New Phenylethanoid Glycosides and Sugar Esters from Ku-Ding-Cha, a Herbal Tea Produced from *Ligustrum purpurascens*

G.-M. She, D. Wang, S.-F. Zeng, C.-R. Yang, and Y.-J. Zhang

ABSTRACT: Ku-Ding-Cha is a kind of herbal tea used commonly in China for its effects on hypertension, fatness, inflammation, and diuresis. The chlorophyll removal fraction from 60% aqueous acetone extract of Ku-Ding-Cha produced from the leaves of *Ligustrum purpurascens* (Oleaceae) exhibited observable antioxidant activity on 1,1-diphenyl-2-picrylhydrazyl (DPPH) radical scavenging assay (SC_{50} = 45 μg/mL). Further detailed phytochemical investigation on this fraction led to the isolation of 2 new phenylethanoid glycosides, ligupurpurosides C-D (1 and 2), and 2 new sugar esters, ligupurpurosides E-F (3 and 4), together with 15 known compounds (5 to 19). All of them, except for 7, 8, 9, 11, and 12, were isolated from Ku-Ding-Cha and its original plants for the first time. The chemical structures were elucidated based on the analysis of spectroscopic data, including 1D, 2D NMR, and FABMS. In addition, the isolated compounds were evaluated for their antioxidant activities by DPPH assay.

Keywords: antioxidant, Ku-Ding-Cha, *Ligustrum purpurascens*, monoterpenoid glycoside, phenylethanoid, sugar ester

Introduction

Recent reports indicate that there is an inverse relationship between the dietary intake of antioxidant-rich foods and the incidence of human diseases (Edwin 1996). There has been increasing interest in searching after natural antioxidants present in fruit, vegetables, herbs, and beverages.

Like green tea, Ku-Ding-Cha, as a kind of herbal tea, is popularly used in the south area of China. It was deemed as beverage of the poor in the past and local people always drink it for clearing away "heat" and "toxic" material in body in the summer instead of tea that exhibits very bitter taste.

The leaves of *Ligustrum purpurascens* Y. C. Yang (Oleaceae) are used as one of the original raw materials of Ku-Ding-Cha. It is a kind of famous herbal tea commonly used in China for its effects on hypertension, fatness, inflammation, and diuresis (He and others 1992). The plant is now cultivated largely in the southwest of Sichuan and the northeast of Yunnan Province, China, for the production of Ku-Ding-Cha. Our previous study has reported the isolation of several phenylethanoid and flavonoids glycosides from the original plants of Ku-Ding-Cha, belonging to the genus *Ligustrum* (He and others 1992, 1994). The antioxidant activities of these compounds against oxidation of human low-density lipoprotein (LDL) were also reported (Chen and others 2000; Wong and others 2001). In addition, a series of triterpenes and triterpenoid glycosides were reported from the other original plants (*Ilex* species) of Ku-Ding-Cha (Ouyang and others 1996a, 1996b, 1997).

The chlorophyll removal fraction from 60% aqueous acetone extract of Ku-Ding-Cha produced from the leaves of *L. purpurascens* exhibited observable antioxidant activity (SC_{50} = 45 μg/mL) on 1,1-diphenyl-2-picryldydrazyl (DPPH) radical scavenging assay. As a part of our ongoing phytochemical investigation of Chinese tea and tea-like plants, and in search of antioxidant intake from plants sources, a detailed study on this fraction was carried out. This led to the isolation of 13 phenylethanoid glycosides (1 and 2, 7 to 17), 4 sugar esters (3 to 6), and 2 monoterpenoid glycosides (18 and 19). Of them, 1 to 4 were new compounds. All the compounds, except 7, 8, 9, 11, and 12, were identified for the first time from Ku-Ding-Cha and its original plant. The antioxidant activities of these compounds on the DPPH radical scavenging assay were also tested. This article presents the details of the study.

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Materials and Methods

General procedures

Optical rotations were measured on a P-1020 Polarimeter (JASCO, Tokyo, Japan). IR spectra were recorded on an IR-450 spectrometer (Shimadzu, Kyoto, Japan) with KBr pellets. ^{1}H and ^{13}C NMR spectra were recorded in CD_3OD with Bruker AM-400 and DRX-500 spectrometers operating at 400 and 500 MHz for ^{1}H, and 100 and 125 MHz for ^{13}C, respectively. Coupling constants are expressed in hertz (Hz) and chemical shifts are given on a δ (ppm) scale with tetramethylsilane as internal standard. Negative ion FABMS and HRFABMS were recorded on an AutoSpec 3000 spectrometer (VG, Manchester, U.K.) with glycerol as the matrix. The HPLC was performed with a Shimadzu LC-10AD HPLC (Tokyo, Japan) on a ZORBAX SB-C_{18} column (4.6 × 250 mm, 5 μm, Agilent), using a UV detector (Shimadzu SPD-M10A). The 1,1-diphenyl-2-picryldydrazyl (DPPH) (Aldrich Chemical Co., St. Louis, Mo., U.S.A.) radical scavenging assay was performed on an Emax precision microplate reader. Column chromatography was performed on Diaion HP20SS (Mitsubishi Chemical Co., Tokyo, Japan), Sephadex LH-20 (Pharmacia Fine Chemical Co. Ltd., Sweden), Chromatorex ODS (Fuji Silysia Chemical Co. Ltd., Aichi, Japan), and

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Silica gel (Qingdao, China). TLC was carried on silica gel G precoated plates (Qingdao Haiyang Chemical Co., Qingdao, China) with benzene-ethyl formate-formic acid (1:7:1) or $CHCl_3$-MeOH-H_2O (8:2:0.2 or 7:3:0.5). The spots were detected by spraying with 2% ethanolic $FeCl_3$ or 10% H_2SO_4 ethanol solution followed by heating.

Plant materials

The herbal tea of Ku-Ding-Cha produced from the leaves of *L. purpurascens* Y. C. Yang was purchased from Yunye Ku-Ding-Cha factory, Yongshan County, Yunnan Province, China, and identified by Prof. Chong-Ren Yang (Kunming Inst. of Botany, Chinese Academy of Sciences).

Extraction and isolation

Ku-Ding-Cha (2.0 kg) produced from the leaves of *L. purpurascens* was extracted with 60% aqueous acetone at room temperature 3 times. After the removal of organic solvent under reduced pressure, the aqueous solution afforded precipitates, which were removed by filtration, and the filtrate was partitioned with ethyl ether to yield ethyl ether and aqueous fraction. The aqueous fraction was concentrated to a small volume (400 mL) and subjected to a Diaion HP20SS column, eluting with H_2O-MeOH (1:0-0:1) and Me_2CO, to afford 11 fractions (fr. A to K). Fr. E (43 g) was separated by column chromatography (CC) on Sephadex LH-20 (H_2O-MeOH, 1:0-0:1) and silica gel ($CHCl_3$-MeOH-H_2O, 9:1:0.1-7:3:0.5) to yield 3 (12 mg), 4 (5 mg), 5 (3 mg), and 6 (4 mg). Repeated CC on Sephadex LH-20, MCI-gel CHP-20P, and Chromatorex ODS, eluting with H_2O-MeOH (1:0-0:1), respectively, gave 1 (2 mg), 2 (10 mg), 8 (30 mg), 10 (2 mg), and 12 (50 mg) from fr. G (62 g), and 9 (52 mg), 11 (44 mg), 13 (17 mg), and 16 (12 mg) from fr. H (47 g). Fr. I (35 g) was applied to CC on silica gel ($CHCl_3$-MeOH-H_2O, 9:1:0.1-7:3:0.5), Sephadex LH-20 (H_2O-MeOH, 1:0-0:1) and MCI-gel CHP-20P (H_2O-MeOH, 1:0-0:1) to afford 7 (4 mg), 14 (28 mg), 15 (3 mg), 17 (18 mg), 18 (32 mg), and 19 (6 mg).

Compound (1). Brown amorphous powder, $[\alpha]_D^{21}$ -58.4° (*c* 0.4, MeOH). IR ν_{max}^{KBr}: 3429, 1726, 1629, 1515, 1456, 1166, and 835 cm^{-1}. ^1H (500 MHz, CD_3OD) and ^{13}C NMR (125 MHz, CD_3OD): see Table 1. Negative FABMS: *m/z* 737 $[M-H]^-$; negative HRFABMS *m/z* 737.2639 $[M-H]^-$, calcd for $C_{35}H_{45}O_{17}$, 737.2656.

Compound (2). Brown amorphous powder, $[\alpha]_D^{21}$ -64.7° (*c* 0.9, MeOH). IR ν_{max}^{KBr}: 3428, 1694, 1629, 1610, 1515, 1443, 1165, and 833 cm^{-1}. ^1H (500 MHz, CD_3OD) and ^{13}C NMR (125 MHz, CD_3OD): see Table 1. Negative FABMS: *m/z* 753 $[M-H]^-$, 607 $[M-147(rhamnosyl)]^-$, 461 $[M-147(rhamnosyl)-146(rhamnosyl)]^-$; negative HRFABMS *m/z* 753.2625 $[M-H]^-$, calcd for $C_{35}H_{45}O_{18}$, 753.2605.

Compound (3). Yellow amorphous powder, $[\alpha]_D^{21}$ -5.4° (*c* 1.0, pyridine). IR ν_{max}^{KBr}: 3426, 1697, 1630, 1605, and 1515 cm^{-1}. ^1H (500 MHz, CD_3OD) and ^{13}C NMR (125 MHz, CD_3OD): see Table 2. Negative FABMS: *m/z* 471 $[M-H]^-$, 325 $[M-147\ (rhamnosyl)]^-$; negative HRFABMS *m/z* 471.1504 $[M-H]^-$, calcd for $C_{21}H_{27}O_{12}$, 471.1502.

Compound (4). Yellow amorphous powder, $[\alpha]_D^{21}$ -64.7° (*c* 1.5, pyridine). IR ν_{max}^{KBr}: 3426, 1696, 1631, 1604, and 1518 cm^{-1}. ^1H (500 MHz, CD_3OD) and ^{13}C NMR (125 MHz, CD_3OD): see Table 2. Negative FABMS: *m/z* 633 $[M-H]^-$, 487 $[M-147(rhamnosyl)]^-$; negative HRFABMS *m/z* 633.2025 $[M-H]^-$, calcd for $C_{27}H_{37}O_{17}$, 633.2030.

HPLC analysis. The powdered Ku-Ding-Cha sample (1.500 g) was ultrasonically extracted with methanol (100 mL) for 30 min. The extract was filtered through a 0.45 μm membrane filter for the HPLC analysis. Ligupurpuroside D (2), osmanthuside B (11), acteoside (12), and ligurobustoside C (18) purified and identified from this study were used as standard references. The optimal mobile phase for the analysis of Ku-Ding-Cha extracts was a binary gradient elution system consisting of acetonitrile (A) and water (B). The gradient program used was as follows: 0 min: 20% A, 15 min: 20% A, and 43 min: 100% A. Column temperature was set at 30 °C. The flow rate was 1.0 mL/min and the injection volume was 10 μL. The UV detection wavelength was monitored at 227 nm. The peaks areas of compounds 2, 11, 12, and 18 were calculated and compared with the authentic samples.

DPPH radical scavenging assay

The DPPH assay was performed as described in our previous study (Wang and others 2005), and ascorbic acid was used as positive control. Scavenging activity was determined by the following equation: % scavenging activity = $100 \times (A_{control} - A_{sample})/A_{control}$. The SC_{50} value was obtained through extrapolation from linear regression analysis and denoted the concentration of sample required to scavenge 50% of DPPH radicals.

Results and Discussion

Identification of compounds 1 to 19

The preliminary DPPH assay showed that the chlorophyll removal fraction from 60% aqueous acetone extract of

Table 1 — ^{13}C (125 MHz) and ^1H (500 MHz) NMR spectroscopic data for 1 and 2 in CD_3OD (δ in ppm, *J* in Hz).

	1		2	
Nr	δ_C	δ_H	δ_C	δ_H
1	130.9		130.5	
2	131.3	7.02 (d, 8.4)	130.9	7.06 (d, 8.6)
3	116.5	6.67 (d, 8.4)	116.1	6.68 (d, 8.6)
4	157.2		156.8	
5	116.5	6.67 (d, 8.4)	116.1	6.68 (d, 8.6)
6	131.3	7.02 (d, 8.4)	130.9	7.06 (d, 8.6)
7	36.8	2.81 (m)	36.4	2.84 (t, 4.8)
8	72.8	3.70 (m)	72.4	3.80 (m)
		4.05 (m)		4.05 (m)
Glc-1′	104.8	4.33 (d, 8.2)	104.3	4.35 (d, 8.2)
2′	76.2	3.30 (m)	75.8	3.39 (m)
3′	83.9	3.50 (m)	83.4	3.54 (m)
4′	70.8	3.65 (m)	70.3	3.70 (m)
5′	75.8	3.48 (m)	75.4	3.40 (m)
6′	64.5	4.50 (dd, 12.0, 2.0)	64.6	4.50 (dd, 12.0, 2.0)
		4.35 (dd, 12.0, 6.0)		4.34 (dd, 12.0, 6.0)
Rha-I-1″	103.6	5.02 (br s)	103.2	5.03 (br s)
2″	72.9	3.93 (br s)	72.4	3.92 (br s)
3″	70.7	3.70 (m)	70.3	3.68 (m)
4″	81.5	3.83 (m)	81.0	3.82 (m)
5″	68.8	4.12 (m)	68.4	4.20 (m)
6″	18.9	1.24 (d, 6.2)	18.5	1.27 (d, 6.3)
Rha-II-1‴	102.8	5.18 (br s)	102.4	5.21 (br s)
2‴	73.4	3.88 (br s)	72.9	3.94 (br s)
3‴	73.3	3.59 (m)	72.8	3.61 (m)
4‴	74.2	3.38 (m)	73.8	3.39 (m)
5‴	70.7	3.70 (m)	70.2	3.68 (m)
6‴	18.2	1.26 (d, 5.6)	17.8	1.31 (d, 6.2)
Acyl-1⁗	127.4		127.6	
2⁗	131.6	7.40 (d, 8.4)	115.0	7.06 (br s)
3⁗	117.3	6.78 (d, 8.4)	146.7	
4⁗	161.9		149.6	
5⁗	117.3	6.78 (d, 8.4)	116.5	6.79 (d, 8.6)
6⁗	131.6	7.40 (d, 8.4)	123.1	6.91 (dd, 8.6)
7⁗	147.3	7.62 (d, 15.8)	147.2	7.59 (d, 15.8)
8⁗	115.3	6.35 (d, 15.8)	114.8	6.31 (d, 15.8)
CO	169.5		169.0	

Ku-Ding-Cha produced from the leaves of *L. purpurascens* had obvious antioxidant activity (IC_{50} = 45.0 μg/mL) than that of the ethyl ether extract (IC_{50} = 415 μg/mL). Repeated CC of this chlorophyll removal fraction on Diaion HP20SS, Sephadex LH-20, silica gel, MCI-gel CHP-20P, and Chromatorex ODS, afforded 19 compounds. Of those, 11 known compounds were identified to be *O*-acylglycoses (5) (Birkofer and others 1969), isocistanoside F (6) (Wu and others 2004), ligurobustosides N (7) (He and others 2003), ligupurpuroside A (8) (He and others 1992), ligupurpuroside B (9) (He and others 1992), echinacoside (10) (Kobayashi and others 1984), osmanthuside B (11) (Sugiyama and Kikuchi 1990), acteoside (12) (He and others 1992), lipedoside A-II (13) (He and others 1994), osmanthuside B_6 (14) (Zhang and others 1998), eutigoside A (15) (Khan and others 1992), campenoside II (16) (Kitagawa and others 1984), osmanthuside A (17) (Sugiyama and Kikuchi 1990), ligurobustoside C (18) (Tian and others 1998), and ligurobustoside I (19) (Tian and others 1998), by comparison of their physical and spectroscopic data with those reported in the literature (Figure 1). The known compounds 5, 6, 10, and 13 to 19 were isolated for the first time from Ku-Ding-Cha and its original plant. The 4 new compounds were named as ligupurpurosides C to F (1 to 4), respectively, and their structures were elucidated as follows.

Ligupurpuroside C (1) was obtained as a brown amorphous powder. Its molecular formula was assigned as $C_{35}H_{46}O_{17}$ on the basis of the ^{13}C NMR data (Table 1) and negative HRFABMS (*m/z* 737.2639 $[M-H]^-$), which was the same as that of ligupurpuroside B (9). The ^1H and ^{13}C NMR spectra of 1 (Table 1) displayed the presence of a *p*-hydroxyphenylethyl unit [δ 6.67, 7.02 (each 2H, d, J = 8.4 Hz), 4.05 (1H, m), 3.70 (1H, m), and 2.81 (2H, m)], a β-D-glucopyranosyl [anomeric H at δ 4.33 (1H, d, J = 8.2 Hz)], two α-L-rhamnopyranosyl [δ 5.02, 5.18 (each 1H, br s), and 1.24 (3H, d, J = 6.2 Hz) and 1.26 (3H, d, J = 5.6 Hz)], and a *p*-coumaroyl [δ 6.78 and 7.40 (each 2H, d, J = 8.4 Hz), 7.62 and 6.35 (each 1H, d, J = 15.8 Hz)] units, suggesting that 1 was a phenylethanoid glycoside. These NMR features were very similar to those of 9, except for the carbon signals arising from the β-D-glucopyranosyl unit. It is observed that the chemical shifts of glucosyl C-3′ and C-6′ were downfield shifted to δ 83.9 and 64.5 in 1, from δ 81.6 and 62.3 in 9, respectively, suggesting that the *p*-coumaroyl group linked at glucosyl C-4′ in 9 was located at the glucosyl C-6′ in 1. This was further confirmed by the HMBC experiment, in which correlations of the glucosyl H-6′ (δ 4.49 and 4.35) with the carbonyl carbon (δ 169.5) of *p*-coumaroyl group were observed. Moreover, the anomeric proton signals at δ 4.33 (H-1′), 5.02 (H-1″), and 5.18 (H-1‴) were correlated with the carbon signals at δ 72.8 (C-8), 83.9 (C-3′), and 81.5 (C-4″), respectively. Accordingly, the structure of ligupurpuroside C (1) was determined to be 2-(4-hydroxyphenyl)-ethyl-[3-*O*-α-L-rhamnopyranosyl(1→4)-α-L-rhamnopyranosyl] [6-*O*-*p*-coumaroyl]-*O*-β-D-glucopyranoside.

Ligupurpuroside D (2) was obtained as a brown amorphous powder, and had the same molecular formula $C_{35}H_{46}O_{18}$ as deduced from the negative HRFABMS (*m/z* 753.2625 $[M-H]^-$). The ^1H and ^{13}C NMR spectra of compounds 2 and 7 (Table 1) were very similar with each other and gave same signals assignable to a *p*-hydroxy- phenylethyl, an (*E*)-caffeoyl, a β-D-glucopyranosyl, and two α-L-rhamnopyranosyl units. Comparison of the NMR data for 2 with 1 indicated that their differences were only at the presence of (*E*)-caffeoyl group in 2 instead of the *p*-coumaroyl group in 1, respectively. The full assignments of sugar signals were carried out by HSQC, ^1H-^1H COSY, and TOCSY experiments. The HMBC correlations of glucosyl H-6′ (δ 4.34 and 4.50) in 2 with the carbonyl carbon at δ 169.0 (2) confirmed that the location of (*E*)-caffeoyl groups were at C-6′ of

Table 2—^{13}C (125MHz) and ^1H (500MHz) NMR spectroscopic data for 3 and 4 in CD_3OD (δ in ppm, *J* in Hz).

	3				4			
Nr	δ_C		δ_H		δ_C		δ_H	
Acyl-1	127.2				127.3			
2	131.4		7.46 (d, 8.5)		115.1		7.05 (br s)	
3	116.2		6.80 (d, 8.5)		147.1			
4	161.5				149.8			
5	116.2		6.80 (d, 8.5)		116.8		6.77 (d, 8.2)	
6	131.4		7.46 (d, 8.5)		123.5		6.96 (d, 8.2)	
7	147.6		7.65 (d, 15.9)		147.9		7.59 (d, 15.9)	
8	115.0		6.34 (d, 15.9)		114.5		6.26 (d, 15.9)	
CO	168.5				168.3			
Glc	**α**	**β**	**α**	**β**	**α**	**β**	**α**	**β**
1′	94.1	98.2	5.10(d, 3.6)	4.52 (d, 7.7)	94.1	98.2	5.10 (d, 3.5)	4.57 (d, 7.7)
2′	71.2	74.7	3.48 (m) 3.48 (m)		71.2	74.8	3.49 (m) 3.49 (m)	
3′	79.2	81.6	4.08(t, 9.2)	3.84 (t, 9.2)	78.9	81.4	4.07 (t, 9.6)	3.89 (t, 9.6)
4′	70.3	70.7	4.93 (t, 9.8)	4.90 (t, 9.8)	70.3	70.7	4.96 (t, 9.7)	4.93 (t, 9.7)
5′	76.2	77.5	3.53 (m) 3.53 (m)		75.9	77.5	3.54 (m) 3.55 (m)	
6′	62.4	62.5	3.59 (m)	3.50 (m)	62.4	62.5	3.58 (m)	3.55 (m)
Rha-I-1″	102.9		5.10 (br s)		103.4		5.05 (br s)	
2″	72.4		3.92 (m)		72.4		3.88 (m)	
3″	72.1		3.58 (m)		70.7		3.56 (m)	
4″	73.9		3.45 (m)		81.4		3.87 (t, 9.3)	
5″	70.3		3.35 (m)		68.8		3.32 (m)	
6″	18.4		1.08 (d, 6.1)		19.2		1.05 (d, 6.2)	
Rha-II-1‴					102.6		5.19 (d, 1.3)	
2‴					72.9		3.90 (m)	
3‴					72.8		3.54 (m)	
4‴					73.9		3.66 (m)	
5‴					70.3		3.39 (m)	
6‴					17.7		1.09 (d, 6.2)	

the glucosyl unit in 2. Therefore, the structures of ligupurpurosides D (2) was determined to be 2-(4-hydroxyphenyl)-ethyl-[3-*O*-α-L-rhamnopyranosyl(1→4)-α-L-rhamnopyranosyl] [6-*O*-(*E*)-caffeoyl]-*O*-β-D-gluco-pyranoside (2).

Ligupurpuroside E (3) was isolated as a yellow powder. The molecular formula was assigned as $C_{21}H_{28}O_{12}$ by the negative HRFABMS (*m/z* 471.1504 [M-H]$^-$). The FABMS also exhibited fragment ion peak at *m/z* 325 [M-147 (rhamnosyl)]$^-$, suggesting the

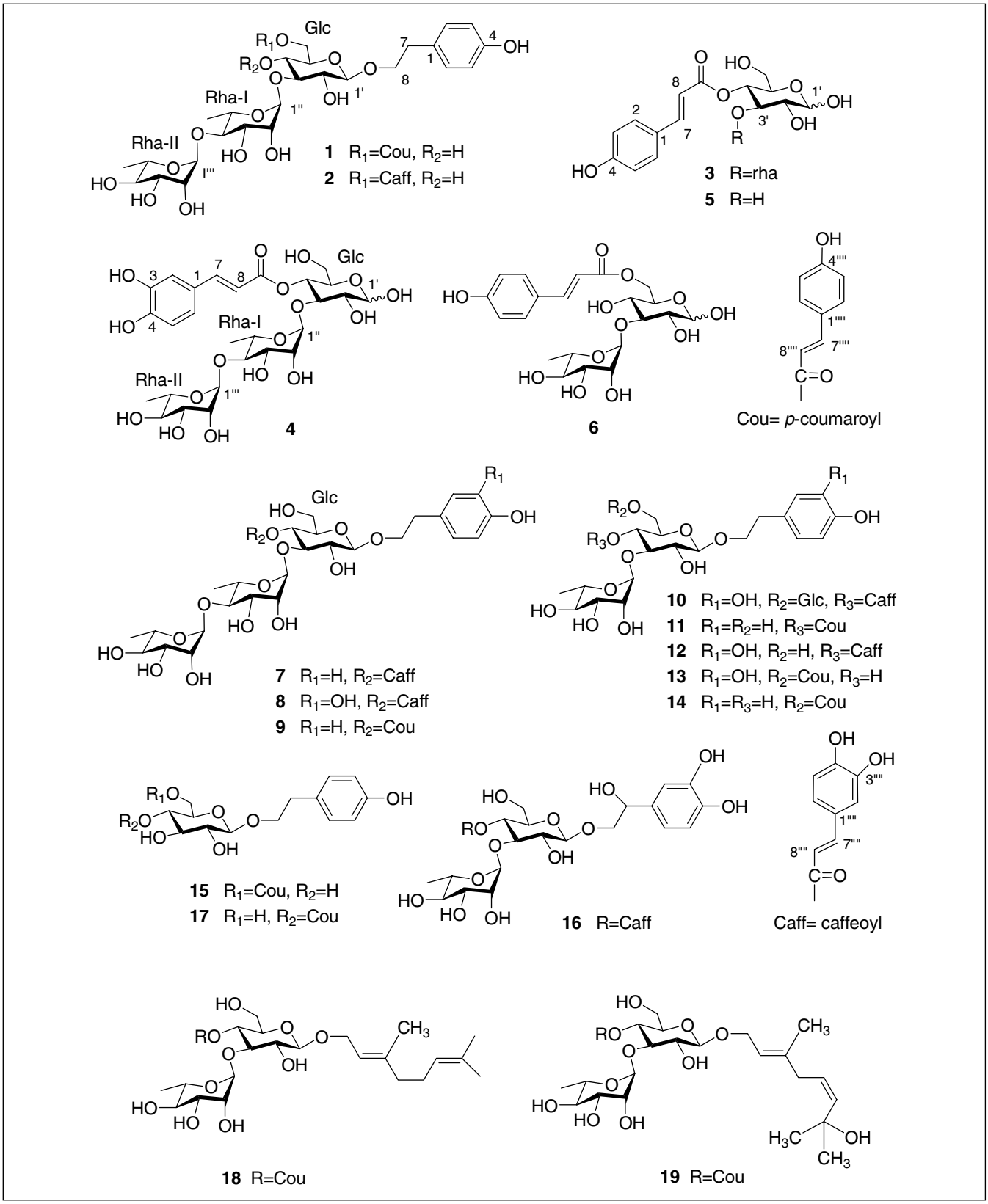

Figure 1—Compounds 1 to 19 isolated from Ku-Ding-Cha produced from *L. purpurascens*.

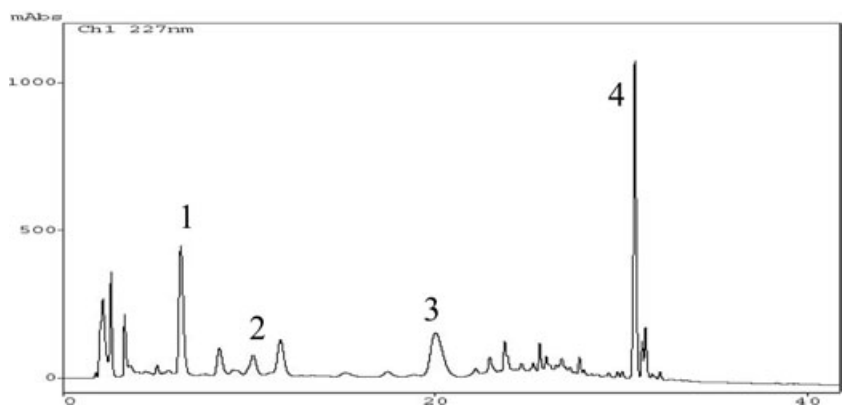

Figure 2—HPLC profiles of MeOH extract of Ku-Ding-Cha produced from *L. purpurascens.* Peaks 1: acetoside (12), 2: ligupurpuroside D (2), 3: osmanthuside B (11), and 4: ligurobustoside C (18).

Table 3—Content of some major compounds (2, 11, 12, and 18) in Ku-Ding-Cha.[a]

Compounds	Content (g/100 g of Ku-Ding-Cha)
2	1.6
11	3.2
12	5.2
18	7.6

[a]Average of 2 separate extractions.

Table 4—DPPH radical scavenging activity of compounds isolated from Ku-Ding-Cha.

Compounds	SC_{50} (μM)[a,b]
Ascorbic acid[c]	10.5 ± 0.3
2	63.5 ± 0.7
3	477 ± 9
4	24.6 ± 0.3
6	149 ± 3
7	58.1 ± 0.4
8	20.2 ± 0.2
9	181 ± 4
10	19.4 ± 0.4
11	293 ± 5
12	16.9 ± 0.3
13	47.8 ± 0.5
14	239 ± 7
16	18.2 ± 0.3
17	149 ± 4
18	500 ± 9
19	530 ± 9

[a]SC_{50}: radical scavenging activity (concentration in μM required for 50% reduction of DPPH radical).
[b]Values represent means ± SD ($n = 3$).
[c]Positive control.

presence of a terminal deoxyhexosyl unit in molecule. The ^{1}H and ^{13}C NMR spectra of 3 (Table 2) showed the presence of a *p*-coumaroyl, and 1/2 α- and 1/2 β-D-glucopyranosyl units. The duplication (α and β forms) of the signals due to glucosyl unit suggested 3 was a pair of D-glucosyl anomeric isomers. Comparison of the NMR data with those of 5 (Table 2) suggested that 3 had a similar structure to that of 5. The only difference between 3 and 5 was the observation of one more set of signals arising from a rhamnosyl unit in 3. Interpretation of the HMBC spectrum revealed correlations from rhamnosyl H-1" (δ 5.10) to glucosyl C-3′ [δ 79.2 (α form) and 81.6 (β form)], indicating that the rhamnosyl unit was linked at C-3′ of the glucosyl unit in 3. Moreover, correlations of glucosyl H-4′ [δ 4.93 (1/2H, t, J = 9.8 Hz, α form) and δ 4.90 (1/2H, t, J = 9.8 Hz, β form)] with carbonyl carbon (δ 168.5) of the *p*-coumaroyl group were observed. Based on the above-mentioned evidence, the structure of compound 3 was determined to be 3-*O*-α-L-rhamnopyranosyl-4-*O*-*p*-coumaroyl-D- glucopyranose.

Ligupurpuroside F (4) was obtained as a yellow amorphous powder and its HRFABMS exhibited a quasi-molecular ion peak at *m/z* 633.2025 $[M-H]^-$ corresponding to a molecular formula $C_{27}H_{38}O_{17}$. The ^{1}H and ^{13}C NMR spectra of 4 (Table 2) displayed signals due to a (*E*)-caffeoyl and two α-L-rhamnopyranosyl units together with a 1/2 α- and a 1/2 β-D-glucopyranosyl units. The NMR features of 4 were closely related to those of ligupurpuroside A (8), except for the absence of signals assignable to the 2-hydroxyl-(4-hydroxyphenyl)-ethyl unit in 4. The duplication (α and β forms) of the signals due to glucosyl unit suggested that 4 was a pair of D-glucosyl anomeric isomers. Location of the (*E*)-caffeoyl group and the sugar sequence were further confirmed by the HMBC experiment, in which correlations of glucosyl H-4′ [δ 4.96 (α form) and 4.93 (β form)] with δ 168.3 (carbonyl carbon of the (*E*)-caffeoyl group), rhamnosyl-I H-1" (δ 5.05) with glucosyl C-3′ [δ 78.9 (α form) and 81.4 (β form)], and rhamnosyl-II H-1"′ (δ 5.19) with rhamnosyl-I C-4" (δ 81.4) were observed. Accordingly, the structure of compound 4 was identified as 3-*O*-[α-L- rhamnopyranosyl(1→4)-α-L-rhamnopyranosyl]-4-*O*-(*E*)-caffeoyl-D-glucopyranose.

Though compounds 3 and 4 as new natural sugar esters were isolated first, from the point of biogenetic view, they might be derived from the relevant phenylethanoid glycosides.

HPLC analysis of Ku-Ding-Cha

The herbal tea of Ku-Ding-Cha was extracted with MeOH and analyzed by HPLC (Figure 2). As shown in Table 3, the HPLC analysis found that the new phenylethanoid glycoside, ligupurpuroside D (2), together with the known glycosides, osmanthuside B (11), acteoside (12), and ligurobustoside C (18), was one of the major compounds in Ku-Ding-Cha, with a content of about 1.6 g, 3.2 g, 5.2 g, and 7.6 g in 100 g of dry tea material, respectively.

DPPH radical-scavenging assay

The antioxidant activity of the isolated compounds was evaluated by DPPH assay and the results are shown in Table 4. Of them, compounds 4, 8, 10, 12, and 16 were the most active ones due to the structure possessed two 3,4-dihydroxylphenyl units, which may play an important role for the antioxidant activity.

Many studies have addressed the health benefit of drinking green tea because it contains 4 major catechin antioxidants. The present results and published data (He and others 1992, 1994) suggest that the phenylethanoid glycosides as main constituents

occurring in the leaves of *L. purpurescens* will promote the reasonable usage of this kind of herbal tea for the human health (Machida and Kikuchi 1992; Tijburg and others 1997; Chen and others 2000; Wong and others 2001).

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