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A novel acylated quercetin tetraglycoside from oolong tea (*Camelia sinensis*) extracts

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Abstract—A novel acylated quercetin tetraglycoside, namely quercetin 3-*O*-(2^G-*p*-coumaroyl-3^G-*O*-β-L-arabinosyl-3^R-*O*-β-D-glucosylrutinoside) was isolated from oolong tea (*Camelia sinensis*) extracts. Structural analysis of this compound was achieved by NMR, TOF-MS and high-resolution FAB-MS. Triglycosyl flavonols have previously been reported from tea leaves and tea seeds however this is the first report of an aromatic acylated and tetraglycosyl flavonol.

Oolong tea is produced from green tea via heating and fermentation processes. It is known to contain over 70 different compounds such as oolonghomobisflavan A, B[1] and theasinensin,[2] which are derived from epigallocatechingallate. In addition, green tea and black tea extracts are also reported to contain myricetin, quercetin or kaempferol glycosides with 1–3 of sugar moieties such as rhamnose, galactose and glucose.[3–5] The present study reports for the first time, the isolation and identification of a novel compound, acylated quercetin tetraglycoside (compound **1**) from oolong tea extracts.

Oolong tea (hot water) extract was fractionated by high-performance liquid chromatography (HPLC) using a reverse phase column (Develosil-ODS-UG, 5 cm i.d. × 50 cm, Nomura Chemical Co. Ltd., Japan) with a flow rate of 32 mL/min and a detector wavelength at 280 nm. The solvent systems used were as follows: an isocratic elution of 20% CH_3CN containing 0.05% trifluoroacetic acid (TFA) in H_2O for 80 min, a linear gradient elution using 20–90% of CH_3CN containing 0.05% TFA in H_2O for 15 min, an isocratic elution of 90% CH_3CN containing 0.05% TFA in H_2O for 50 min. The hydrophobic fraction was eluted at between 130–140 min. One gram of the hydrophobic fraction was further fractionated by MCI-gel CHP 20P (25 mm i.d. × 450 mm, pore size; 75–150 μm, Mitsubishi Chemical Co., Japan), and eluted with 20%, 40% and 60% aqueous MeOH and 70% aqueous acetone, successively. Furthermore, the fraction eluted with 60% aqueous MeOH was fractionated by preparative HPLC to isolate **1**. Preparative HPLC was performed on a 10 mm i.d. × 250 mm stainless steel column filled with develosil ODS-HG-5 (Nomura Chemical, Co. Ltd., Japan) monitored at 280 nm. The solvent system used was as follows: a linear gradient elution for 40 min from 20% to 40% solvent B (90% CH_3CN containing 0.01% TFA) in solvent A (0.01% TFA in H_2O) at a flow rate of 4 mL/min. ^{1}H NMR, ^{13}C NMR, ^{1}H{^{13}C}-HSQC, ^{1}H{^{13}C}-HMBC, TOCSY, 1D-HOHAHA and DQF-COSY spectra of **1** were obtained on a DMX-750 spectrometer (BRUKER BIOSPIN, Germany). Compound **1** was dissolved in 100% CD_3OD. The residual ^{1}H and ^{13}C signals of CD_3OD were used as the internal standard (δ 3.30 for ^{1}H, δ 48.97 for ^{13}C, respectively). The mass spectrum of **1** was obtained using a nano ESI-Q-TOF MS, equipped with a Z-spray ion source (Micromass, Manchester, UK) in positive mode. High-resolution fast-atom bombardment mass spectrometry (HRFAB-MS) of **1** was recorded on a JEOL 700T FABMS (JEOL, Japan) with nitrobenzene matrix in positive mode.

Keywords: Oolong tea; *Camelia sinensis*; Flavonol glycoside.
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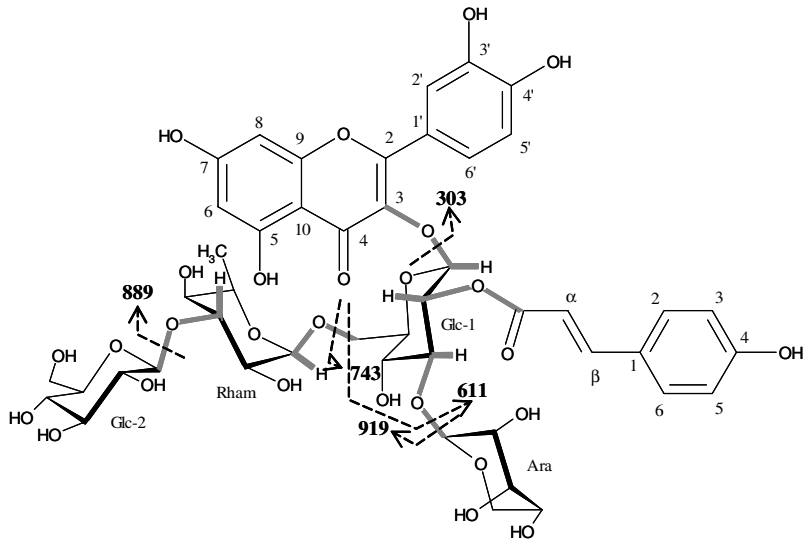

Figure 1. Structure of **1**. The bold gray lines indicate significant $^{1}H\{^{13}C\}$-HMBC correlations. The arrowheads and bold numbers indicate MS–MS fragments by TOF-MS.

Sugar analysis of **1** was carried out as follows. One point five milligrams of **1** was dissolved in 10 μL of DMSO and then heated in boiling water for 20 min with 200 μL of 1 N HCl. The hydrolyzed solution was filtered with filter paper (pore size; 0.45 μm). The filtrate was analyzed by HPLC with two different columns, a Shimpack-SPR-Pb (7.8 mm i.d. × 250 mm, Shimadzu Co. Ltd., Japan) or a Shodex Ionpak KS-801 (8 mm i.d. × 300 mm). The solvent system was 100% H_2O with a flow rate of 0.8 mL/min at 80 °C. The detector was a Shimadzu RID-10A. The sugar composition was identified by comparing retention times with that of authentic samples, which were as follows; D-glucose: 12.78 and 13.60 min, L-arabinose: 16.32 and 15.68 min, L-rhamnose: 14.66 and 13.89 min (Shimpack-SPR-Pb and Shodex Ionpak KS-801, respectively).

The above HCl hydrolysate was diluted with 50% CH_3CN and submitted to aglycone analysis. HPLC was conducted using a Develosil C30-UG-5 column (4.6 mm i.d. × 150 mm, Nomura Chemical Co. Ltd., Japan), at a flow rate of 0.6 mL/min; the solvent system used was as follows; a linear gradient elution from 18% to 63% of CH_3CN containing 0.1% TFA in H_2O over a 10 min period, then 5 min of isocratic elution of 63% CH_3CN containing 0.1% TFA in H_2O. The photodiode array detector SPD-M10A (Shimadzu Co. Ltd., Japan) was monitored from 250 to 400 nm. Under these conditions, the retention time and λ_{max} of quercetin was 10.2 min and 370 nm, respectively while *p*-coumaric acid was 7.5 min and 308 nm, respectively.

Compound **1** showed UV–vis absorption maxima in 100% MeOH at 355 nm ($\log \varepsilon = 4.242$), 317 nm ($\log \varepsilon = 4.459$), 267 nm ($\log \varepsilon = 4.335$), 259 nm ($\log \varepsilon = 4.327$).[6] The hydrolysis products showed that **1** contained quercetin and *p*-coumaric acid. The ^{1}H NMR spectrum showed two signals due to an A ring of quercetin at δ 6.16 (1H, d, $J = 2.0$ Hz) and 6.35 (1H, d, $J = 2.0$ Hz), and three signals due to a B ring at δ 6.88 (1H, d, $J = 8.4$ Hz), 7.55 (1H, dd, $J = 8.4$, 2.1 Hz) and 7.59 (1H, d, $J = 2.1$ Hz). In addition, two kinds of signals appeared at δ 6.81 (2H, d, $J = 8.6$ Hz) and 7.45 (2H, d, $J = 8.6$ Hz), and two other signals showed vicinal coupling each other at δ 6.36 (1H, d, $J = 15.9$ Hz) and 7.68 (1H, d, $J = 15.9$ Hz). These results indicated the presence of a *p*-coumaric acid skeleton. Compound **1** showed quasi-molecular ion at m/z $[M+H]^+$ 1051 and MS–MS products (m/z 919, 889, 743, 611 and 303) in its TOF-MS (Fig. 1). The molecular formula of **1** was determined to be $C_{47}H_{54}O_{27}$ as revealed by HRFAB-MS [m/z 1051.2938 $[M+H]^+$, $\Delta + 0.8$ mmu]. The molecular weight of **1**, the presence of quercetin and *p*-coumaric acid and the presence of four anomeric ^{1}H and ^{13}C signals in the NMR indicate the possibility that it includes four sugars. Furthermore, the sugar analysis of hydrolyzed **1** showed glucose, arabinose and rhamnose at the mole ratio of 2:1:1. Such results therefore suggest that **1** should consist of the following components; quercetin, *p*-coumaric acid, arabinose, rhamnose and two glucoses. In the 1D-HOHAHA of **1**, the following six signals were resonated when the signal at δ 5.54 (1H, d, $J = 8.0$ Hz) was irradiated; δ 5.22 (1H, dd, $J = 9.0$, 8.0 Hz), 3.85 (1H, t, $J = 9.0$ Hz), 3.49 (1H, t, $J = 9.0$ Hz), 3.51 (1H, m,), 3.87 (1H, br s, $J = 12.0$ Hz), 3.53 (1H, dd, $J = 12.0$, 6.0 Hz). The coupling constant of these six signals indicated five protons, which have axial configuration with each other and the methylene protons. These signals were assigned as β-D-glucose 1 (Glc-1). Similarly, assignments of other sugars obtained by irradiating other anomeric protons (δ 4.33, 4.61 and 4.45) are shown in Table 1. In the HMBC, three cross peaks were observed between Glc-1 H-3 (δ 3.85) and Ara C-1 (δ 105.29), Glc-1 H-6 (δ 3.53 and 3.87) and Rham C-1 (δ 102.29), Rham H-3 (δ 3.63) and Glc-2 C-1 (δ 106.58), respectively. Thus, the connection of Glc-1 ($6 \rightarrow 1$) [($3 \rightarrow 1$) Ara] Rham ($3 \rightarrow 1$) Glc-2 was suggested. In addition, HMBC indicated an ether bond of quercetin 3-*O*-β-Glc-1 and ester bond between H-2 of Glc-1 and carbonyl carbon (δ 168.66) of *p*-coumaric acid. The

Table 1. ^{1}H and ^{13}C NMR spectral data of **1**

	^{1}H (δ)	J (Hz)	^{13}C (δ)		^{1}H (δ)	J (Hz)	^{13}C (δ)
Quercetin				Glc-1	C-3 of quercetin		
2	—		158.87	1	5.54	d 8.0	100.98
3	—		134.95	2	5.22	dd 9.0, 8.0	74.53
4	—		179.02	3	3.85	t 9.0	84.33
5	—		163.05	4	3.49	t 9.0	70.16
6	6.16	d 2.0	99.80	5	3.51	m	76.83
7	—		16.67	6a	3.87	br d 12.0	68.49
8	6.35	d 2.0	94.80	6b	3.53	dd 12.0, 6.0	
9	—		158.40	Glc-2	C-3 of Rham		
10	—		105.85	1	4.45	d 7.8	106.58
1′	—		123.17	2	3.27	dd 9.0, 8.0	75.47
2′	7.59	d 2.1	117.50	3	3.39	t 9.0	70.89
3′	—		145.89	4	3.44	t 9.0	77.51
4′	—		149.65	5	3.28	m	77.51
5′	6.88	d 8.4	116.15	6a	3.72	dd 12.0, 4.0	62.07
6′	7.55	dd 8.4, 2.1	123.45	6b	3.77	dd 12.0, 2.0	
p-Coumaric acid				Rham	C-6 of Glc-1		
1	—		127.32	1	4.61	br s	102.29
2	7.45	d 8.6	131.28	2	3.98	dd 3.0,1.5	71.27
3	6.81	d 8.6	116.74	3	3.63	dd 10.0, 3.0	83.07
4	—		161.24	4	3.45	t 10.0	72.53
5	6.81	d 8.6	116.74	5	3.53	dd 10.0, 6.0	69.49
6	7.45	d 8.6	131.28	6	1.12	d 6.0	17.94
α	6.36	d 15.9	115.16	Ara	C-3 of Glc-1		
β	7.68	d 15.9	147.23	1	4.33	d 7.0	105.29
C=O			168.66	2	3.53	dd 10.0, 7.0	72.18
				3	3.48	dd 10.0, 1.0	73.88
				4	3.77	m	69.49
				5a	3.57	dd 12.0, 1.0	67.17
				5b	3.89	dd 12.0, 3.0	

Solvents: CD_3OD.

lower field shift of H-2 of Glc-1 indicated that H-2 of Glc-1 was acylated with *p*-coumaric acid.

Based on the above data, **1** was identified as quercetin 3-*O*-(2^{G}-*p*-coumaroyl-3^{G}-*O*-β-L-arabinosyl-3^{R}-*O*-β-D-glucosylrutinoside) (Fig. 1). In contrast, isolation of quercetin 3-glucosyl(1 → 3)-rutinoside from tea leaves was reported in 1991.[7] Other flavonol glycosides such as *C*-arabinosylated flavone, apigenin 6,8-di-*C*-arabinoside[8] and a flavonol galactoside[9,10] have also been isolated from tea leaves. In this paper, we detail the novel isolation of an arabinosyl flavonol, which was found to comprise of three kinds of sugar moieties, glucose, rhamnose and arabinose. It is suggested that tea has many kinds of polyphenols because of its various glycosylation patterns.

Evaluation of 1,1-diphenyl-2-picrylhydrazyl (DPPH) radical scavenging activity of **1** was measured.[11] Compound **1** showed comparatively strong antioxidant activity although lower than that of quercetin (Table 2). Based on the previous report,[12] which suggested that quercetin was liberated as aglycone from its glucosides due to hydrolysis by β-glucosidase isolated from rat small intestinal mucosa of duodenum, it was expected that **1** might exhibit stronger activity in vivo as quercetin. Furthermore, it was assumed that **1** would be absorbed into body easily and could be a better antioxidant because it was readily soluble to water while quercetin was insoluble.

Table 2. Antioxidant activity of **1**

	EC_{50}(nmol/mL)[a]
Compound **1**	16.2
Quercetin	8.6
p-Coumaric acid	377.8
α-Tocopherol	27.4

[a] The effective concentration of antioxidant necessary to decrease the initial DPPH radical by 50%.

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References and notes

1. Hashimoto, F.; Nonaka, G.; Nishioka, I. *Chem. Pharm. Bull.* **1989**, *37*, 3255–3263.
2. Hashimoto, F.; Nonaka, G.; Nishioka, I. *Chem. Pharm. Bull.* **1988**, *36*, 1676–1684.
3. Finger, A.; Engelhardt, U. H.; Wray, V. *Phytochem.* **1991**, *30*, 2057–2060.
4. Sekine, T.; Arai, Y.; Ikegami, F.; Fujii, Y.; Shindo, S. *Chem. Pharm. Bull.* **1993**, *41*, 1184–1187.
5. Sekine, T.; Arita, J.; Yamaguti, A.; Saito, K.; Okonogi, S.; Morisaki, N.; Iwasaki, S.; Murakoshi, I. *Phytochem.* **1991**, *30*, 991–995.
6. The UV–vis spectrum (600–250 nm) was measured using a JASCO V-520 UV–vis spectrophotometer, 0.197 mg of compound **1** was dissolved in 3 mL of MeOH.
7. Andreas, F.; Ulrich, H. E. *J. Sci. Food Agric.* **1991**, *55*, 313–321.
8. Chaboud, A.; Raynaud, J.; Debourcieu, L. *J. Nat. Prod.* **1986**, *49*, 1145.
9. Andreas, F.; Ulrich, H. E.; Victory, W. *Phytochem.* **1991**, *30*, 2057–2060.
10. Jacqoues, K. *Phytochem.* **1967**, *6*, 317–318.
11. A test sample (ethanol solution, 2 mL) was added to 0.5 mM DPPH in ethanol (1 mL) mixed with a 50 mM 2-monopholinoethanesulfonic acid (MES) buffer (pH 7.4, 2 mL), and the mixture was shaken vigorously and kept at 25 °C for 30 min. The DPPH radical scavenging activity is expressed as the ratio of the relative decrease in the absorbance of the test sample mixture at 517 nm to that of the 1 mM butylhydroxytoluene (BHT) solution. DPPH radical scavenging activity (%) = {(ethanol alone)−(test sample)}/{(ethanol alone)−(BHT)} × 100.
12. Ioku, K.; Pongpiriyadacha, Y.; Konishi, Y.; Takei, Y.; Nakatani, N. *Biosci. Biotechnol. Biochem.* **1998**, *62*, 1428–1431.