Novel Antiallergic Catechin Derivatives Isolated from Oolong Tea

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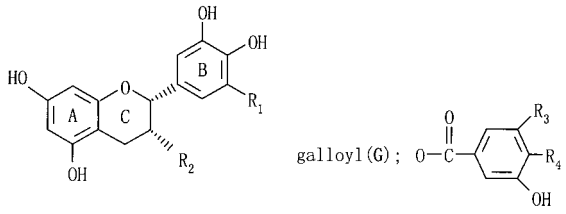
Two catechin derivatives (C-1 and C-2) with potent antiallergic activity were isolated from Taiwanese oolong tea by HPLC techniques. From NMR and FAB-MS analyses, the structures of C-1 and C-2 were elucidated as (−)-epigallocatechin 3-*O*-(3-*O*-methyl)gallate and (−)-epigallocatechin 3-*O*-(4-*O*-methyl)gallate, respectively. The oolong tea leaves contained 0.34% (dry weight) C-1 and 0.20% C-2. Traces of C-2 were detected in only 1 of 15 varieties of green tea tested. C-1 was detected in 13 of 15 green tea varieties; C-1 was most concentrated in tea cultivars classified as Assam hybrids (0.50−0.82% of dry weight). Quantitative analyses of green tea, oolong tea, and black tea manufactured from same batches of tea leaves showed that neither catechin derivative was produced during the fermentation process. Oral doses of C-1 and C-2 (5−50 mg/kg) significantly inhibited type I allergic (anaphylactic) reactions in mice sensitized with ovalbumin and Freund's incomplete adjuvant. These inhibitory effects exceeded that of the major tea catechin, (−)-epigallocatechin 3-*O*-gallate, which has known antiallergic properties.

Keywords: *Tea; catechin; antiallergic reaction; O-methylated galloyl epigallocatechin; EGCG*

INTRODUCTION

Tea (*Camellia sinensis*), one of the most popular beverages, contains various beneficial constituents. Several in vitro and in vivo studies have demonstrated that constituents of tea exhibit biological and pharmacological properties. These constituents have been reported to act in ways that are antiallergic (Kakegawa et al., 1985; Wagner, 1989; Ohmori et al., 1995; Shiozaki et al., 1997; Matsuo et al., 1997), antioxidative (Khan et al., 1992; Yoshino et al., 1994; Sano et al., 1995; Serafini et al., 1996), antimutagenic/anticarcinogenic (Wang et al., 1989; Jain et al., 1989; Yamane et al., 1991; Yen and Chen, 1995), antiatherosclerotic (Miura et al., 1994), and antibacterial (Fukai et al., 1991). Catechins apparently play a significant role in these actions. The major catechins in tea leaves are (−)-epigallocatechin 3-*O*-gallate (EGCG), (−)-epigallocatechin (EGC), (−)-epicatechin 3-*O*-gallate (ECG), and (−)-epicatechin (EC) (Figure 1). Epicatechins account for up to 15% (w/w) of the dry leaves; EGCG comprises 6−10% of the dry weight.

Epidemiological studies (Marks and Marks, 1993; Altman and Chiaramonte, 1997) have shown that the incidence of food allergy is increasing worldwide. Food allergy is generally classified as an immediate hypertensive (type I) allergy. Shiozaki et al. (1997) recently reported that EGCG strongly inhibited type I allergic reactions in rats. The release of chemical mediators (such as histamine and leukotrienes) from mast cells, which is activated by specific allergens and IgE, triggers type I allergic diseases (Pearce et al., 1984). Matsuo et al. (1997) also reported that, among tea catechins,



Catechins	R_1	R_2	R_3	R_4
(−)-Epigallocatechin-3-*O*-gallate (EGCG)	OH	G	OH	OH
(−)-Epicatechin-3-*O*-gallate (ECG)	H	G	OH	OH
(−)-Epigallocatechin (EGC)	OH	OH	–	–
(−)-Epicatechin (EC)	H	OH	–	–
(−)-Epigallocatechin-3-*O*-(3-*O*-methyl)gallate (C-1)	OH	G	OCH_3	OH
(−)-Epigallocatechin-3-*O*-(4-*O*-methyl)gallate (C-2)	OH	G	OH	OCH_3

Figure 1. Chemical structures of tea epicatechins and epicatechin derivatives.

EGCG most strongly inhibited histamine release from rat peritoneal exudate cells including mast cells when the cells were stimulated with a calcium ionophore or compound 48/80. The concentrations of the major catechins in oolong (semifermented) tea and in black (fermented) tea are lower than those in green (unfermented) tea, because these catechins are oxidized by polyphenol oxidase during fermentation. On the other hand, Yamamoto et al. (1996) demonstrated that infusions of oolong tea manufactured in Taiwan significantly inhibited the release of histamine from mouse mast cells and that the inhibitory activity exceeded that of green tea infusions prepared from the cultivar Yabukita.

In this paper, we report two novel antiallergic catechins isolated from Taiwanese oolong tea (Tong ting) and the concentrations of these catechins in various tea cultivars.

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MATERIALS AND METHODS

Chemicals. The allergen ovalbumin (OVA) was purchased from Sigma (St. Louis, MO), and Freund's incomplete adjuvant (FIA) and diphenhydramine hydrochloride were purchased from Wako Pure Chemical Co. Ltd. (Osaka, Japan). EGCG was prepared from green tea, and its purity (~98%) was confirmed by ^{1}H NMR. Other epicatechins (EC, ECG, EGC) and gallocatechin (GC) were purchased from Kurita Co. (Tokyo, Japan).

Tea. Samples of Tong ting oolong tea, which is manufactured from tea leaves cultivated in central Taiwan, were obtained from markets in Japan and Taiwan. All other teas were cultivated at the plantation of the National Research Institute of Vegetables, Ornamental Plants and Tea in Kanaya, Shizuoka. Freshly picked tea leaves were dried in a microwave oven and stored in a refrigerator before analysis. The extraction and isolation of antiallergic catechins (C-1 and C-2) were performed according to the method previously reported (Miyase et al., 1992).

Analysis of Catechins. The major catechins (EGCG, ECG, EC, EGC, GC), C-1, and C-2 in tea leaves were determined according to the modified HPLC method of Umegaki et al. (1996) as described previously. Briefly, tea catechins were extracted from 100 mg of tea leaf powder by 10 mL of acetonitrile/distilled water (1:1) at room temperature, and the sample solution was used for HPLC analysis. Aliquots of the solution were applied to a Develosil PhA-5 analytical column (4.6 mm × 250 mm; Nomura Chemicals, Seto, Japan) held at 30 °C. The mobile phase, consisting of 0.1 M NaH_2PO_4 buffer (pH 2.5)/acetonitrile (85:15) containing 0.1 mM EDTA2Na was driven at 1.0 mL/min. The catechins were detected electrochemically at an applied potential of 0.6 V.

Assay of Antiallergic Activity. The in vivo type I antiallergic activity of tea catechins was determined using mouse abdominal wall (AW method) according to Kataoka et al. (1997), with slight modification. Briefly, male *dd*Y mice (Japan SLC, Hamamatsu, Japan) at 5 weeks of age were sensitized intraperitoneally with a 1:1 mixture of OVA (2 mg/mL of N-saline) and FIA. The catechin sample (or distilled water as control) was administered orally to mice 9 days after initial exposure to OVA. Sixty minutes after the administration of the sample, a 0.1 mL solution of Evans blue dye (10 mg/mL of N-saline) was injected intravenously. The 60-min interval was chosen to obtain maximum activity on the basis of preliminary experiments with EGCG and the derivatives at doses of 10−50 mg/kg. Within 5 min after the injection of dye, the abdominal skin of the mice was detached under ether anesthesia, without injury to the abdominal wall. Five minutes after injection of the dye, 50 μL of OVA solution (5 μg/site) was injected in the exposed abdominal wall. The mouse was killed by cervical dislocation 7 min after the challenge, and then the abdominal wall was removed. The area of the abdominal wall permeated by blue dye was measured using a densitograph with spot image processing software (Atto AE-6920, Tokyo, Japan). Antiallergic activity was expressed as the inhibition ratio compared with control.

Statistical Analysis. The results were expressed as mean ± SEM, and the statistical significance was determined by the nonparametric Mann−Whitney U test.

RESULTS

Extraction and Isolation of Compounds 1 (C-1) and 2 (C-2). As shown in Figure 2, two novel antiallergic catechins were isolated from infusions of Taiwanese oolong tea. Three hundred grams of Tong ting oolong tea was extracted with 500 mL of boiling water for 30 min. The extract was passed through a porous polymer gel (Mitsubishi Diaion HP-20) column (9 × 40 cm). After the column had been washed with water, the adsorbed material was eluted with water/methanol (1:1) to give a pale brown residue (31 g). The residue was chromatographed on a silica gel column (9 × 25 cm) and eluted with chloroform/methanol/water (82:16:2) and subsequently with chloroform/methanol/water (40:50:10) to yield six fractions (A−F). Fraction C (2.657 g) was rechromatographed on a reversed-phase (ODS) column (5 × 50 cm, × 2) and eluted with water/acetonitrile (88:12) to give 241 mg of C-1 and 101 mg of C-2.

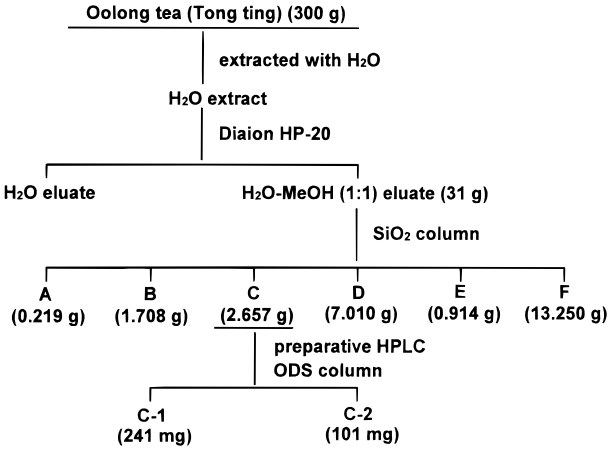

Figure 2. Flow sheet for extraction and purification of C-1 and C-2 from oolong tea.

Structure of Catechin Derivatives. Compound C-1 was obtained as an amorphous powder. The ^{1}H NMR spectrum was similar to that of EGCG (Davis et al., 1996) except for the presence of a methoxyl proton signal at δ 3.82 (3H, s) and two aromatic proton signals at δ 7.06 (1H, d, J = 2 Hz) and 7.12 (1H, d, J = 2 Hz). On irradiation of the methoxyl proton signal, nuclear Overhauser effect was observed at the aromatic proton signal at δ 7.06. From these data C-1 was assumed to be (−)-epigallocatechin 3-*O*-(3-*O*-methyl)gallate and was assigned by comparison of the reported NMR data (Davis et al., 1996; Saijo, 1982). Compound C-2 was obtained as an amorphous powder. The FAB-MS of C-2 showed a pseudo molecular ion $[M + H]^+$ at m/z 473, consistent with a molecular formula of $C_{23}H_{20}O_{11}$. The ^{1}H NMR spectrum was also similar to that of EGCG and showed a methoxyl proton signal at δ 3.83 (3H, s), two methine proton signals at δ 5.06 (1H, br s) and 5.54 (1H, m $W_{1/2}$ = 9 Hz), methylene proton signals at δ 2.93 (1H, dd, J = 17.5, 2.5 Hz) and 3.04 (1H, dd, J = 17.5, 4.5 Hz), and four aromatic proton signals at δ 6.03 (1H, d, J = 2.5 Hz), 6.05 (1H, d, J = 2.5 Hz), 6.64 (2H, br s), and 7.00 (2H, s). The methoxyl proton signal was correlated to the carbon signal at δ 60.5 in the HMQC spectrum, suggesting that the methoxyl group was located at the hindered site by di-ortho-substitution (Kaufman et al., 1989). In the HMBC spectrum, the methoxyl proton signal was correlated to the carbon signal at δ 140.5 and the equivalent aromatic proton signal at δ 7.00 was correlated to the carbon signals at δ 140.5 and 166.0, which were assigned to an ester carbonyl carbon. The carbonyl carbon signal was correlated to a methine proton signal at δ 5.54, which was assigned to H-3 of the catechin skeleton. Therefore, the structure of C-2 was decided to be (−)-epigallocatechin 3-*O*-(4-*O*-methyl)-gallate (see Figure 1).

Type I Antiallergic Activity of Tea Catechins. A significant antigen-specific anaphylactic reaction was detected 9 days after sensitization with OVA and the adjuvant. Oral administration of EGCG inhibited the anaphylactic reaction induced in mouse abdominal wall by 12.6, 26.9, and 22.1% after a single dose of 10, 50, or 100 mg/kg, respectively (Figure 3). The strongly inhibitory effects of C-1 and C-2 were dose-dependent; C-1

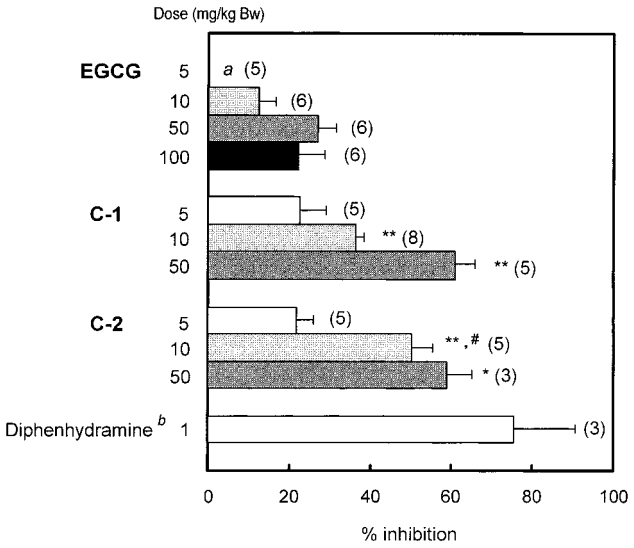

Figure 3. Effect of orally administered catechin on the anaphylactic reaction in *dd*Y mice. Values are mean ± SEM. Figures in parentheses indicate number of mice. [a]Not effective [b]Diphenhydramine, H1 blocker, was used as positive control. *Significantly different from the corresponding EGCG group at a dose of 10 or 50 mg/kg (*, $P < 0.05$; **, $P < 0.01$). #Significantly different from the corresponding C-1 group at a dose of 10 mg/kg (#, $P < 0.05$).

Table 1. Catechin Contents of Various Tea Leaves

	% of dry wt of tea leaves[a]							
tea variety	EGCG	EGC	ECG	EC	C	GC	C-1	C-2
green tea								
Yabukita	9.22	3.45	1.78	0.97	0.30	0.18	ND[b]	ND
Asahi	6.94	4.05	2.37	1.36	0.60	0.41	0.06	ND
Hatsumomiji	9.23	7.77	2.33	1.75	0.32	0.46	ND	ND
Takachiho	6.70	4.39	2.68	1.75	0.40	0.40	0.02	ND
Seishin taipan	6.60	3.62	1.22	0.72	0.15	0.19	0.22	0.10
Izumi	11.35	3.98	2.69	1.00	0.41	0.49	0.03	ND
Sansashiran	7.23	4.31	1.71	1.06	0.28	0.26	0.17	ND
Benihomare	10.09	4.43	2.36	1.08	0.24	0.30	0.50	ND
Benifuji	10.11	3.67	1.59	0.76	0.26	0.32	0.82	ND
Benifuuki	9.73	3.97	2.93	1.14	0.32	0.43	0.78	ND
Ohba oolong	6.98	3.66	1.14	0.83	0.34	0.31	0.23	ND
Seishin oolong	6.88	3.24	1.05	0.76	0.29	0.24	0.18	ND
Taiwan lineage 1	8.47	4.23	2.92	1.50	0.33	0.24	0.03	ND
Taiwan lineage 2	7.27	5.16	1.88	1.31	0.47	0.45	0.24	ND
IND113	12.12	5.64	3.32	1.27	0.53	1.16	0.24	ND
oolong tea								
Tong ting	6.58	6.12	0.82	0.79	0.07	0.36	0.34	0.20

[a] Values are means of duplicate determinations. Detection limits calculated for some catechins were ~0.003−0.014% (S/N = 3). [b] ND, not detected.

and C-2 were significantly more inhibitory than corresponding doses of EGCG. In particular, C-2 was a potent inhibitor (with 50% inhibitory effect) at a dose of ~10 mg/kg.

Catechin Content of Tea Leaves. The concentrations of C-1, C-2, and catechin in various tea leaves were examined by HPLC analysis with ECD detection (Table 1). C-1 represented 0.02−0.82% of dry weight in Tong ting oolong tea and in 13 of the 15 green teas tested. The cultivars Benihomare, Benifuji, and Benifuuki, classified as Assam hybrids, contained more C-1 than the other tea cultivars, and only the cultivars Seishin taipan and Tong ting oolong tea contained C-2. Under these analytical conditions, neither catechin derivative was detected in the cultivar Yabukita, which accounts for >90% of the green tea manufactured in Japan. All tea leaves shown in Table 1 were unfermented, except

Table 2. Changes in Tea Catechins during the Process of Fermentation

	% of dry wt of tea leaves[a]							
tea variety	EGCG	EGC	ECG	EC	C	GC	C-1	C-2
Benihomare								
green tea	9.74	4.58	2.27	1.11	0.18	0.24	0.58	ND[b]
oolong tea	9.20	3.52	2.02	0.90	0.21	0.22	0.47	ND
black tea	0.34	0.13	0.05	0.08	0.02	0.07	ND	ND
Benifuji								
green tea	11.84	4.28	1.95	0.86	0.26	0.26	1.06	ND
oolong tea	9.11	3.26	1.52	0.77	0.29	0.26	0.77	ND
black tea	0.24	0.08	0.06	0.06	0.02	0.04	0.04	ND
Izumi								
green tea	13.11	4.76	2.91	1.06	0.36	0.36	0.04	ND
oolong tea	10.99	3.83	2.51	0.86	0.33	0.39	0.02	ND
black tea	0.48	0.09	0.10	0.10	0.05	0.07	0.02	ND

[a] Values are means of duplicate determinations. Detection limits calculated for some catechins were ~0.003−0.014% (S/N = 3). [b] ND, not detected.

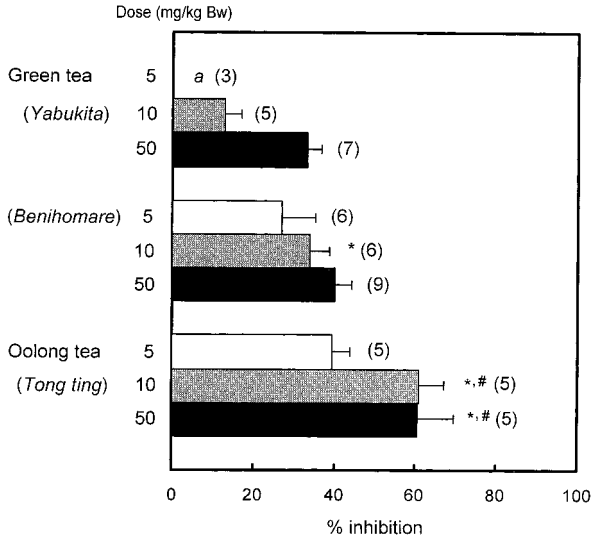

Figure 4. Effect of orally administered catechin mixture on the anaphylactic reaction in *dd*Y mice. Values are mean ± SEM. Figures in parentheses indicate number of mice. [a]Not effective. * Significantly different from the corresponding Yabukita group at a dose of 10 or 50 mg/kg (*, $P < 0.01$). # Significantly different from the corresponding Benihomare group at a dose of 10 or 50 mg/kg (#, $P < 0.05$).

Tong ting oolong. Neither C-1 nor C-2 was produced during fermentation of tea leaves (Table 2); neither compound was produced in green tea, oolong tea, or black tea manufactured from the same batches of tea leaves of each cultivar Benihomare, Benifuji, and Izumi. This finding suggests that the catechins occur naturally in these cultivars.

Antiallergic Activity of Catechin Mixture. The effects of the catechin mixture on the anaphylactic reaction are shown in Figure 4. The samples were catechin mixtures fractionated from the tea cultivars shown in Table 1. Tong ting oolong tea had the most inhibitory effect, then Benihomare green tea, and then Yabukita green tea. There were relatively small differences between the effects of the mixture and single, corresponding doses of pure C-1, C-2, or EGCG, although the mixture contained catechins that have little or no antiallergic activity.

DISCUSSION

Tea catechins are known to inhibit histamine release from mast cells activated by cross-linking of specific

allergen and IgE (Pearce, 1984). Some reports about the relationship between the inhibitory activity of histamine release and the chemical structure of catechin suggest that the gallo-type structure in catechins plays an important role in the inhibitory effect on histamine release (Ohmori et al., 1995; Matsuo et al., 1997). EGCG, which has two gallo-type structures, exerts a stronger effect than ECG or EGC, which each have one gallo-type structure. The importance of the hydroxy moiety on the B ring is also evident from the results of the comparison between EC and EGC (Ohmori et al., 1995). The two antiallergic catechins presented in this study were *O*-methylated forms of the galloyl moiety in EGCG, and their antiallergic activities were much higher than that of EGCG. Although this is a preliminary in vitro study, C-2 strongly inhibited histamine release from IgE/antigen-stimulated mouse mast cells (PT-18 cells); 45% inhibition resulted at a concentration of 50 μg/mL reaction mixture, but only 22% inhibition resulted from EGCG given at 100 μg/mL. The inhibitory effect of C-1 is about the same as that of EGCG. These results suggest that the 3-*O*- or 4-*O*-methylation of the galloyl moiety in EGCG might enhance the antiallergic response.

EGCG binds readily with plasma protein (Sazuka et al., 1996), and EGCG in plasma and bile partly forms the dimerized products (Tomita et al., 1998). When EGCG and the methylated forms (500 μg/mL) were incubated with fresh mouse plasma at 37 °C, ~50% of the EGCG was degraded during a 15-min incubation. However, during the same period, only 10% of the methylated forms such as C-1 and C-2 were degraded (data not shown). We speculate that the higher antiallergic activity of the *O*-methylated forms of EGCG might be associated with stability in vivo, but further investigations are needed. The administration of the tea catechin mixture resulted in a strong antiallergic effect (see Figure 4). The magnitude of antiallergic effect was similar to or slightly less than that elicited by the administration of pure EGCG, C-1, or C-2 at the same dose (see Figure 3). Chen et al. (1997) reported pure EGCG is eliminated more readily from the body than EGCG in decaffeinated green tea extract. They speculated that other constituents in the tea extract compete with EGCG for binding to plasma and tissue proteins and also for metabolic enzymes. Our results support their hypothesis that reduction in pharmacological activities that results from binding with plasma and tissue protein can be attenuated by the competitive binding reaction among polyphenols, such as those in the catechin mixture (see Figure 4).

Recently, Piskula and Terao (1998) detected the *O*-methylated form of EC in rat plasma after epicatechin administration. It is well-known that catechol-*O*-methyl transferase catalyzes *O*-methylation of various plant polyphenols (Guldberg and Marsden, 1975), and we have found that C-1 and C-2 were produced when EGCG was incubated with mouse liver homogenate or authentic catechol-*O*-methyltransferase in the presence of *S*-adenosylmethionine. Interestingly, more C-2 than C-1 was produced (data not shown). Therefore, EGCG in vivo may also be partly converted to C-2 and C-1 by the methyltransferase, and these catechin complexes may have antiallergic properties.

In conclusion, we demonstrated that two *O*-methylated galloyl epigallocatechins have potent antiallergic effects and that their concentrations differ markedly among tea cultivars. Tea extracts that contain these constituents may be effective in the treatment of allergic disorders.

ABBREVATIONS USED

C-1, (−)-epigallocatechin 3-*O*-(3-*O*-methyl)gallate; C-2, (−)-epigallocatechin 3-*O*-(4-*O*-methyl)gallate; EGCG, (−)-epigallocatechin 3-*O*-gallate; EGC, (−)-epigallocatechin; ECG, (−)-epicatechin 3-*O*-gallate; EC, (−)-epicatechin; GC, (+)-gallocatechin; C, (+)-catechin; OVA, ovalbumin; FIA, Freund's incomplete adjuvant; AW method, abdominal wall method.

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