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Synthesis, Structure Analyses, and Characterization of Novel Epigallocatechin Gallate (EGCG) Glycosides Using the Glucansucrase from *Leuconostoc mesenteroides* B-1299CB

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In this study, three epigallocatechin gallate glycosides were synthesized by the acceptor reaction of a glucansucrase produced by *Leuconostoc mesenteroides* B-1299CB with epigallocatechin gallate (EGCG) and sucrose. Each of these glycosides was then purified, and the structures were assigned as follows: epigallocatechin gallate 7-*O*-α-D-glucopyranoside (EGCG-G1); epigallocatechin gallate 4′-*O*-α-D-glucopyranoside (EGCG-G1′); and epigallocatechin gallate 7,4′-*O*-α-D-glucopyranoside (EGCG-G2). One of these compounds (EGCG-G1) was a novel compound. The EGCG glycosides exhibited similar or slower antioxidant effects, depending on their structures (EGCG ≥ EGCG-G1 > EGCG-G1′ > EGCG-G2), and also manifested a higher degree of browning resistance than was previously noted in EGCG. Also, EGCG-G1, EGCG-G1′, and EGCG-G2 were 49, 55, and 114 times as water soluble, respectively, as EGCG.

KEYWORDS: ***Leuconostoc mesenteroides*** **glucansucrase; EGCG; glycoside; acceptor reaction**

INTRODUCTION

Green tea, a popular and commonly consumed beverage in Asia, is an important source of flavonoids called catechins. The green tea catechins, specifically epigallocatechin gallate (ECGC), epigallocatechin (EGC), epicatechin gallate (ECG), and epicatechin (EC), have been extensively studied for their utility as bioactive substances for use as antioxidant and anticancer agents (*1*, *2*). EGCG is the most abundant of the green tea catechins (*3*). It exhibits potent antioxidant (*4*), anticancer (*5*), antitumorigenic (*6*), and antibacterial (*7*) effects and also appears to play a part in the prevention of dental caries (*8*) and in the regulation of plasma lipid levels (*9*). However, EGCG is only minimally water soluble and is readily degraded in aqueous solutions (*10*). Due to these disadvantages, the use of EGCG in the food and cosmetic industries remains somewhat limited. To overcome this problem, a great deal of research has been done concerning the glycosylations of a variety of polyphenols (*10*−*12*). The resultant transglycosylated compounds have sometimes exhibited increased solubility in water, increased stability against light or oxidation, improved taste qualities, and stronger tyrosinase inhibitory effects (*10*−*13*). When improved in such ways, glycosylated bioactive substances have proven to be much more useful as food additives and cosmetics.

For glycosylated polyphenols, however, it is quite difficult to determine their bioavailability. Hollman and Katan found that human absorption of the quercetin glycosides (52%) from onions is far better than that of the pure aglycon (24%) (*14*). Researchers proposed that glycosylation might be an important factor in its bioavailability, but critically significant factors were the number and positions of the glucose moieties (*15*).

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Recently, enzymatic transglycosylation has been employed in the modification of natural bioactive compounds, in an effort to improve their physicochemical qualities. Lee et al. (*16*) reported that glycosylated ascorbic acids exhibited rather effective antioxidant properties, exerting a preventative effect against lipid oxidation, and also reported that they exhibited a synergistic effect superior to that observed for normal ascorbic acid. Gilly et al. (*17*) also demonstrated that the glycosylation of resveratrol resulted in a strengthening of this compound's preventative effect against enzymatic oxidation. Glycosylated naringin was 250 times more water soluble than naringin and 10 times less bitter (*18*). Li et al. (*19*) reported that the solubility of glycosylated puerarin was 14−168 times higher than that of puerarin. Glycosylated catechin was determined to be quite stable against ultraviolet (UV) radiation, although catechin could be degraded fairly readily (*11*).

Glucosyltransferases (GTFs) generated by *Leuconostoc mesenteroides* and *Streptococcus mutans* synthesize dextrans or glucans, using sucrose (*20*). Robyt et al. (*21*) demonstrated that glucosyltransferases can transfer the glucose originating from sucrose to other carbohydrates and then induce oligosaccharide synthesis. This reaction is known as the *acceptor reaction*, and the carbohydrates added in the reaction are referred to as *acceptors* (*22*). In the presence of a variety of acceptors, GTFs can transfer mono-dp, di-dp, or higher dp glucooligosaccharides to the acceptor, via the formation of diverse glycosidic linkages (*23*).

The dextransucrase isolated from *L. mesenteroides* NRRL B-1299 has been shown to synthesize two forms of dextrans: fraction L, which is precipitated by 38% ethanol and contains 27% α-1,2 and 1% α-1,3 branched glucan; and fraction S, which is precipitated by 40% ethanol and contains 35% α-1,2 branch glucan (*24*−*26*). Dols et al. (*27*) also conducted an acceptor reaction, using B-1299 in the presence of maltose and sucrose. In this case, a primarily α-1,6-linked glucooligosaccharide was formed, which also featured α-1,2 branch linkages. Recently, a series of acceptor reactions have been conducted in a variety of studies, using various polyphenols with the glucansucrase from *L. mesenteroides*. Yoon et al. (*28*) synthesized two acarbose analogues using glucansucrases from *L. mesenteroides* B-512FMC and B-742CB. Kim et al. (*29*) reported that such acarbose glycosides exerted marked inhibitory effects against α-glucosidase, α-amylase, and cyclomaltodextrin glucanosyltransferase. Seo et al. (*23*) synthesized a variety of salicin and phenol glycoside structures by carrying out of glucansucrase acceptor reactions. That group determined that the salicin glycosides exerted an inhibitory effect against blood coagulation. *L. mesenteroides* NRRL B-1299CB is a constitutive mutant, developed via the EMS method (*30*). 1299CB glucansucrase in culture media, therefore, does not form a complex with dextran, thereby ensuring higher acceptor reaction efficiency.

In this study, we investigated the enzymatic synthesis of EGCG glycosides, in which a D-glucopyranosyl residue is attached to the 7-hydroxyl group of the A ring (EGCG-G1) or to the 4′-hydroxyl group of the B ring (EGCG-G1′), as well as both the 7- and 4′-hydroxyl groups of EGCG (EGCG-G2), using the glucansucrase from *L. mesenteroides* B-1299CB. EGCG-G1 was enzymatically synthesized for the first time in this study, and EGCG-G1′ and EGCG-G2 were previously synthesized by Nanjo et al. from the reaction of cyclodextrin glucanotransferase of *Bacillus stearothermophilus* with α-cyclodextrin (*32*). All of these EGCG glycosides exhibited distinct physical and biochemical properties.

MATERIALS AND METHODS

Materials. EGCG, dimethyl-d_6 sulfoxide (DMSO-d_6), 1,1-diphenyl-2-picrylhydrazyl (DPPH), and 2,5-dihydroxybenzoic acid (DHB) were obtained from the Sigma Chemical Co. The Sephadex LH-20 gel was acquired from Amersham Biosciences (Uppsala, Sweden).

Enzyme Preparation. *L. mesenteroides* B-1299CB was grown at 28 °C on LM medium containing 2% (w/v) glucose as a carbon source. The LM medium used in this study consisted of 4 g of yeast extract, 2 g of peptone, 0.2 g of $MgSO_4·7H_2O$, 0.01 g of $FeSO_4·7H_2O$, 0.01 g of NaCl, 0.01 g of $MnSO_4·H_2O$, 0.015 g of $CaCl_2·2H_2O$, and 2 g of K_2HPO_4 per liter of deionized water. After fermentation, the culture was harvested, centrifuged, and concentrated, using 30K cutoff hollow fibers (Millipore).

Glucansucrase Activity Assays. Enzyme activity was assayed via the incubation of the enzyme for different reaction periods at 28 °C with 100 mM sucrose, using 20 mM sodium acetate (pH 5.2) as a substrate. The standard assay mixtures consisted of 200 μL of 200 mM sucrose and 200 μL of an enzyme solution. Each of the enzyme reaction samples was spotted onto a Whatman K5 TLC plate (Whatman Inc, Clifton, NJ). The TLC plates were developed twice in an acetonitrile/water (85:15, v/v) solvent system. Each of the carbohydrates was visualized by dipping the plates into 0.3% (w/v) *N*-(1-naphthyl)-ethylenediamine and 5% (v/v) H_2SO_4 in methanol, followed by 10 min of heating at 121 °C (*33*). The quantity of fructose released from the sucrose was then analyzed using the NIH Image Program, using the standard materials (*34*). One unit of glucansucrase activity, for the purposes of this study, was defined as the quantity of enzyme required to produce 1 μmol of fructose per minute at 28 °C and a pH of 5.2, in 20 mM sodium acetate buffer.

Glucosylation of EGCG. The reaction mixture (250 mL) was consisted of 0.2% EGCG (500 mg), 80 mM sucrose (6.84 g), and B-1299CB glucansucrase (2.4 units/mL). The mixture was incubated at 28 °C for 6.5 h, after which the sucrose had been depleted. The reaction mixture was then boiled for 5 min to halt the enzyme reaction.

Analysis of Acceptor Reaction Product by Thin-Layer Chromatography (TLC). TLC was conducted at room temperature, using a silica gel 60 F_{254} TLC plate (Merck Co.). One microliter of the reaction digests was then spotted onto the silica gel plate, and the plate was developed using a solvent mixture of ethyl acetate/acetic acid/water (3:1:1, v/v/v). The developed plate was then dried and visualized as was previously described (*33*).

Purification of EGCG Acceptor Reaction Products. The reaction digests (250 mL) were then subjected to Sephadex LH-20 column (47 × 200 mm) chromatography. The transfer products were washed in distilled water (total 3 L, flow rate = 1 mL/min) to remove the sugars (dextran, fructose, and glucose) from the reaction digest and then successively eluted with 70% (v/v) ethanol (1 L). The eluant (containing the EGCG glycosides) was concentrated at 47 °C with a rotary evaporator and then subjected to HPLC on an LC-10AD instrument (Shimadzu, Kyoto, Japan) under the following conditions: reverse column, 400 × 3.9 mm i.d. μ-Bondapak C_{18} (Waters); mobile phase, 23% methanol; flow rate, 0.5 mL/min; room temperature; detection, RID-10A model RI detector (Shimadzu).

Matrix-Assisted Laser Desorption Ionization Time of Flight Mass Spectrometry (MALDI-TOF-MS) Analysis. The purified EGCG glycosides (3 mg/mL) were diluted with deionized water and then mixed with DHB (1 mg/mL) dissolved in acetonitrile, at a 1:1 ratio (v/v). The mixed solution (1 μL) was then spotted onto a stainless steel plate and slowly dried at room temperature. The mass spectrum was obtained using a Voyager DE-STR MALDI-TOF mass spectrometer (Applied Biosystems). Mass spectra were obtained in positive linear mode with delayed extraction (average of 75 laser shots), with an acceleration voltage of 65 kV.

Nuclear Magnetic Resonance (NMR) Analysis. About 2−3 mg of the purified EGCG glycosides was dissolved in DMSO-d_6 (250 μL) and then placed into 3 mm NMR tubes. The NMR spectra were obtained on a Unity Inova 500 spectrometer (Varian Inc.), operating at 500 MHz for ^1H and at 125 MHz for ^{13}C, at 25 °C. Linkages between EGCG and glucose were evaluated using the spectra obtained via homonuclear

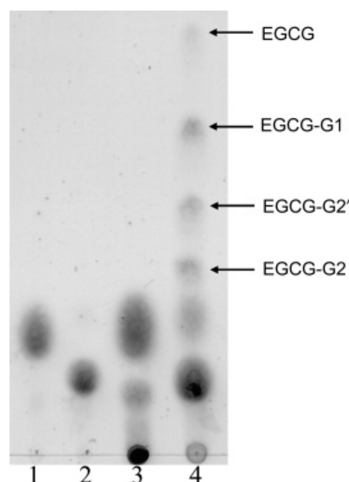

Figure 1. Thin-layer chromatogram of the glucansucrase acceptor reaction digests: lane 1, glucose; lane 2, sucrose; lane 3, enzyme reaction digest (without EGCG); lane 4, enzyme reaction digest with EGCG. Arrows indicate EGCG acceptor reaction products. Because of EGCG inhibition effect of glucansucrase, the amounts of sucrose left in lane 4 were more than those in lane 3.

correlation spectroscopy (COSY), heteronuclear single quantum coherence (HSQC), and heteronuclear multiple bond correlation (HMBC).

Antioxidant Activity. The antioxidant activities of the EGCG or of each of the EGCG glycosides were assessed via DPPH radical scavenging (*35*). Each sample (10, 12.5, 25, 50, 100, 200 μM) was dissolved in ethanol (30 μL) and mixed thoroughly with a 100 μM DPPH ethanol solution (270 μL). After 10 min of maintenance at room temperature in darkness, the absorbance of the mixture was monitored at 517 nm on a SmartSpec 3000 spectrophotometer (Bio-Rad). DPPH radical-scavenging activity was determined according to the decrease in absorbance of the DPPH radical in the sample, as compared to that observed with a blank (ethanol). SC_{50} designates the concentration of the sample in which the levels of DPPH radicals were reduced by 50%.

Browning-Resistant Effect of EGCG and Its Glycosides. Browning resistance after UV irradiation in an aqueous system was evaluated in water (1.0 mL) containing 0.25% (w/v) EGCG or the EGCG glycosides. The sample solutions were then exposed to UV irradiation at a distance of 10 cm from the 254 nm, 10 W, G10T8-AN UV source (Germicidal, Sankyo Denki) for 24 h at room temperature. Increases in absorbance at 460 nm were then determined with a SmartSpec 3000 spectrophotometer (Bio-Rad).

Water Solubility Analysis. All excess EGCG and EGCG glycosides were mixed in 200 μL of water in an Eppendorf tube, at room temperature. A 3510R-DTH ultrasonic cleaner (Branson, Danbury, CT) was used to maximize solubility. After 1 h of sonication at room temperature, each of the samples was diluted and then filtered through a 0.45 μm MFS membrane (Adventec, Pleasanton, CA) for HPLC analysis, to determine the concentrations. A model 1525 HPLC system, connected to a 400 × 3.9 mm i.d. μ-Bondapak C_{18} column (Waters, Milford, MA) and a model 2487 UV detector (Waters) at 280 nm were utilized to quantify the amounts of EGCG and EGCG glycosides. The mobile phase consisted of 23% methanol and was conducted via the isocratic method, with a flow rate of 0.5 mL/min. The concentrations of the EGCG and EGCG glycosides were calculated as was described previously by Li et al. (*19*).

RESULTS AND DISCUSSION

Acceptor Reaction of EGCG and Purification of EGCG Glycosides. After conducting the acceptor reaction using *L. mesenteroides* NRRL B-1299 glucansucrase with EGCG and sucrose, we detected three reaction products via TLC analysis (**Figure 1**, lane 4). After conducting Sephadex LH-20 chromatography followed by HPLC purification (**Figure 2**), we obtained the following yields for EGCG-G1, EGCG-G2, and

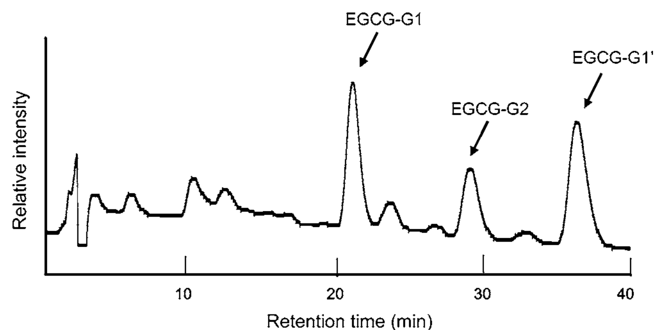

Figure 2. HPLC chromatogram of EGCG glycosides after Sephadex LH-20 column chromatography.

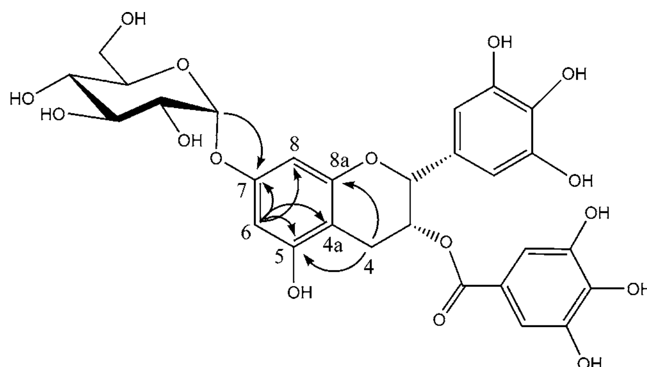

Figure 3. HMBC correlations of EGCG-G1.

EGCG-G1′: 45.4 mg (9.1% of EGCG), 99.5 mg (19.9%), and 43.5 (8.7%) mg, respectively.

Structural Determination of EGCG Glycosides. The numbers of glucose units attached to the purified EGCG-G1, EGCG-G1′, and EGCG-G2 were verified via MALDI-TOF MS analysis. The molecular weights of the glycosides had increased above that of EGCG by exactly one glucose residue addition; EGCG-G1 and EGCG-G1′ contained one attached glucose, and EGCG-G2 contained two attached glucose residues. The glucosidic linkages were determined via ^{1}H, ^{13}C, ^{1}H-COSY, HSQC, and HMBC analyses, and the results are summarized in **Table 1**.

EGCG-G1. The molecular ions of EGCG-G1 were observed at *m/z* 643 $(M + Na)^+$. In **Table 1**, a doublet signal at 5.28 ppm (J = 3.5 Hz) was assigned to the anomeric proton, showing that only one glucosyl residue is α-linked to the EGCG. Almost all of the carbon signals assigned to the EGCG moiety were identical to those of EGCG, except for the assignment of signals at 100.9 ppm to C-4a, at 96.4 ppm to C-6, and at 97.5 ppm to C-8. These signals showed downfield shifts of 3.3, 1.9, and 1.8 ppm, indicating that the transferred glucosyl residue had been attached to C-7 in the EGCG. In our HMBC data, the H-6 was observed at 6.18 ppm, and the couplings occurred with C-4a, C-5, C-7, and C-8 of the A ring; the H-4 was observed at 3.01 and 2.73 ppm, and the couplings occurred with C-5 and C-8a of the A ring (**Figure 3**). These correlations precisely assigned the carbon signals (C-5, C-7, and C-8a) of the A ring. After that, the C-1‴ of the glucosyl residue was observed at 98.6 ppm, and the coupling appeared to have occurred between proton H-1‴ of the glucosyl residue and the C-7 of the EGCG (**Figure 4A**). According to these results, we determined that the structure of EGCG-G1 could be most appropriately referred to as epigallocatechin gallate-7-*O*-α-D-glucopyranoside (**2**) (**Figure 5**).

EGCG-G1′. The molecular ions of EGCG-G1′ were observed at *m/z* 643 $(M + Na)^+$. In **Table 1**, a doublet signal at 4.87

Table 1. ^{13}C and ^{1}H NMR Data of EGCG and EGCG Analogues

	EGCG (δ)		EGCG-G1 (δ_1)			EGCG-G1′ (δ_2)			EGCG-G2 (δ_3)		
C position	δ_C	δ_H	δ_1C	($\delta_1 - \delta$)	δ_H	δ_2C	($\delta_2 - \delta$)	δ_H	δ_3C	($\delta_3 - \delta$)	δ_H
EGCG											
2	76.6	4.95 (br s)	77.1	(0.5)	5.02 (br s)	76.3	(−0.3)	5.04 (br s)	76.9	(0.3)	5.08 (br s)
3	68.2	5.36 (br s)	68.3	(0.1)	5.41 (br s)	67.9	(−0.3)	5.42 (br s)	68.1	(−0.1)	5.45 (br s)
4	25.9	2.92 (dd) (J = 4.5, 17 Hz)	26.3	(0.4)	3.01 (dd) (J = 4.5, 17 Hz)	25.7	(−0.2)	2.96 (dd) (J = 4.5, 17 Hz)	26.3	(−0.4)	3.01 (dd) (J = 4.5, 17 Hz)
		2.65 (br d) (J = 17 Hz)	26.3	(0.4)	2.73 (br d) (J = 17 Hz)			2.69 (br d) (J = 17 Hz)			2.75 (br d) (J = 17 Hz)
4a	97.6		100.9	(3.3)		97.4	(−0.2)		100.9	(3.3)	
5	155.8		156.0	(0.2)		155.4	(−0.4)		155.8	(0.0)	
6[a]	94.5	5.82 (d) (J = 2.5 Hz)	96.4	(1.9)	6.18 (d) (J = 2 Hz)	94.4	(−0.1)	5.87 (d) (J = 2 Hz)	96.5	(1.0)	6.19 (d) (J = 2 Hz)
7	156.7		157.1	(0.4)		156.6	(−0.1)		157.1	(0.4)	
8[a]	95.7	5.93 (d) (J = 2.5 Hz)	97.5	(1.8)	6.23 (d) (J = 2 Hz)	95.6	(−0.1)	5.97 (d) (J = 2 Hz)	97.6	(1.9)	6.24 (d) (J = 2 Hz)
8a	156.7		156.9	(0.2)		156.5	(−0.2)		156.9	(0.2)	
1′	128.8		128.9	(0.1)		135.1	(6.3)		135.3	(6.5)	
2′/6′	105.7	6.40 (s)	105.9	(0.2)	6.45 (s)	105.5	(−0.2)	6.51 (s)	105.9	(0.2)	6.53 (s)
3′/5′	145.6		146.1	(0.5)		150.2	(4.6)		150.7	(5.1)	
4′	132.5		132.8	(0.3)		133.3	(0.8)		133.8	(1.3)	
1′′	119.5		119.6	(0.1)		119.2	(−0.3)		119.5	(0.0)	
2′′/6′′	108.8	6.81 (s)	109.1	(0.3)	6.84 (s)	108.7	(−0.1)	6.84 (s)	109.1	(0.1)	6.84 (s)
3′′/5′′	145.8		145.9	(0.1)		145.5	(−0.3)		145.9	(0.1)	
4′′	138.7		139.0	(0.3)		138.6	(−0.1)		139.1	(0.4)	
COO–	165.4		165.7	(0.3)		165.1	(−0.3)		165.6	(0.2)	
glucose (-G1/-G1′)											
1′′′			98.6		5.28 (d) (J = 3.5 Hz)	104.0		4.87 (d) (J = 4 Hz)	98.6		5.29 (d) (J = 4 Hz)
2′′′			72.1		3.35 (dd) (J = 3.5, 9.5 Hz)	71.8		3.41 (dd) (J = 4, 9.5 Hz)	72.1		3.36 (dd) (J = 3.5, 9.5 Hz)
3′′′			73.5		3.62 (m)	73.2		3.62 (m)	73.5		3.62 (m)
4′′′			70.3		3.21 (dd) (J = 9.5, 9.5 Hz)	69.3		3.21 (dd) (J = 9.5, 9.5 Hz)	70.3		3.23 (m)
5′′′			74.1		3.51 (m)	73.9		3.98 (m)	74.1		3.50 (m)
6′′′			61.1		3.60/3.54 (m)	60.1		3.60/3.54 (m)	61.1)		3.60/3.54 (m
glucose (-G2)											
1′′′′									104.3		4.87 (d) (J = 3.5 Hz)
2′′′′									72.2		3.41 (dd) (J = 3.5, 9.5 Hz)
3′′′′									73.5		3.62 (m)
4′′′′									69.7		3.26 (m)
5′′′′									74.3		3.53 (m)
6′′′′									60.5		3.60/3.63 (m)

[a] Positions are changeable with each other.

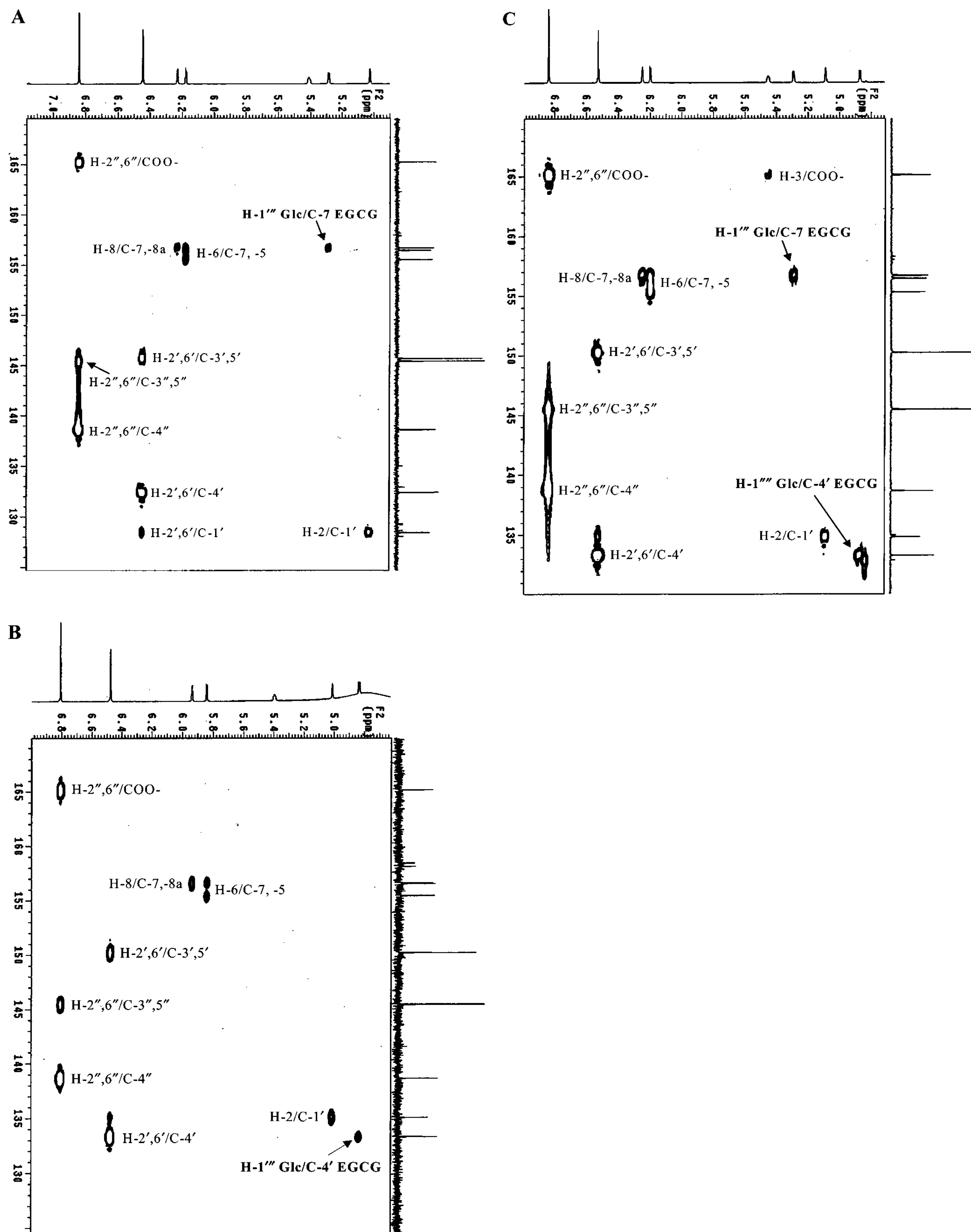

Figure 4. HMBC NMR spectra of EGCG-G1 (**A**), EGCG-G1′ (**B**), and EGCG-G2 (**C**) recorded in DMSO-d_6 at 25 °C. The NMR peaks are designated by the particular proton/carbon position on each product.

ppm ($J = 4$ Hz) was assigned to the anomeric proton, thereby showing that only one glucosyl residue was α-linked to EGCG, similarly to EGCG-G1. Almost all of the carbon signals assigned to the EGCG moiety were identical to those of EGCG, except for the assignment of signals at 135.1 ppm to C-1′ and at 150.2 ppm to C-3′/5′. These signals evidenced downfield shifts of 6.3 and 4.6 ppm, respectively, demonstrating that the transferred glucosyl residue had been attached to C-4′ in the EGCG. In

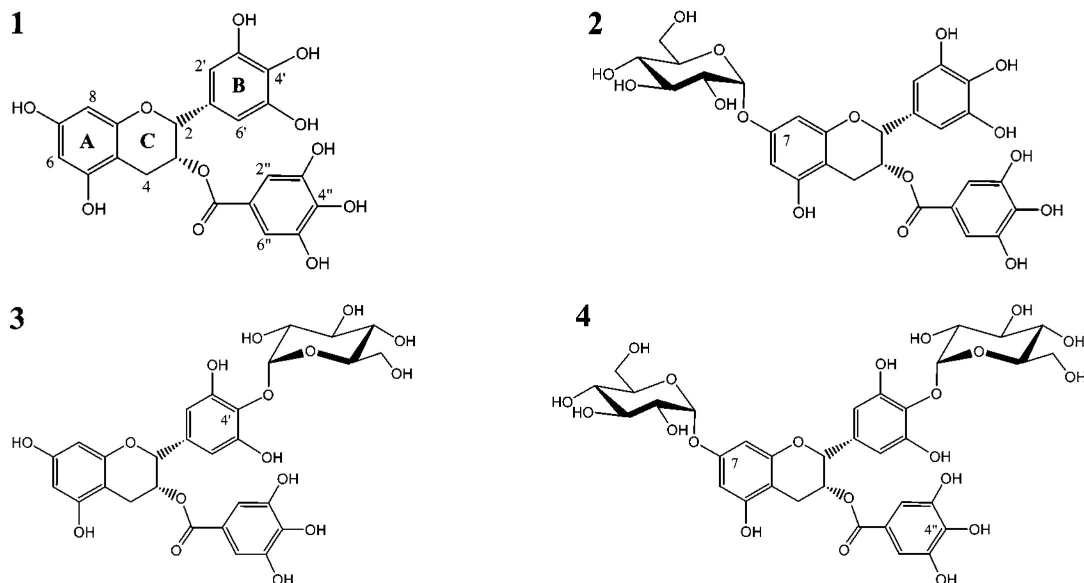

Figure 5. Structures of EGCG (**1**), EGCG-G1 (**2**), EGCG-G1′ (**3**), and EGCG-G2 (**4**).

our HMBC data, the carbon signals of the A ring of EGCG-G1′ were shown as EGCG-G1. Furthermore, the C-1‴ of the glucosyl residue was observed at 104.0 ppm, and the coupling appeared to have occurred between the H-1‴ proton of the glucosyl residue and the C-4′ of the EGCG (**Figure 4B**).

According to these results, the structure of EGCG-G1 is commensurate with the name epigallocatechin gallate-4′-*O*-α-D-glucopyranoside (**3**) (**Figure 5**).

EGCG-G2. The molecular ions of EGCG-G2 were observed at *m/z* 805 (M + Na)$^+$. In **Table 1**, two doublet signals at 4.87 ppm (J = 3.5 Hz) and 5.29 ppm (J = 4 Hz) were assigned to the anomeric protons, thereby indicating that two glucosyl residues were α-linked to EGCG. Almost all of the carbon signals assigned to the EGCG moiety were identical to those of EGCG, except for the assignment of signals at 100.9 ppm to C-4a, at 96.5 ppm to C-6, at 97.6 ppm to C-8, at 135.3 ppm to C-1′, and at 150.7 ppm to C-3′/5′. These signals evidenced downfield shifts of 3.3, 1.0, 1.9, 6.5, and 5.1 ppm, respectively, and indicate that the two glucoses had been attached to the C-7 and C-4′ of EGCG, respectively. By comparing the carbon signals of EGCG-G1 and EGCG-G2, it can be seen that the carbon signals of EGCG-G2 were almost same in their assignments. According to our HMBC data, the carbon signals of the A ring of EGCG-G2 were shown as EGCG-G1 and EGCG-G2. Furthermore, the couplings appeared to have occurred between the H-1‴ proton of the glucosyl residue and the C-7 of EGCG and also between the H-1⁗ proton of the other glucosyl residue and the C-4′ of EGCG (**Figure 4C**). These results indicate that two α-glucosidic linkages (α-1→7 and α-1→4′) were formed during the acceptor reaction and that EGCG-G2 should appropriately be referred to as epigallocatechin gallate-7,4′-*O*-α-D-glucopyranoside (**4**) (**Figure 5**).

One of the EGCG glycosides (EGCG-G1) described in this study is reported here for the first time. However, EGCG-G2 was reported previously by Nanjo et al. (*32*), who used the cyclodextrin glucanotransferase of *B. stearothermophilus.* EGCG-G1′ was also reported by Kitao et al. (*10*), who used the sucrose phosphorylase from *L. mesenteroides* in their study. That group synthesized two EGCG glycosides, epigallocatechin gallate-4′-*O*-α-D-glucopyranoside and epigallocatechin gallate-4′,4″-*O*-α-D-glucopyranoside. Both of the glycosides were formed via the attachment of glucose to the B ring or to the gallate residue.

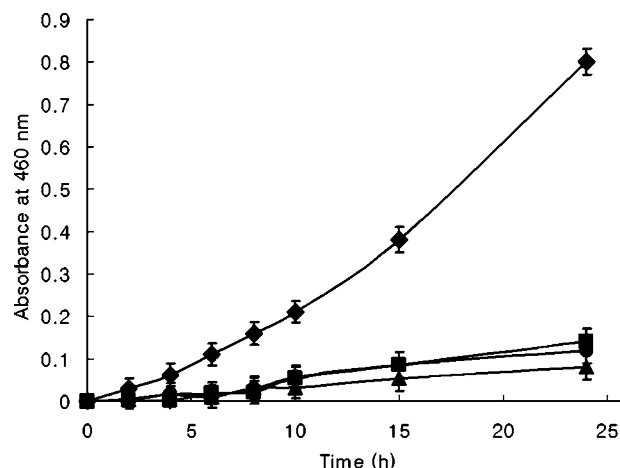

Figure 6. Browning resistance to UV irradiation of EGCG and EGCG glycosides. Each sample (0.25%, 1 mL), EGCG (□), EGCG-G1 (■), EGCG-G1′ (●), and EGCG-G2 (▲), was exposed to UV irradiation at a distance of 10 cm for 24 h at room temperature. Increases in absorbance at 460 nm were then determined.

Catechin glycoside was also formed via the attachment of glucose to the B ring, as an α-linkage (*11*, *12*).

Browning-Resistant Effect. Catechins in water are markedly susceptible to degradation and browning via UV irradiation (*11*). **Figure 6** illustrates the browning-resistant properties of EGCG glycosides after UV irradiation. EGCG exhibited rapid browning. However, the EGCG glycosides [EGCG-G1 (■), EGCG-G1′ (●), EGCG-G2 (▲)] browned at a very slow rate, even after 24 h of irradiation [EGCG-G1 (17.5% browning comopared withthat of EGCG), EGCG-G1′ (15% browning compared with EGCG), EGCG-G2 (10.1% browning compared with EGCG)]. The catechin glycoside (3′-*O*-α-D-glucopyranoside) synthesized via transglycosylation with sucrose phosphorylase also exhibited a browning-resistant quality (*11*), thus indicating that the glycosylation of the compounds conferred a degree of stability on the EGCG (catechins) with regard to UV irradiation, even though the glycosylation positions and linkages differed between the resultant compounds.

Antioxidant Activity. EGCG and its glycosides exhibited differing antioxidant effects, depending on their structural configurations. The SC_{50} of EGCG-G1, according to the results

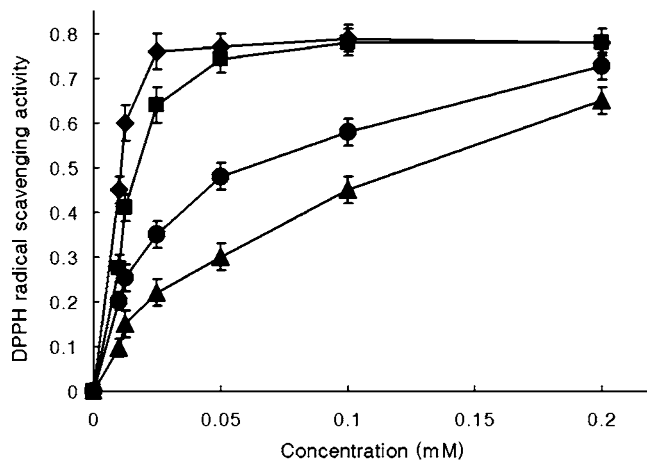

Figure 7. DPPH radical-scavenging activities of EGCG and EGCG glycosides: EGCG (□); EGCG-gG1 (■); EGCG-AgG2 (▲); EGCG-BgG2 (●). Each sample (30 μL of 10, 12.5, 25, 50, 100, or 200 μM) was mixed with 100 μM 1,1-diphenyl-2-picrylhydrazyl (270 μL) in darkness at room temperature for 10 min, and the absorbance was monitored at 517 nm. Each value is the mean ± standard deviation ($n = 3$).

Table 2. Solubilities of EGCG and EGCG Glycosides

sample	solubility in water[a] (mM)	relative solubility
EGCG	5.12 ± 2.21	1
EGCG-G1	253.92 ± 6.53	49
EGCG-G1′	281.85 ± 7.30	55
EGCG-G2	584.39 ± 12.65	114

[a] Mean ± standard deviation ($n = 3$).

of DPPH radical-scavenging measurements (**Figure 7**), was 1.1 μM, a level of activity comparable with that of purified EGCG (SC_{50} = 0.9 μM). However, the SC_{50} values for EGCG-G1′ and EGCG-G2 were 2.8 and 8 μM, both lower than that of purified EGCG. The glucosyl residue attached to the 7-hydroxyl moiety in the A ring exhibited a more profound antioxidant activity than did the EGCG-G′ in which the glucosyl residue was attached to the 4′-hydroxyl moiety in the B ring. Thus, the 4′-hydroxyl moiety in the B ring must play an important role in DPPH radical scavenging, whereas the 7-hydroxyl moiety in the A ring has a lesser influence on antioxidant activity, as was previously concluded by Valcic et al. (*36*). According to Nanjo et al., the SC_{50} values of EGCG, epigallocatechin gallate-4′-*O*-α-D-glucoside, epigallocatechin gallate-3′-*O*-α-D-glucoside, epigallocatechin gallate-7,3′-*O*-α-D-glucoside, and epigallocatechin gallate-4′,4″-*O*-α-D-glucoside were 1.2 (*33*) or 1.8 μM (*37*), 1.8 μM (*33*), 1.0 (*33*) or 9.9 (*37*) μM, 1.2 (*33*) or 4.7 (*37*) μM, and 22 μM (*33*), respectively (*33*, *37*). These values showed a pattern similar to that of our results. From these results, we confirmed that the *o*-trihydroxyl group in the B ring and the galloyl moiety are the most important structural properties for scavenging activity on the DPPH radicals.

Effects of Glycosylation on Water Solubility. We also conducted a comparison of the water solubilities of the EGCG and its glycosides. The solubility of EGCG was 5 mM, whereas the solubilities of EGCG-G1, EGCG-G1′, and EGCG-G2 were 253, 281, and 584 mM, or 49, 55, and 114 times as high as that of EGCG. Kitao et al. (*11*) also reported an increase in the solubility of glycosylated catechins (3′-*O*-α-D-glucopyranoside) by a factor of 50-fold, as compared to that of unglycosylated catechin. **Table 2** shows that the attachment of a glucosyl residue to EGCG resulted in an increase in the water solubility of the EGCG glycosides and that the number of attached glucosyl residues plays a clear role in water solubility.

In this study, we demonstrated the synthesis of one novel EGCG glycoside (EGCG-G1), which contains a glucosyl residue in the A ring of EGCG, and also described, in part, the biochemical properties of the resultant compounds. Furthermore, the observed increases in water solubility and browning resistance should further broaden the industrial applications in which the EGCG glycosides can be employed. Currently, research is underway to optimize yield increases of specific EGCG glycosides, via glucansucrase engineering and alterations in bioreactor design. Because EGCG has been the focus of great interest for its bioavailability, the EGCG glycosides should be expected to eventually be useful as materials for use in food additives and cosmetics. For EGCG glycosides, however, bioavailability studies such as absorption or antioxidant activity in plasma and tissue have not been carried out. Thus, further studies regarding the bioavailability of the EGCG glycosides are in progress.

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