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# Tea catechins and flavonoids from the leaves of *Camellia sinensis* inhibit yeast alcohol dehydrogenase

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ABSTRACT

Four new quercetin acylglycosides, designated camelliquercetisides A–D, quercetin 3-*O*-[α-L-arabinopyranosyl(1→3)][2-*O*″-(*E*)-*p*-coumaroyl][β-D-glucopyranosyl(1→3)-α-L-rhamnopyranosyl(1→6)]-β-D-glucoside (**17**), quercetin 3-*O*-[2-*O*″-(*E*)-*p*-coumaroyl][β-D-glucopyranosyl(1→3)-α-L-rhamnopyranosyl(1→6)]-β-D-glucoside (**18**), quercetin 3-*O*-[α-L-arabinopyranosyl(1→3)][2-*O*″-(*E*)-*p*-coumaroyl][α-L-rhamnopyranosyl (1→6)]-β-D-glucoside (**19**), and quercetin 3-*O*-[2-*O*″-(*E*)-*p*-coumaroyl][α-L-rhamnopyranosyl(1→6)]-β-D-glucoside (**20**), together with caffeine and known catechins, and flavonoids (**1**–**16**) were isolated from the leaves of *Camellia sinensis*. Their structures were determined by spectroscopic (1D and 2D NMR, IR, and HR-TOF-MS) and chemical methods. The catechins and flavonoidal glycosides exhibited yeast alcohol dehydrogenase (ADH) inhibitory activities in the range of $IC_{50}$ 8.0–70.3 μM, and radical scavenging activities in the range of $IC_{50}$ 1.5–43.8 μM, measured by using the 1,1-diphenyl-2-picrylhydrazyl (DPPH) radical.

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## 1. Introduction

Green tea from the plant *Camellia sinensis* has a long history of use as a social, medicinal, and health drink.[1,2] The medicinal effects of green tea are mainly attributed to caffeine, theanine, catechins, and polyphenols.[3–6] These compounds have been shown to exhibit antibacterial, anticarcinogenic, and antioxidant properties and play a protective role against cognitive deficits and coronary heart disease.[7–10] A crude extract of green tea and its main components, catechins and caffeine, have been found to reduce the concentration of ethanol and acetaldehyde in the blood and liver of ICR mice.[11] Consumed ethanol is mostly metabolized to acetaldehyde by NAD-dependent alcohol dehydrogenase (ADH) and further to acetic acid by NAD-dependent aldehyde dehydrogenase (ALDH), mostly in the liver (Fig. 1). Accumulation of acetaldehyde in the body is considered to be the main cause of hangovers, which are characterized by unpleasant symptoms such as headache, nausea, vomiting, thirst, dizziness, vertigo, and other decreased sensory abilities, after the heavy drinking of alcohol.[12,13] The intermediate acetaldehyde is potently toxic, mutagenic, and carcinogenic. It is believed to be a major factor in the development of alcohol-associated cancers, liver and pancreatic diseases, as well as coronary heart disease.[14–17] Oxidation of ethanol to acetaldehyde is thought to be a convenient and useful means of preventing alcoholic disorders. However, little research has been performed on the chemical ingredients of green tea that interact with the alcohol metabolizing enzymes ADH and ALDH. Recently, we have reported isolation and characterization of triterpenoidal saponins from the green tea root, which showed strong inhibitory effects against ADH.[18] In an ongoing search for biologically active compounds from medicinal plants,[19,20] we have found that the extract of tea leaves strongly inhibits ADH. Here, we report the isolation and characterization of four new acylated quercetin glycosides, named camelliquercetisides A–D (**17**–**20**), along with 16 known isolates from the tea leaves. Their ADH-inhibitory and 1,1-diphenyl-2-picrylhydrazyl (DPPH) radical scavenging activities are reported.

## 2. Results and discussion

The extract of green tea leaves (*Camellia sinensis*) was suspended in $H_2O$ and partitioned successively with *n*-hexane and *n*-BuOH. The *n*-BuOH layer was subjected to multiple chromatographic purification steps including reversed-phase $C_{18}$ flash column chromatography and preparative $C_{18}$ HPLC to produce four new (**17**–**20**) and sixteen known compounds (**1**–**16**) (Fig. 2).

Compounds **1**–**16** were identified by comparison of their spectroscopic data with those reported in the literature, as (−)-epigallocatechin (EGC) (**1**),[21] caffeine (**2**), (+)-catechin (**3**),[21] (−)-epigallocatechin gallate (EGCG) (**4**),[21] (−)-epicatechin gallate (ECG) (**5**),[21] myricetin 3-*O*-β-D-galactopyranoside (**6**),[22] myricetin 3-*O*-β-D-glucopyranoside (**7**),[23] (−)-epicatechin 3-*O*-(3′-*O*-methyl)

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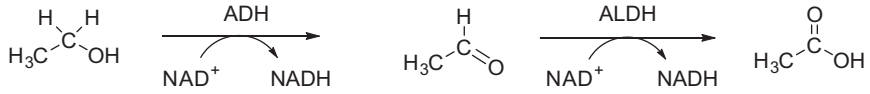


**Figure 1.** Metabolic pathway for alcohol: ADH, alcohol dehydrogenase; ALDH, aldehyde dehydrogenase; $NAD^+$, nicotinamide adenine dinucleotide; and NADH, a reduced form of $NAD^+$.

gallate (**8**),[24] (−)-epiafzelechin gallate (**9**),[25] (−)-epigallocatechin 3-*O*-(*Z*)-*p*-coumaroate (**10**),[26] (−)-epigallocatechin 3-*O*-(*E*)-*p*-coumaroate (**11**),[27] quercetin 3-*O*-β-D-glucopyranosyl(1→3)-α-L-rhamnopyranosyl(1→6)-β-D-glucopyranoside (**12**),[28] quercetin 3-*O*-β-D-glucopyranosyl(1→3)-α-L-rhamnopyranosyl(1→6)-β-D-galactopyranoside (**13**),[28] kaempferol 3-*O*-β-D-glucopyranosyl(1→3)-α-L-rhamnopyranosyl(1→6)-β-D-galactopyranoside (**14**),[28] kaempferol 3-rutinoside (**15**),[29] and kaempferol 3-*O*-β-D-galactopyranoside (**16**).[22]

Compounds **17–20** were obtained as pale brown solids. The molecular formula of **17** was determined to be $C_{47}H_{54}O_{27}$ based on its HR-TOF-MS (*m*/*z* 1051.3065 $[M+H]^+$, positive ESI mode). The $^1H$ NMR and $^1H$–$^1H$ COSY data of **17** suggested the presence of quercetin, *trans*-4-hydroxycinnamic [(*E*)-*p*-coumaric] acid, and four monocarbohydrate moieties: $\delta_H$ 7.59 (1H, d, *J* = 2.0 Hz), 7.54 (1H, dd, *J* = 8.4, 2.0 Hz), and 6.87 (1H, d, *J* = 8.4 Hz) for a 3,4-disubstitution on the B ring of the quercetin unit; $\delta_H$ 6.33 (1H, br s) and 6.13 (1H, br s) for the A ring; $\delta_H$ 7.67 (1H, d, *J* = 16.0 Hz), 7.44 (2H, d, *J* = 8.4 Hz), 6.79 (2H, d, *J* = 8.4 Hz), and 6.39 (1H, d, *J* = 16.0 Hz) for a *p*-coumaric acid moiety; and $\delta_H$ 5.56 (1H, d, *J* = 8.0 Hz), 4.61 (1H, br s), 4.45 (1H, d, *J* = 7.6 Hz), and 4.33 (1H, d, *J* = 6.8 Hz) for the anomeric protons of four carbohydrate units. Additionally, the corresponding anomeric carbons of the carbohydrates were observed at $\delta$ 101.0, 102.3, 105.9, and 105.3 in the $^{13}C$ NMR spectra, assigned with assistance from the HSQC experiment. Acid hydrolysis of **17** followed by TLC analysis identified glucose, rhamnose, and arabinose as the carbohydrate moieties. Their absolute configurations were determined to be D-, L-, and L-, respectively, from HPLC analysis of the chiral-derivatized sample of the hydrolysate using (*R*)- or (*S*)-(−)-1-phenylethylamine.[30,31] The HMBC correlations established the glycosidic linkages as follows: $\delta_H$ 5.56 (H-1, Glc1) to $\delta_C$ 134.9 (C-3 of quercetin unit), $\delta_H$ 4.61 (H-1, Rha) to $\delta_C$ 68.5 (C-6, Glc1), $\delta_H$ 4.45 (H-1, Glc2) to $\delta_C$ 83.1 (C-3, Rha), $\delta_H$ 4.33 (H-1, Ara) to $\delta_C$ 84.5

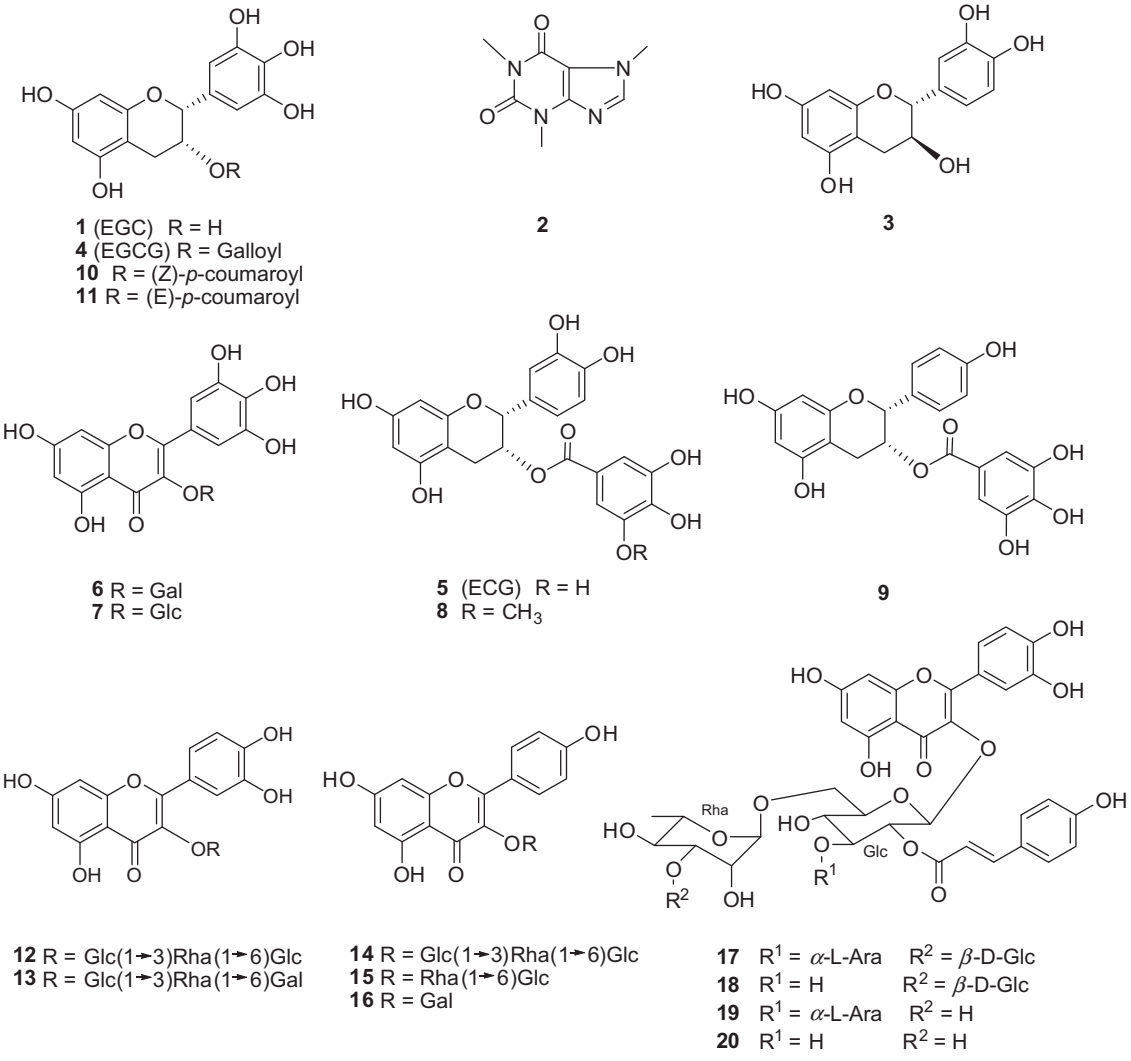


**Figure 2.** Chemical structures of isolates **1**–**20** from the leaves of *C. sinensis*.

(C-3, Glc1), $\delta_H$ 3.87 (H-3, Glc1) to $\delta_C$ 105.3 (C-1, Ara), $\delta_H$ 3.61 (H-3, Rha) to $\delta_C$ 105.9 (C-1, Glc2), and $\delta_H$ 3.88 and 3.52 (H-6, Glc1) to $\delta_C$ 102.3 (C-1, Rha). 3-O-glycosylation at the quercetin moiety was further evidenced by the upfield shift of C-3 ($\Delta\delta$ 2.1 ppm) and the downfield shift of both C-2 ($\Delta\delta$ 11.9 ppm) and C-4 ($\Delta\delta$ 2.2 ppm), compared to those of unsubstituted quercetin.[32] The attachment of a *p*-coumaroyl group to C-2 of the Glc1 moiety was established from the HMBC correlation [H-2 ($\delta$ 5.22, Glc1) to the carbonyl carbon ($\delta$ 168.6, coumaroyl group)]. It is notable that the H-2 of Glc1 was downfield-shifted compared to that of Glc2 due to the ester linkage. The configurations of the two glucopyranosyl, rhamnopyranosyl, and arabinopyranosyl units were established as β, β, α, and α, respectively, from their respective coupling constants ($J_{H1-H2}$ 8.0, 7.6, ~0, 6.8 Hz). Thus, the structure of **17** was determined to be quercetin 3-*O*-[α-L-arabinopyranosyl(1→3)][2-*O*″-(*E*)-*p*-coumaroyl][β-D-glucopyranosyl(1→3)-α-L-rhamnopyranosyl(1→6)]-β-D-glucoside, designated camelliquercetiside A.

Compound **18** showed similar $^1$H and $^{13}$C NMR spectral patterns to **17** except that the arabinopyranosyl unit was not observed. The molecular formula was determined to be $C_{42}H_{46}O_{23}$ from HR-TOF-MS analysis ([M+H]$^+$ *m/z* 919.2536). The configurations of the two glucopyranosyl units and the rhamnopyranosyl unit were established as β, β, and α, respectively, from their respective coupling constants ($J_{H1-H2}$ 8.0, 7.6, and ~0 Hz). Thus, the structure of **18** was established as quercetin 3-*O*-[2-*O*″-(*E*)-*p*-coumaroyl][β-D-glucopyranosyl(1→3)-α-L-rhamnopyranosyl(1→6)]-β-D-glucoside, camelliquercetiside B. The presence of the carbohydrate units was confirmed from the analysis of acid hydrolysate and their connectivity was confirmed from the interpretation of HMBC spectral data.

Compound **19**, with a molecular formula of $C_{41}H_{44}O_{22}$ determined by the HR-TOF-MS analysis, exhibited similar NMR spectroscopic patterns compared to **17**. However, the terminal glucopyranosyl moiety in **17** was not observed in **19**. The linkage of the arabinopyranosyl unit to the glucopyranosyl unit was confirmed from the HMBC [H-3 ($\delta$ 3.85, Glc) to C-1 ($\delta$ 105.3, Ara) and H-1 (Ara) to C-3 (Glc)] and the ROESY correlations [NOE between H-3 ($\delta$ 3.85, Glc) and H-1 ($\delta$ 4.32, Ara)]. The α-configuration of the arabinopyranosyl unit was confirmed from the coupling constant ($J$ = 6.8 Hz) of H-1 at $\delta$ 4.32. Thus, the structure of **19** was determined to be quercetin 3-*O*-[α-L-arabinopyranosyl(1→3)][2-*O*″-(*E*)-*p*-coumaroyl][α-L-rhamnopyranosyl(1→6)]-β-D-glucoside, camelliquercetiside C.

Compound **20** showed two anomeric proton signals at $\delta$ 5.52 ($J$ = 8.0 Hz) and 4.56 (br s) with the respective carbon signals at $\delta$ 100.9 and 102.3, which indicated the presence of two carbohydrate moieties. The molecular formula was determined to be $C_{36}H_{36}O_{18}$ from the HR-TOF-MS analysis. Acid hydrolysis and chiral derivatization identified the two monosaccharides as D-glucose and L-rhamnose. From the spectroscopic data (COSY, HSQC, and HMBC spectra) (Table 1), compound **20** was characterized as quercetin 3-*O*-[2-*O*″-(*E*)-*p*-coumaroyl][α-L-rhamnopyranosyl(1→6)]-β-D-glucoside, camelliquercetiside D.

ADH activities of isolates **1**–**20** were evaluated using the yeast ADH enzyme.[33] These isolates showed strong inhibitory effects on ADH in the $IC_{50}$ range of 8.0–70.3 μM, except caffeine **2**, whereas disulfiram, an alcohol aversive drug, known to be an excellent inhibitor of both ADH and ALDH,[34] inhibited ADH at an $IC_{50}$ of 247.9 μM (Table 2). Isolate **11** (EGC coumarate) was found to be the strongest and appeared to be more effective than **1** (EGC), which is likely due to an additional *p*-hydroxycinnamoyl group. The enhanced effect of an additional *p*-hydroxycinnamoyl on the inhibition of ADH was also observed in **18**, as compared to **1**. Attachment of an arabinopyranosyl group at the C-3 position of Glc did not seem to show a considerable effect (**17** vs **18**; **19** vs **20**). All flavonoidal glycosides, including newly isolated quercetin glycosides **17–20**, were more or less comparable to tea catechins. It is notable none of the isolates showed any significant ALDH-inhibitory or activating activities (data not shown), unlike disulfiram. The isolates were also tested for radical scavenging activities using the 1,1-diphenyl-2-picrylhydrazyl (DPPH) radical (Table 2). The new isolates **17**–**20** exhibited moderately good radical-scavenging activities in the $IC_{50}$ range of 19.8–29.2 μM, while tocopherol (Vit. E) showed an $IC_{50}$ of 11.4 μM. The DPPH-radical scavenging activities of the isolates do not seem to positively correlate with the ADH activities. The yeast ADH used in this study is a metallodehydrogenase containing stoichiometric amounts of zinc and having a high specificity towards ethanol.[33,35–37] The ADH-inhibitory effects of the isolates may be related to their metal-chelating ability as in zinc-chelating pyrazoles and *o*-phenanthroline.[38,39] Interestingly, phytophenols in whiskey were reported to depress alcohol metabolism through ADH inhibition.[40] Green tea and tea products are proven to be rich in catechins and flavonoidal polyphenols by numerous quantifying techniques.[41,42]

In the present study, we have demonstrated that catechins and flavonoids including four new quercetin glycosides isolated from tea leaves showed strong yeast ADH-inhibitory activities as well as DPPH-radical scavenging activities. Although yeast ADH may not behave like human ADHs and oval or colonic bacterial ADHs,[33,37,43] consumption of green tea or tea extract products could help prevent alcohol metabolism and the release of toxic acetaldehyde (or even detrimental formaldehyde, a metabolite of methanol), alleviating alcohol hangovers and reducing a risk of developing alcohol-related health disorders.

## 3. Experimental

### 3.1. General experimental procedures

Melting points were measured on a Fisher melting point apparatus and are uncorrected. Optical rotations were measured on a Perkin Elmer 341-LC polarimeter. IR spectra were recorded on a Perkin Elmer BX FT-IR spectrometer. NMR spectra were recorded on a Varian Mercury 400 spectrometer with standard pulse sequences operating at 400 MHz for $^1$H NMR and 100 MHz for $^{13}$C NMR. Chemical shifts, measured in ppm, were referenced to solvent peaks ($\delta_H$ 3.31 and $\delta_C$ 49.15 for $CD_3OD$). HR-TOF-MS spectra were recorded in the positive ESI mode on a Waters LCT Premier mass spectrometer coupled with a Waters AQUITY HPLC. Flash column chromatography was conducted on a $C_{18}$ column (40–63 μm, 90 id × 180 mm, Merck). Medium-pressure liquid chromatography (MPLC) was conducted on reversed-phase $C_{18}$ silica gel (Cosmosil 40 $C_{18}$ Prep, 40 id × 320 mm) at 5 mL/min. Preparative $C_{18}$ HPLC was performed on a Waters 600 model system equipped with a photodiode array UV detector 996 using a Varian Dynamax HPLC column (Microsorb 100-5, $C_{18}$, 21.4 id × 250 mm) at 7 mL/min with a UV detection at 220 or 270 nm. HPLC analyses of the (*S*)-(−)-1-phenylethylamine derivatives of the hydrolysate of flavonoidal glycosides and standard monosaccharides were performed on a Varian Chromsep HPLC column (Microsorb 100-5, $C_{18}$, 4.6 id × 250 mm) with a 0.8 mL/min gradient elution of 30 to 46% aqueous MeCN for 60 min at 220 nm. TLC was performed on precoated silica gel plates (Kieselgel 60, $F_{254}$, Merck). Spots were detected by staining with a solution of *p*-anisaldehyde-sulfuric acid in methanol, followed by heating. Optical density for a 96-well microplate was measured on a Tecan Sunrise microplate reader at 340 and 517 nm for the ADH and DPPH assays, respectively. (*R*)-1-Phenylethylamine, (*S*)-(−)-1-phenylethylamine, $NaBH_3CN$, ADH (alcohol dehydrogenase from *Saccharomyces cerevisiae*, EC 1.1.1.1, >90% protein, 331 units/mg), β-NAD$^+$ (β-nicotinamide adenine dinucleotide, oxidized form), ethanol (99.5%), and tetraethyl-

**Table 1**
$^1$H and $^{13}$C NMR spectroscopic data of compounds **17**–**20** in $CD_3OD$[a]

| Position | 17 | | 18 | | 19 | | 20 | |
|---|---|---|---|---|---|---|---|---|
| | $\delta_c$ | $\delta_H$ ($J$ in Hz) | $\delta_c$ | $\delta_H$ ($J$ in Hz) | $\delta_c$ | $\delta_H$ ($J$ in Hz) | $\delta_c$ | $\delta_H$ ($J$ in Hz) |
| *Quercetin* | | | | | | | | |
| 2 | 158.8 | — | 158.7 | — | 158.7 | — | 158.7 | — |
| 3 | 134.9 | — | 134.9 | — | 134.8 | — | 134.9 | — |
| 4 | 178.8 | — | 178.9 | — | 178.8 | — | 178.9 | — |
| 5 | 162.9 | — | 162.9 | — | 162.9 | — | 162.9 | — |
| 6 | 99.8 | 6.13, br s | 99.8 | 6.15, d (2.0) | 99.8 | 6.14, d (2.0) | 99.8 | 6.15, d (1.6) |
| 7 | 165.5 | — | 165.5 | | 165.6 | — | 165.5 | — |
| 8 | 94.8 | 6.33, br s | 94.8 | 6.34, d (1.6) | 94.8 | 6.33, d (2.0) | 94.8 | 6.33, d (1.6) |
| 9 | 158.3 | — | 158.2 | — | 158.2 | — | 158.2 | — |
| 10 | 105.5 | — | 105.6 | — | 105.8 | — | 105.8 | — |
| 1′ | 123.2 | — | 123.2 | — | 123.1 | — | 123.2 | — |
| 2′ | 117.5 | 7.59, d (2.0) | 117.5 | 7.59, d (2.0) | 117.4 | 7.57, d (2.0) | 117.4 | 7.57, br s |
| 3′ | 145.8 | — | 145.7 | — | 145.8 | — | 145.8 | — |
| 4′ | 149.5 | — | 149.5 | — | 149.6 | — | 149.5 | — |
| 5′ | 116.2 | 6.87, d (8.4) | 116.1 | 6.87, d (8.4) | 116.1 | 6.87, d (8.4) | 116.1 | 6.86, d (8.0) |
| 6′ | 123.4 | 7.54, dd (8.4, 2.0) | 123.5 | 7.55, d (8.4, 1.6) | 123.5 | 7.55, d (8.4, 2.0) | 123.5 | 7.55, dd (8.4, 2.0) |
| *p-coumaric acid* | | | | | | | | |
| 1 | 127.3 | — | 127.2 | — | 127.3 | — | 127.2 | — |
| 2, 6 | 131.3 | 7.44, d (8.4) | 131.2 | 7.44, d (8.4) | 131.3 | 7.44, d (8.8) | 131.2 | 7.44, d (8.4) |
| 3, 5 | 116.7 | 6.79, d (8.4) | 116.8 | 6.80, d (8.8) | 116.8 | 6.80, d (8.4) | 116.8 | 6.80, d (8.4) |
| 4 | 161.1 | — | 161.1 | — | 161.1 | — | 161.1 | — |
| $\alpha$ | 115.1 | 6.39, d (16.0) | 115.2 | 6.37, d (16.0) | 115.1 | 6.38, d (16.0) | 115.2 | 6.38, d (16.0) |
| $\beta$ | 147.2 | 7.67, d (16.0) | 146.9 | 7.68, d (16.0) | 147.2 | 7.66, d (16.0) | 146.9 | 7.67, d (16.0) |
| C=O | 168.6 | — | 168.5 | — | 168.6 | — | 168.5 | — |
| *Glc1* | | | | | | | | |
| 1 | 101.0 | 5.56, d (8.0) | 101.0 | 5.50, d (8.0) | 100.8 | 5.56, d (8.0) | 100.9 | 5.52, d (8.0) |
| 2 | 74.6 | 5.22, t (8.6) | 75.8 | 5.04, t (8.6) | 74.6 | 5.21, dd (9.6, 8.0) | 75.8 | 5.03, t (8.8) |
| 3 | 84.5 | 3.87 | 76.3 | 3.62, t (9.2) | 84.5 | 3.85, t (8.4) | 76.3 | 3.61, t (9.0) |
| 4 | 70.2 | 3.50 | 71.0 | 3.40, dd (9.2, 8.8) | 70.3 | 3.48 | 71.7 | 3.37, t (8.4) |
| 5 | 76.8 | 3.54, m | 77.6[b] | 3.43, m | 77.0 | 3.49, m | 77.4 | 3.40, m |
| 6 | 68.5 | 3.88, dd (12.0, 2.4) | 68.6 | 3.87, br d (10.8) | 68.3 | 3.87 | 68.4 | 3.87, br d (10.0) |
| | | 3.52 | | 3.51 | | 3.46 | | 3.45 |
| *Glc2* | | | | | | | | |
| 1 | 105.9 | 4.45, d (7.6) | 105.9 | 4.45, d (7.6) | | | | |
| 2 | 75.6 | 3.27, t (8.0) | 75.5 | 3.28, t (8.4) | | | | |
| 3 | 77.6[b] | 3.42, t (9.0) | 77.3 | 3.44, m | | | | |
| 4 | 71.0 | 3.39, t (8.8) | 71.7 | 3.39, dd (9.2, 8.8) | | | | |
| 5 | 77.6[b] | 3.26, m | 77.6[b] | 3.26, m | | | | |
| 6 | 62.2 | 3.74, dd (12.0, 2.0) | 62.2 | 3.76, dd (11.6, 2.0) | | | | |
| | | 3.71, dd (12.0, 4.4) | | 3.70, dd (11.6, 4.0) | | | | |
| *Rha* | | | | | | | | |
| 1 | 102.3 | 4.61, br s | 102.2 | 4.60, br s | 102.4 | 4.57, br s | 102.3 | 4.56, br s |
| 2 | 71.3 | 3.99, dd (3.2, 1.6) | 71.4 | 3.97, br s | 72.2 | 3.68, dd (3.2, 1.6) | 72.2 | 3.66, dd (3.1, 1.7) |
| 3 | 83.1 | 3.61, dd (9.2, 3.2) | 83.2 | 3.61, t (9.2 | 72.3[b] | 3.54 | 72.3 | 3.54, dd (10.0, 3.2) |
| 4 | 72.6 | 3.45, t (9.2) | 72.6 | 3.45 | 74.0[c] | 3.27, t (9.6) | 74.0 | 3.26, t (9.2) |
| 5 | 69.56 | 3.51, m | 69.5 | 3.52, m | 69.9 | 3.45, m | 69.8 | 3.48, m |
| 6 | 18.2 | 1.13, d (6.0) | 18.2 | 1.12, d (6.0) | 18.1 | 1.13, d (6.4) | 18.1 | 1.13, d (6.0) |
| *Ara* | | | | | | | | |
| 1 | 105.3 | 4.33, d (6.8) | | | 105.3 | 4.32, d (6.8) | | |
| 2 | 72.3 | 3.53 | | | | 72.3[b] | 3.53 | |
| 3 | 74.0 | 3.47, dd (8.8, 3.6) | | | 74.0[c] | 3.47, dd (8.8, 3.6) | | |
| 4 | 69.57 | 3.77, m | | | | 69.9 | 3.77 | |
| 5 | 67.3 | 3.85 | | | | 67.3 | 3.84, m | |
| | | 3.56 | | | | | 3.56, m | |

[a] $^1$H and $^{13}$C NMR measured at 400 and 100 MHz, respectively. The assignments are based on $^1$H–$^1$H COSY, TOSCY, HSQC, HMBC, and ROESY experiments. Overlapped signals are reported without designating multiplicity.
[b,c] Overlapped within the same columns

thiuram disulfide (disulfiram, 97%) were purchased from Sigma–Aldrich Chemical Co.

### 3.2. Plant material

Green leaves of *Camellia sinensis* were freshly picked from a 20-year-old Yabukita cultivar in Jeju Island, a southern island of the Republic of Korea, in May, 2005, and used for extraction after immediate steaming and air-drying (also available in a local market as Uksoocha). A voucher specimen (CS78) was deposited at the Natural Product Chemistry Laboratory, Department of Chemistry, Kongju National University, Republic of Korea.

### 3.3. Extraction and isolation

The leaves of *Camellia sinensis* (1.2 kg) were sequentially extracted with 80% aqueous MeOH (3 × 10 L, 1 day each time) and $CH_2Cl_2$–MeOH (1:1, 10 L, 7 days) at room temperature. The combined extracts were concentrated under reduced pressure. The resulting dark green residue (585 g) was suspended in $H_2O$ and

**Table 2**
Alcohol dehydrogenase (ADH) and radical scavenging (DPPH) activities of compounds **1–20**

| Compound | Activity ($IC_{50}$, μM)[a] | | Compound | Activity ($IC_{50}$, μM)[a] | |
|---|---|---|---|---|---|
| | ADH | DPPH | | ADH | DPPH |
| **1** | 16.5 ± 2.1 | 16.2 ± 0.2 | **12** | 19.7 ± 1.5 | 7.7 ± 0.4 |
| **2** | 196.7 ± 26.8 | >100 | **13** | 41.0 ± 4.1 | 30.1 ± 0.2 |
| **3** | 21.1 ± 1.3 | 4.3 ± 0.1 | **14** | 17.6 ± 1.3 | >100 |
| **4** | 42.8 ± 3.2 | 8.3 ± 0.4 | **15** | 40.4 ± 3.4 | >100 |
| **5** | 65.5 ± 4.1 | 1.5 ± 0.0 | **16** | 70.3 ± 7.7 | >100 |
| **6** | 18.2 ± 1.9 | 3.5 ± 0.0 | **17** | 17.3 ± 1.3 | 19.8 ± 0.1 |
| **7** | 19.9 ± 1.5 | 28.0 ± 2.1 | **18** | 13.7 ± 0.6 | 22.0 ± 0.3 |
| **8** | 44.1 ± 2.1 | 10.8 ± 0.2 | **19** | 46.7 ± 5.3 | 31.9 ± 1.4 |
| **9** | 44.3 ± 2.2 | 2.8 ± 0.0 | **20** | 41.5 ± 1.1 | 29.2 ± 0.1 |
| **10** | 22.1 ± 0.1 | 26.4 ± 0.3 | Disulfiram[b] | 247.9 ± 10.8 | |
| **11** | 8.0 ± 0.5 | 43.8 ± 2.9 | Tocopherol[b] | | 11.4 ± 1.6 |

[a] The experiments were performed in triplicate, and the average values are expressed with the standard errors of the mean.
[b] Disulfiram: tetraethylthiuram disulfide; tocopherol: Vit. E.

extracted with *n*-hexane (6.4 L) and then with *n*-BuOH (4.8 L). A portion (100 g) of the *n*-BuOH extract (360 g) was chromatographed on a $C_{18}$ flash column using a 10% stepwise gradient elution of increasing MeOH concentration in $H_2O$ (4 L each) to afford eleven fractions. A portion (1.5 g) of the active 50% MeOH eluate (5.7 g) was fractionated on a reversed-phase MPLC (30–60% MeOH) to give ten subfractions (1–10). Subfractions 2 (53.0 mg), 3 (83.7 mg), 5 (330 mg), and 6 (85.7 mg) were purified to provide compounds **1** (15.0 mg, $t_R$ 16.0 min), **2** (10.0 mg, $t_R$ 19.5 min), and **3** (8.3 mg, $t_R$ 20.6 min) from subfraction 2, compounds **4** (7.2 mg, $t_R$ 26.7 min) and **5** (57.0 mg, $t_R$ 30.4 min) from subfraction 3, compounds **6** (24.2 mg, $t_R$ 59.0 min), **7** (9.4 mg, $t_R$ 61.0 min), **8** (33.2 mg, $t_R$ 63.7 min), **9** (9.9 mg, $t_R$ 65.0 min), **10** (14.8 mg, $t_R$ 68.0 min), and **11** (43.5 mg, $t_R$ 70.5 min) from subfraction 5, and compounds **12** (14.5 mg, $t_R$ 75.5 min) and **13** (17.0 mg, $t_R$ 77.7 min) from subfraction 6 by preparative $C_{18}$ HPLC (0.05% trifluoroacetic acid-buffered gradient elution of 10–15% aqueous MeCN for 20 min and 15–28% aqueous MeCN for 80 min). Compounds **14** (73.4 mg, $t_R$ 26.0 min), **15** (4.83 mg, $t_R$ 29.5 min), and **16** (2.41 mg, $t_R$ 30.6 min) were obtained from subfraction 7 (121.8 mg) by preparative $C_{18}$ HPLC (0.05% trifluoroacetic acid-buffered gradient elution of 22–28% aqueous MeCN for 60 min). The 80% MeOH eluate (1.05 g, a deep brown solid) from the flash column was fractionated by reversed-phase $C_{18}$ MPLC (60–100% MeOH) to afford ten subfractions. Subfraction 5 (160 mg, a brown solid, 70% MeOH eluate) was purified by preparative $C_{18}$ HPLC (0.05% trifluoroacetic acid-buffered gradient elution of 10–15% aqueous MeCN for 10 min and 15–28% aqueous MeCN for 80 min) to afford compounds **17** (30.1 mg), **18** (15.4 mg), **19** (15.1 mg), and **20** (12.7 mg) with a $t_R$ of 53.08, 56.6, 59.5 and 65.0 min, respectively.

### 3.4. Camelliquercetiside A, quercetin 3-*O*-[α-L-arabinopyranosyl(1→3)][2-*O*″-(*E*)-*p*-coumaroyl][β-D-glucopyranosyl(1→3)-α-L-rhamnopyranosyl(1→6)]-β-D-glucoside (17)

A pale brown solid; mp 228–230 °C; $[\alpha]_D^{20}$ −83.9 (*c* 0.13, MeOH); $^1$H ($CD_3OD$, 400 MHz) and $^{13}$C NMR ($CD_3OD$, 100 MHz) data, Table 1; HR-TOF-MS (positive ESI mode) *m/z* 1051.3065 $[M+H]^+$ (calcd for $C_{47}H_{54}O_{27}$+H, 1051.2931).

### 3.5. Camelliquercetiside B, quercetin 3-*O*-[2-*O*″-(*E*)-*p*-coumaroyl][β-D-glucopyranosyl(1→3)-α-L-rhamnopyranosyl(1→6)]-β-D-glucoside (18)

A pale brown solid; mp 220–222 °C; $[\alpha]_D^{20}$ −92.65 (*c* 0.07, MeOH); UV ($CH_3OH$) $\lambda_{max}$ (log ε) 355 (4.55), 316 (4.46), 267 (4.37), 257 (4.35) nm; IR (neat) $\nu_{max}$ 3361, 1734, 1649, 1602, 1512, 1440, 1356, 1198, 1166 $cm^{-1}$; $^1$H ($CD_3OD$, 400 MHz) and $^{13}$C NMR ($CD_3OD$, 100 MHz) data, Table 1; HR-TOF-MS (positive ESI mode) *m/z* 919.2536 $[M+H]^+$ (calcd for $C_{42}H_{46}O_{23}$+H, 919.2508).

### 3.6. Camelliquercetiside C, quercetin 3-*O*-[α-L-arabinopyranosyl(1→3)][2-*O*″-(*E*)-*p*-coumaroyl][α-L-rhamnopyranosyl(1→6)]-β-D-glucoside (19)

A pale brown solid; mp 218–221 °C; $[\alpha]_D^{20}$ −87.0 (*c* 0.1, MeOH); UV ($CH_3OH$) $\lambda_{max}$ (log ε) 356 (4.56), 316 (4.45), 267 (4.33), 256 (4.31) nm; IR (neat) $\nu_{max}$ 3352, 1734, 1647, 1602, 1514, 1440, 1356, 1195, 1166 $cm^{-1}$; $^1$H ($CD_3OD$, 400 MHz) and $^{13}$C NMR ($CD_3OD$, 100 MHz) data, Table 1; HR-TOF-MS (positive ESI mode) m/*z* 889.2434 $[M+H]^+$ (calcd for $C_{41}H_{44}O_{22}$+H, 889.2402).

### 3.7. Camelliquercetiside D, quercetin 3-*O*-[2-*O*″-(*E*)-*p*-coumaroyl][α-L-rhamnopyranosyl(1→6)]-β-D-glucoside (20)

A pale brown solid; mp 188–191 °C; $[\alpha]_D^{20}$ −101.54 (*c* 0.13, MeOH); UV ($CH_3OH$) $\lambda_{max}$ (log ε) 354 (4.67), 315 (4.59), 268 (4.46), 257 (4.45) nm; IR (neat) $\nu_{max}$ 3347, 1735, 1654, 1598, 1512, 1442, 1354, 1200, 1166 $cm^{-1}$; $^1$H ($CD_3OD$, 400 MHz) and $^{13}$C NMR ($CD_3OD$, 400 MHz) data, Table 1; HR-TOF-MS (positive ESI mode) *m/z* 757.2017 $[M+H]^+$ (calcd for $C_{36}H_{36}O_{18}$H, 757.1980).

### 3.8. Hydrolysis and identification of the sugar moieties in 17–20

Flavonoidal glycosides were hydrolyzed and analyzed according to the literature precedent.[18] Briefly, glycoside (**17**, 2 mg) was heated in a 1 M HCl solution (2 mL, 1:1 dioxane–$H_2O$) at 80 °C for 2 h. The resulting mixture was concentrated and partitioned between water (2 mL) and butanol (2 mL). The aqueous layer was analyzed by TLC (70:27:3 $CH_2Cl_2$–MeOH–$H_2O$) and was identified to contain glucose ($R_f$ 0.24), arabinose ($R_f$ 0.32), and rhamnose ($R_f$ 0.43), based on comparison with authentic samples. The aqueous layer was reacted with (*S*)-(−)-1-phenylethylamine (2 mg) and $NaBH_3CN$ (8 mg) followed by acetylation with acetic anhydride (0.2 mL) and pyridine (0.2 mL). After drying, the resulting dark brown residue was analyzed by HPLC. The carbohydrates were identified as L-arabinose ($t_R$, 28.1 min), D-glucose ($t_R$ 38.3 min), and L-rhamnose ($t_R$ 42.3 min) by comparing their retention times with those of (*S*)-(−)-1-phenylethylamine derivatives of authentic carbohydrates, while (*R*)-(+)-1-phenylethylamine derivatives of authentic carbohydrates were detected at $t_R$, 26.7 min, 35.5 min, and 39.2 min, respectively. The same procedure was applied for the identification of the sugar moieties in the other isolates.

### 3.9. Alcohol dehydrogenase (ADH) assay

ADH activity was measured according to the literature precedent.[18,19] Briefly, stock solutions of samples in DMSO were serially diluted with a phosphate buffer solution (PBS, 10 mM, pH 7.4). Ethanol (20 μL, 25% in PBS), β-$NAD^+$ (20 μL, 1.87 mM in PBS), and the test solutions (20 μL) were added to a 96-well plate (120 μL, PBS) and allowed to equilibrate for 5 min at 24 °C. After an ADH solution (20 μL, 1180 unit/L in PBS) was added, the mixture was incubated at 24 °C for 2 h. The absorbance at 340 nm was measured at intervals of 5 min for a period of 2 h to measure the amount of NADH produced. The 50% inhibitory concentration ($IC_{50}$, μg/mL) of the sample was expressed as the sample concentration at which the amount of NADH produced was reduced by 50%, compared to the control (PBS) at 2 h. Tetraethylthiuram disulfide (disulfiram) was used as the positive control, and the experiments were performed in triplicate.

### 3.10. DPPH radical scavenging activity assay

Measurement of the 1,1-diphenyl-2-picrylhydrazyl (DPPH) radical scavenging activity was performed according to the literature precedent.[20] Briefly, the solutions of serially diluted samples (20 μL) in MeOH were added to an EtOH solution (80 μL) of DPPH (59 μg/mL) in a 96-well plate. After incubation for 30 min at room temperature, the absorbance of the wells was measured at 517 nm with a microplate reader. Radical scavenging activity was expressed in terms of $IC_{50}$ (the concentration required for a 50% decrease in the absorbance of the DPPH control solution).

### Supplementary data

Supplementary data associated with this article can be found, in the online version, at doi:10.1016/j.bmc.2012.02.002.

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