, Japan) and flavonoids (Table 1) were obtained from Funakoshi (Tokyo, Japan). The reactivity of the simple phenolic compounds and the flavonoids with formaldehyde was examined. A reaction mixture containing 10 mM sample solution (1 ml), 0.1% formaldehyde solution (0.05 ml), and 100 mM phosphate-citrate buffer pH 6.0 (8.95 ml) was incubated for 15 min at 25°C. The residual formaldehyde was analyzed according to the method described above.

Table 1. Reactivity of simple phenolic compounds and flavonoids with formaldehyde

Compounds	Reduction rate of formaldehyde (%)
Simple phenolic compounds	
Gallic acid	5.8
Pyrogallol	2.1
Catechol	2.6
Resorcinol	0.04
Phloroglucinol	28.4
Orcinol	4.9
m-Cresol	1.8
Esculetin	4.9
3,5-Xylenol	3.0
4-Methylresorcinol	4.9
2-Methylresorcinol	1.0
Resorcinol dimethyl ether	12.6
Flavonoids	
Flavonol	
Quercetin	3.2
Quercitrin	10.0
Rutin	0.2
Morin	3.1
Dihydroflavonol (taxifolin)	6.5
Flavanone (naringenin)	2.9
Flavone	
Luteolin	1.6
Apigenin	9.8
Isoflavone (genistein)	10.0
Calcone (butein)	1.7
Anthocyanidin	
Cyanidin chloride	16.5
Fisetinidin chloride	12.8
Tea catechin	
(+)-C	26.6
(−)-EC	34.2
(−)-ECg	89.4
(−)-EGC	34.6
(−)-EGCg	87.6

(+)-C, (+)-catechin; (−)-EC, (−)-epicatechin; (−)-ECg, (−)-epicatechin gallate; (−)-EGC, (−)-epigallocatechin; (−)-EGCg, (−)-epigallocatechin gallate

Results and discussion

We first examined the reactivity of the principal green tea catechins (Fig. 1) with formaldehyde at room temperature. As shown in Table 2, tea catechins were found to have reactivity with formaldehyde. Nongalloylated catechins [(+)-C, (−)-EC, (−)-EGC] were found to have the same degree of reactivity. These results suggested that the number of hydroxyl groups in the B-ring hardly affected the reactivity with formaldehyde. In contrast, (−)-EGCg and (−)-ECg, which have a galloyl moiety at the C-3 position, showed much higher reactivity than did the nongalloylated catechins.

To further understand the reactivity of tea catechins with formaldehyde, we examined the reactivity of various kinds of simple phenolic compounds and flavonoids (Table 1). The structure of these simple phenolic compounds mimics in part the structure of tea catechins. Flavonoids were classified into seven groups on the basis of the variations of the C-ring structure, as shown in Fig. 3. Although some simple phenolic compounds were found to react easily with formaldehyde at high temperature,[18] at room temperature only phloroglucinol showed reactivity, as shown in Table 1. This reactivity of phloroglucinol was found to be of the same degree as that of the nongalloylated catechins [(+)-C, (−)-EC, (−)-EGC]. This result suggests that the reactivity of tea catechins with formaldehyde at room temperature is due to the phloroglucinolic A-ring structure. However, in the case of flavonoids, even though some had a phloroglucinolic A-ring structure, all of those tested were found to have lower reactivity than tea catechins. One of the major differences between flavonoids and tea catechins is that flavonoids have a carbonyl group at the C-4 position (Figs. 1, 3). It is generally known that the carbonyl group has an electron-attractive property. Therefore, it is thought that the electron-attractive property of the carbonyl group at the C-4 position may decrease the A-ring charge density and weaken the nucleophilicity of the flavonoids. In the same way, the lowered reactivity observed with anthocyanidins may be due to the electron-attractive property of the oxonium structure. These results suggest that at least two factors are necessary for the existence of reactivity with formaldehyde at room temperature. One factor is the presence of phloroglucinolic A-ring structure, and the other is the absence of an electron-attractive group, such as a carbonyl group, which decreases A-ring charge density.

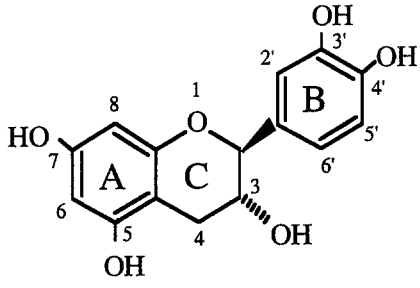
It remained to be explained why the attachment of galloyl moiety enhanced the reactivity of nongalloylated catechins with formaldehyde at room temperature. To examine the influence of the galloyl group, we attempted to

Table 2. Reactivity of green tea catechins with formaldehyde

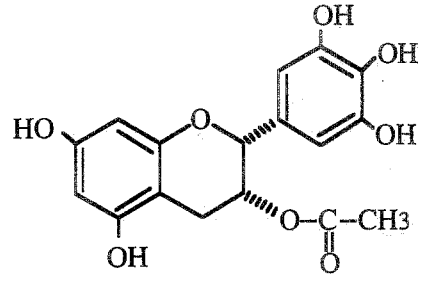
Catechin	Reduction rate of formaldehyde (%)	
	F:C = 1:1	F:C = 1:2
(+)-C	16.8	30.5
(−)-EC	15.2	43.1
(−)-ECg	59.6	86.4
(−)-EGC	19.9	38.7
(−)-EGCg	54.8	81.5

F:C, molar ratio of formaldehyde to tea catechin; (+)-C, (+)-catechin, (−)-EC, (−)-epicatechin; (−)-ECg, (−)-epicatechin gallate; (−)-EGC, (−)-epigallocatechin; (−)-EGCg, (−)-epigallocatechin gallate

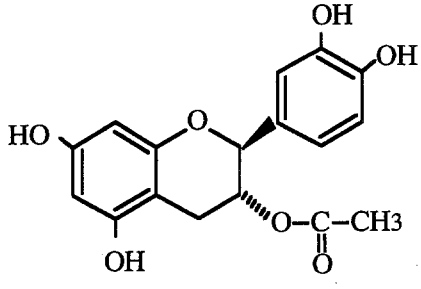
Fig. 2. Structures of (−)-catechin and 3-*O*-acyl derivatives of (−)-catechin and (−)-epigallocatechin



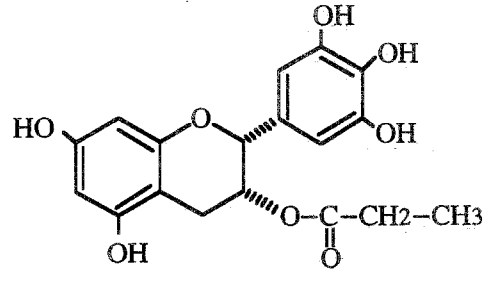
(-)-Catechin



(-)-3-*O* -acetylepigallocatechin



(-)-3-*O* -acetylcatechin



(-)-3-*O* -propionylepigallocatechin

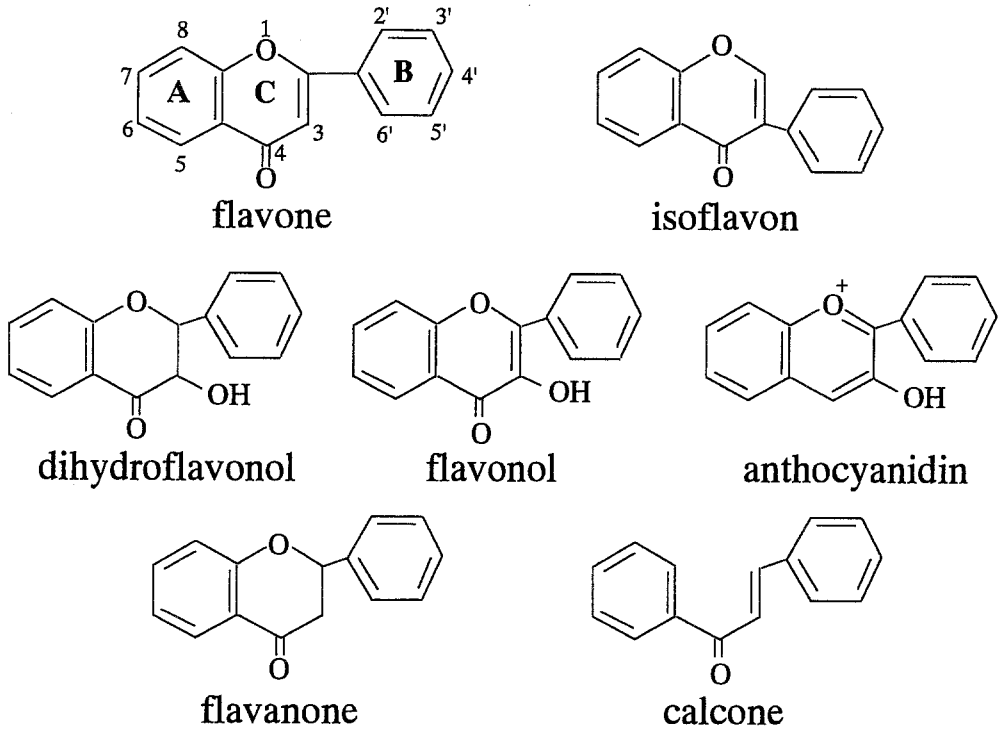


Fig. 3. Structures of flavonoid skeletons

Table 3. Reactivity of (−)-catechin, (−)-epigallocatechin, and their derivatives with formaldehyde

Compounds	Reduction rate of formaldehyde (%)	
	F:C = 1:1	F:C = 1:2
(−)-Catechin	15.2	29.8
(−)-3-*O*-Acetylcatechin	12.1	19.5
(−)-Catechin gallate	43.9	71.2
(−)-Epigallocatechin	21.8	35.1
(−)-3-*O*-Acetylepigallocatechin	13.6	20.9
(−)-3-*O*-Propionylepigallocatechin	15.6	23.2
(−)-Epigallocatechin gallate	55.3	78.9

F:C, molar ratio of formaldehyde to (−)-catechin, (−)-epigallocatechin, or their derivatives

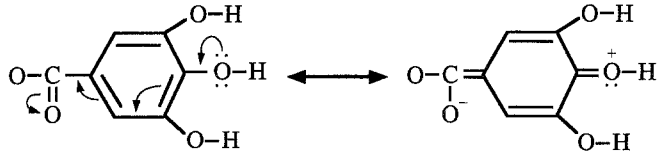


Fig. 4. Mesomeric effect of galloyl ester

compare the reactivity of 3-*O*-acyl derivatives of (−)-C and (−)-EGC (Fig. 2) with that of 3-*O*-galloylated (−)-C and (−)-EGC. As shown in Table 3, acetylated (−)-C was found to have lower reactivity with formaldehyde than (−)-C. Similarly, (−)-EGC derivatives, acetylated (−)-EGC, and propionylated (−)-EGC were also found to have lower reactivity than (−)-EGC; and acetylated (−)-EGC showed lower reactivity than propionylated (−)-EGC. The strength of the electron-attractive property of these functional groups is generally known to be in the order of acetyl group > propionyl group > hydroxyl group. Thus it seems that the electron-attractive property of these acyl groups may result in the decreased reactivity of (−)-C and (−)-EGC with formaldehyde. On the other hand, the galloyl group was found to enhance the reactivity of nongalloylated catechins even though it is also an acyl group generally known to have an electron-attractive property. It could be expected that in the case of the galloyl group movement of a lone pair of electrons of the hydroxyl group at the *para* position would occur by mesomerism, as shown in Fig. 4, whereby an electron donative property would be induced. Thus the electronic property of the galloyl group might be somewhat different from that of the other acyl groups tested in this study, but it is still unclear why the galloyl group attached to the C-3 position enhances the reactivity of nongalloylated catechins such as (−)-C, (−)-EC, and (−)-EGC.

Not only are (−)-ECg and (−)-EGCg characteristic compounds in green tea, they also comprise 60%–70% of the total catechins contained in green tea. In this study it was proved that (−)-ECg and (−)-EGCg, which have a galloyl moiety at the C-3 position, have excellent reactivity with formaldehyde even at room temperature; and these findings suggest the possibility of utilizing tea catechins as a formaldehyde scavenger. Formaldehyde is recognized as one of the main compounds that cause health problems known as "sick building syndrome." Thus, although further investigation is necessary to determine appropriate applications of green tea catechins in building materials, it is expected that the ability of tea catechins to remove formal-

dehyde from the air will be of particular use in the building industry.

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References

1. Matsumura T (1996) Indoor air pollution by chemical compounds (in Japanese). Taiki Kankyo Gakkaishi 31:A154–A164
2. Umeda H (1986) Environmental pollution by formaldehyde (in Japanese). Rep Environ Sci Inst Hyogo Prefect 18:1–14
3. Miyazaki T (1993) Indoor pollution by poisonous gas (in Japanese). Heat Piping Air Condition 12:73–78
4. Herrick FW, Bock LH (1958) Thermosetting, exterior-plywood type: adhesives from bark extracts. For Prod J 8:269–274
5. Takano R, Karasawa R (1981) Production of natural-type adhesive with bark extract (in Japanese). Mokuzai Gijutsu 47:1–6
6. Kiatgrajai P, Wellons JD, Gollob L, White JD (1982) Kinetics of polymerization of (+)-catechin with formaldehyde. J Org Chem 47:2913–2917
7. Hills WE, Urbach G (1959) The reaction of (+)-catechin with formaldehyde. J Appl Chem 9:474–482
8. Fechtal M, Riedl B, Calve L (1993) Modeling of tannins as adhesives. Holzforschung 47:419–424
9. McGraw GW (1988) Reactions at the A-ring of proanthocyanidins. In: Hemingway RW, Karchesy JJ (eds) Chemistry and significance of condensed tannins. Plenum, New York, pp 227–248
10. Tobiason FL, Hoff LA (1988) Mndo molecular orbital analyses of models for proanthocyanidin-methylolphenol reactions. In: Hemingway RW, Karchesy JJ (eds) Chemistry and significance of condensed tannins. Plenum, New York, pp 205–226
11. Nanjo F, Goto K, Seto R, Suzuki M, Sakai M, Hara Y (1996) Scavenging effects of tea catechins and their derivatives on 1,1-diphenyl-2-picrydrazyl radical. Free Rad Biol Med 21:895–902
12. Matsumoto N, Okushio K, Hara Y (1998) Effect of black tea polyphenols on plasma lipids in cholesterol-fed rats. J Nutr Sci Vitaminol 44:337–342
13. Seto R, Nakamura H, Nanjo F, Hara Y (1997) Preparation of epimers of tea catechins by heat treatment. Biosci Biotechnol Biochem 61:1434–1439
14. Hara Y (1998) Influence of tea catechins on the digestive tract. J Cell Biochem Suppl 27:52–58
15. Shimamura T, Hara Y (1993) Inhibition of the infectivity of influenza virus by tea polyphenols. Antiviral Res 21:289–299
16. Sakai M, Suzuki M, Nanjo F (1994) Japanese patent H06279430
17. Carey MA, Persinger HE (1972) Liquid chromatographic determination of traces of aliphatic carbonyl compounds and glycols as derivatives that contain the dinitrophenyl group. J Chromatogr Sci 10:537–543
18. Pizzi A, Horak RM, Ferreira D, Roux DG (1979) Condensates of phenol, resorcinol, and pyrogallol as model compounds of flavonoids A- and B-rings with formaldehyde. Cellulose Chem Technol 13:753–761