Flavonol Glycosides in Leaves of Two *Diospyros* Species

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Fourteen flavonol glycosides including two new compounds were isolated from the leaves of two *Diospyros* plants (*D. cathayensis* and *D. rhombifolia*). The structures of isolated compounds were determined by spectroscopic analysis. The scavenging activity of 1,1-diphenyl-2-picrylhydrazyl (DPPH) radical of the isolated compounds was also investigated.

Key words flavonol glycoside; *Diospyros cathayensis*; *Diospyros rhombiholia*; structure determination; DPPH (1,1-diphenyl-2-picrylhydrazyl) radical scavenging activity

The genus *Diospyros* belongs to the Family Ebenaceae and comprises about 500 species distributed in the tropical and temperate zone.[1)] Although many studies about quinone compounds in the *Diospyros* plants have been reported, there are few reports about constituents in leaves in spite of those medicinal use. In the previous report, we described the isolation of flavonol glycosides including three new compounds and their scavenging activity of 1,1-diphenyl-2-picrylhydrazyl (DPPH) radical.[2)] *D. cathayensis* Steward and *D. rhombifolia* Hemsl distribute in China.[3)] Although both of plants are medicinally used,[4)] chemical constituents are unclear. Upon continued study of phenolic constituents in leaves of *Diospyros* plants, we describe the isolation and structure determination of flavonol glycosides including two new flavonol glycosides with unique sugar chains in the leaves of two *Diospyros* plants in this report. The scavenging activity of DPPH radical of isolated compounds was also investigated.

Results and Discussion

MeOH extracts of the dried leaves of *D. cathayensis* and *D. rhombifolia* were subjected to chromatography on reversed phase ODS column. Further repeated purification of the fractions on reverse phase ODS, Sephadex LH 20 and preparative TLC resulted in the isolation of **1**—**12** from *D. cathayensis*, **7**, **13** and **14** from *D. rhombifolia*.

Compound **13**, a pale yellow solid, exhibited an $[M-H]^-$ ion peak at *m*/*z* 769 in the negative ion FAB-MS, indicating the molecular weight to be 770. The molecular formula of $C_{33}H_{38}O_{21}$ was established by the HR-FAB-MS $[M-H]^-$ at *m*/*z* 769.1826. The EI-MS fragments (*m*/*z* 286, 153, 121), UV absorption and ^{1}H-NMR [δ 5.89 (1H, d, J=2.0 Hz, H-6), 6.07 (1H, d, J=2.0 Hz, H-8), 6.77 (2H, d, J=9.0 Hz, H-3′, 5′), 7.96 (2H, d, J=9.0 Hz, H-2′, 6′)] indicated that **13** had a kaempferol as aglycone. Analysis of ^{1}H- and ^{13}C-NMR (Table 1) spectrum by ^{1}H–^{1}H COSY, ^{13}C–^{1}H COSY and HMBC spectrum showed the presence of a β-glucuronopyranose ($J_{1'',2''}$=7.1 Hz) , a β-glucopyranose ($J_{1''',2'''}$=7.7 Hz) and an α-rhamnopyranose ($J_{1'''',2''''}$=1.0 Hz) in **13** as sugar components. In the HMBC spectrum of **13** (Fig. 2), significant long range correlations were observed between H-1‴/C-2″ and H-1⁗/C-3″, which led to determine the sugar chain to be (2-*O*-α-rhamnopyranosyl-3-*O*-β-glucopyranosyl)-β-glucuronic acid. Furthermore the anomeric proton of glucuronic acid (H-1″) was correlated to C-3 of the kaempferol. Consequently, the structure of **13** was determined to be kaempferol 3-*O*-β-(2″-*O*-α-rhamnopyranosyl-3″-*O*-β-glucopyranosyl)-β-glucuronopyranoside.

Compound **14**, a pale yellow solid, exhibited an $[M-H]^-$ ion peak at *m*/*z* 901 in the negative ion FAB-MS, indicating the molecular weight to be 902. The molecular formula of $C_{39}H_{50}O_{24}$ was established by the HR-ESI-MS $[M+Na]^+$ at *m*/*z* 925.2554. The UV absorptions and ^{1}H-NMR spectral

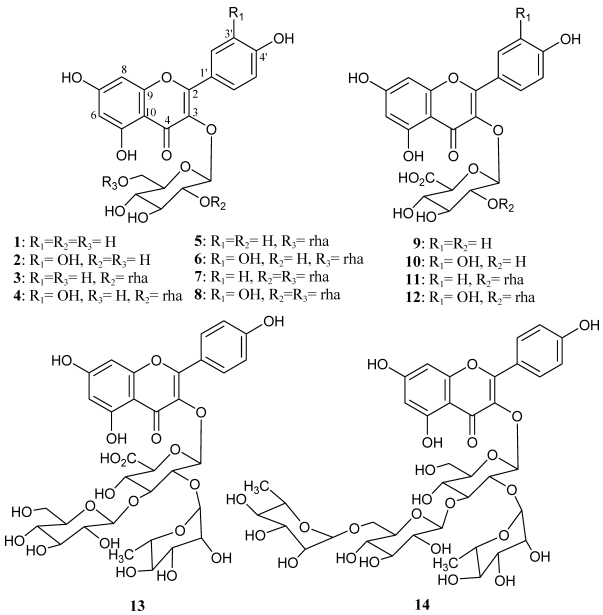

Fig. 1

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Table 1. ^{1}H- and ^{13}C-NMR Spectral Data of Compounds **13** and **14**

No.	**13**[a]		No.	**14**[b]	
	^{1}H	^{13}C		^{1}H	^{13}C
2		154.34	2		158.47
3		132.14	3		133.89
4		176.23	4		178.72
5		160.90	5		162.87
6	5.89 d (2.0)	100.30	6	6.08 d (2.0)	101.38
7		171.62	7		170.48
8	6.07 d (2.0)	94.58	8	6.25 d (2.0)	95.87
9		154.84	9		158.84
10		101.51	10		104.61
1′		120.68	1′		123.23
2′,6′	7.96 d (9.0)	130.49	2′,6′	8.00 d (9.0)	132.07
3′,5′	6.77 d (9.0)	115.23	3′,5′	6.87 d (9.0)	116.23
4′		160.23	4′		161.39
glc-A			glc 1		
1″	5.63 d (7.1)	98.35	1″	5.63 d (7.0)	100.40
2″	3.64 dd (8.0, 7.1)	77.44	2″	3.72 dd (7.0, 8.0)	79.99
3″	3.72 dd (9.0, 8.0)	83.30	3″	3.75 t (8.0)	87.59
4″	3.42 dd (10.0, 9.0)	70.22	4″	3.79 m	70.32
5″	3.31 d (10.0)	74.42	5″	3.38 m	78.09
6″		171.62	6″	3.68 dd (11.6, 5.0)	62.56
				3.90 dd (11.6, 2.4)	
rha			rha 1		
1‴	5.14 d (1.0)	100.95	1‴	5.24 d (1.0)	103.17
2‴	3.81 dd (4.3,1.0)	70.55	2‴	4.06 dd (3.6, 1.0)	72.28
3‴	3.45 dd (10.0, 4.3)	70.50	3‴	3.77 dd (9.5, 3.6)	72.28
4‴	3.12 t (10.0)	71.86	4‴	3.33 t (9.5)	74.07
5‴	3.74 dq (10.0, 6.1)	68.54	5‴	4.08 dq (9.5, 6.4)	70.09
6‴	0.77 d (6.1)	17.23	6‴	0.97 d (6.4)	17.51
glc			glc 2		
1⁗	4.45 d (7.7)	102.11	1⁗	4.58 d (8.0)	102.38
2⁗	3.08 dd (9.0, 7.7)	73.23	2⁗	3.26 dd (9.0, 8.0)	75.08
3⁗	3.22 t (9.0)	76.47	3⁗	3.39 t (9.0)	76.62
4⁗	3.14 t (9.0)	69.68	4⁗	3.41 t (9.0)	69.76
5⁗	3.16 m	76.80	5⁗	3.30 m	78.20
6⁗	3.50 dd (11.7, 5.0)	60.61	6⁗	3.39 m	68.33
	3.72 br d (11.7)			3.83 br d (10.6)	
			rha 2		
			1⁗′	4.50 d (1.6)	102.38
			2⁗′	3.59 dd (3.6, 1.6)	72.10
			3⁗′	3.48 dd (9.8, 3.6)	72.28
			4⁗′	3.23 t (9.8)	73.85
			5⁗′	3.42 m	69.76
			6⁗′	1.09 d (6.2)	17.87

a) Measured in DMSO-d_6. ^{1}H (600 MHz); ^{13}C (150 MHz). *b*) Measured in methanol-d_4. ^{1}H (500 MHz); ^{13}C (125 MHz).

data indicated that **14** was a kaempferol glycoside. The detail analysis of ^{1}H-NMR by HH COSY and HOHAHA spectra showed the presence of two β-glucopyranose and two α-rhamnopyranose moieties in **14**. Significant long range correlations in the HMBC spectrum (Fig. 3) were observed between H-1‴/C-2″, H-1⁗/C-3″ and H-1⁗′/C-6⁗. In addition, the anomeric proton of β-glucose (H-1″) was correlated to C-3 of the kaempferol. Therefore, the structure of **14** was determined to be kaempferol 3-*O*-[2″-*O*-α-rhamnopyranosyl-3″-*O*-(6⁗-*O*-α-rhamnopyranosyl-β-glucopyranosyl)]-β-glucopyranoside. Although the absolute configuration of the sugars has not been determined, **13** and **14** were new flavonol glycoside with unique sugar chains.

On the basis of ^{1}H- and ^{13}C-NMR spectral analysis, and direct comparison with the authentic samples (**1**, **2**, **6**), compounds **1**—**12** were identified with kaempferol 3-*O*-β-glucopyranoside (astragalin: **1**), quercetin 3-*O*-β-glucopyranoside (iso quercitrin: **2**), kaempferol 3-*O*-(2″-*O*-α-rhamnopyranosyl)-β-glucopyranoside (**3**), quercetin 3-*O*-(2″-*O*-α-rhamnopyranosyl)-β-glucopyranoside (**4**), kaempferol 3-*O*-(6″-O-α-rhamonpyranosyl)-β-glucopyranoside (**5**), quercetin 3-*O*-(6″-*O*-α-rhamnopyranosyl)-β-glucopyranoside (rutin: **6**), kaempferol 3-*O*-(2″,6″-di-α-rhamnopyranosyl)-β-glucopyranoside [clitorin (maurtianin): **7**],[4] quercetin 3-*O*-(2″,6″-di-α-rhamnopyranosyl)-β-glucopyranoside (manghaslin, **8**),[5] kaempferol 3-*O*-β-glucuronopyranoside (**9**), quercetin 3-*O*-β-glucuronopyranoside (**10**), kaempferol 3-*O*-(2″-α-rhamnopyranosyl)-β-glucuronopyranoside (**11**) and quercetin 3-*O*-(2″-α-rhamnopyranosyl)-β-glucuronopyranoside (**12**), respectively. Although compounds **7** and **8** have been isolated from various sources,[6] it is the first isolation from the genus *Diospyros*. Furthermore, compounds **11** and **12** had been once isolated from *Alchemilla speciosa*.[7] Detail assignment of the ^{1}H- and ^{13}C-NMR spectrum of compounds **11** and **12** were achieved currently by 2D NMR (^{13}C–^{1}H COSY and HMBC spectrum) in this time as described in experimental part.

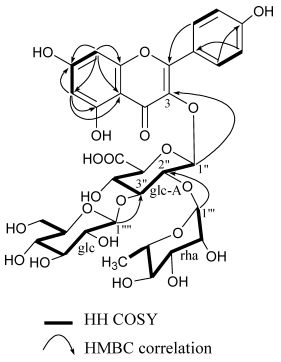

Fig. 2

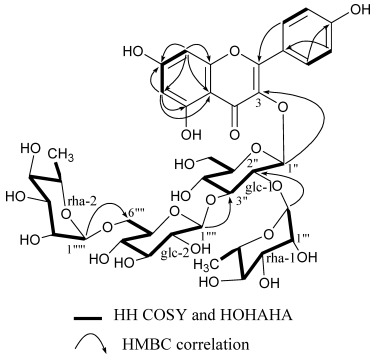

Fig. 3

The scavenging activity (Sc_{50}) of the MeOH extracts of *D. cathayensis* and *D. rhombifolia* were found to be 230, 940 μg/ml, respectively. Although the scavenging activities of extracts were not so strong, the quercetin glycosides such as **4**, **6**, **8**, **10** and **12** have shown strong activities (**4**: 3.6, **6**: 3.6, **8**: 5.6, **10**: 1.5 and **12**: 5.0 μM, respectively).

Experimental

General Experimental Procedures ^{1}H- and ^{13}C-NMR spectra were recorded on LA300 (^{1}H at 300 MHz and ^{13}C at 75 MHz, JEOL), α-500 (500 MHz at ^{1}H and 125 MHz at ^{13}C, JEOL) and JNM-ECA 600 (^{1}H at 600 MHz and ^{13}C at 150 MHz, JOEL) spectrometers. Chemical shift were shown as δ values with trimethylsilane (TMS) as an internal reference. Peak

multiplicities were quoted in Hz. The EI-MS, negative FAB-MS, HR-FAB-MS and ESI-MS were recorded on JMSDX-300, GMS-T100LC and JMS-700T (JOEL) spectrometers. UV spectrum was recorded on 2200 UV spectrophotometer (Shimadzu) and optical rotation was measured on P-1020 polarimeter. Sephadex LH-20, Fuji Silysia Chemical Chromatorex ODS (100—200, mesh) and Sep-Pak C_{18} Cartridges ODS were used for column chromatography. Kiesel-gel 60 F_{256} (Merck) 0.25 mm was used for analytical and 0.5 mm for preparative TLC.

Plant Material *Diospyros cathayensis* was cultivated at the Botanical Gardens of Setsunan University in Japan, and the leaves were collected in September 2003. *D. rhombifolia* was cultivated at the Kyoto Botanical Garden and the leaves were also collected in November 2002. These voucher specimens have been deposited in Gifu Prefectural Institute of Health and Environmental Sciences, Gifu, Japan.

The dried leaves of *D. cathayensis* (1.3 kg) were extracted with MeOH (5 l×weekly×3) at room temperature. After concentration of the solvent, MeOH extract (150 g) was obtained. A part of the extract (145 g) was chromatographed on ODS (Chromatorex) eluted with H_2O followed by increasing concentration of MeOH to give 14 fractions. Fraction 13 (450 mg: 50% MeOH) was chromatographed on Sep Pack C_{18} cartridge. Fraction of 30% MeOH elution was further chromatographed on Sephadex LH 20 column eluted with MeOH. The flavonoid contained fractions were purified by preparative TLC using $CHCl_3$–MeOH–H_2O (65 : 35 : 10: lower phase) to give **1** (12 mg), **2** (18 mg), **3** (22 mg) and **4** (25 mg) as pure form. Fraction 12 (345 mg: 50% MeOH) was fractionated with Sephadex LH 20 column (MeOH). The flavonoid contained fraction was further purified with Sep Pak C_{18} cartridge with 40% MeOH, and purified with preparative TLC developed with $CHCl_3$–MeOH–H_2O (65 : 35 : 10; lower phase) to give **5** (31 mg) and **6** (27 mg) as pure form. Fraction 9 (30% MeOH) was fractionated with Sephadex LH 20 with 10% MeOH. The resulting flavonoid fraction was purified by Sephadex LH 20 with 30% MeOH and finally purified by preparative TLC developed with $CHCl_3$–MeOH–H_2O (65 : 40 : 10) to give **9** (60 mg). By the same procedure of isolation of **9**, fraction 3 (10% MeOH) gave **10** and fraction 5 (10% MeOH) gave **11** (60 mg) and **12** (45 mg), respectively. The dried leaves of *D. rhombifolia* (1 kg) were extracted with MeOH (5 l×1 week×3) at room temperature to give extract (104 g). A part of the MeOH extract (100 g) was subjected to ODS column eluted water followed by MeOH–H_2O mixtures to divide in 17 fractions. Fraction 12 (50% MeOH) was repeatedly fractionated by Sephadex LH 20 with 10% MeOH followed by 30% MeOH. The flavonoid containing fraction was further purified by preparative TLC with $CHCl_3$–MeOH–H_2O (65 : 40 : 10) to give **7** (15 mg) and **14** (15 mg), respectively. Fraction 6 (10% MeOH) was purified by the same manner of isolation of **7** and **10** to give **13** (10 mg).

Compound **11**: A yellow solid; $[\alpha]_D$ −71° (c=0.1, MeOH); UV (MeOH) λ_{max} (log ε): 210 (3.5), 266 (3.4), 348 (3.2); Negative ion FAB-MS $[M-H]^-$ m/z 607. ^{1}H-NMR (300 MHz, CD_3OD) aglycone moiety: δ: 6.09 (1H, d, J=2.0 Hz, H-6), 6.26 (1H, d, J=2.0 Hz, H-8), 6.86 (2H, d, J=9.0 Hz, H-3′, 5′), 8.01 (2H, d, J=9.0 Hz, H-2′, 6′); sugar moieties δ: 5.77 (1H, d, J=7.2 Hz, H-1″), 3.57 (1H, m, H-4″), 3.59 (1H, m, H-3″), 3.61 (1H, d, J=10.1 Hz, H-5″), 3.66 (1H, dd, J=8.1, 7.2 Hz, H-2″); δ: 0.95 (3H, d, J=7.2 Hz, H-6‴), 3.33 (1H, dd, J=9.5, 9.5 Hz, H-4‴), 3.77 (1H, dd, J=9.5, 3.3 Hz, H-3‴), 3.99 (1H, dq, J=9.5, 7.2 Hz, H-5‴), 4.01 (1H, dd, J=3.5, 1.1 Hz, H-2‴), 5.23 (1H, d, J=1.1 Hz, H-1‴). ^{13}C-NMR (75 MHz, CD_3OD) aglycone moiety: δ: 158.11 (C-2), 134.29 (C-3), 179.26 (C-4), 162.87 (C-5), 100.55 (C-6), 168.28 (C-7), 95.25 (C-8), 158.56 (C-9), 105.22 (C-10), 123.08 (C-1′), 132.17 (C-2′, 6′), 116.12 (C-3′, 5′), 161.24 (C-4′); sugar moiety: (glucuronic acid) δ: 100.29 (C-1″), 79.93 (C-2″), 77.12 (C-3″), 73.77 (C-4″), 76.68 (C-5″), 176.27 (C-6″); (rhamnose) δ 102.58 (C-1‴), 72.32 (C-2‴), 72.23 (C-3‴), 74.03 (C-4‴), 69.94 (C-5‴), 17.50 (C-6‴).

Compound **12**: A yellow solid; $[\alpha]_D$ −56° (c=0.1, MeOH); UV (MeOH) λ_{max} (log ε): 212 (3.6), 256 (3.5), 355 (3.3); Negative ion FAB-MS $[M-H]^-$ m/z 623; ^{1}H-NMR (300 MHz, CD_3OD) aglycone moiety: δ: 6.11 (1H, d, J=2.0 Hz, H-6), 6.27 (1H, d, J=2.0 Hz, H-8), 7.68 (1H, d, J=2.0 Hz, H-2′), 6.85 (1H, d, J=8.4 Hz, H-5′), 7.47 (1H, dd, J=8.4, 2.0 Hz, H-6′); sugar moieties: δ 5.83 (1H, d, J=7.3 Hz, H-1″), 3.70 (1H, m, H-2″), 3.59 (1H, dd, J=7.4, 7.2 Hz, H-3″), 3.61 (1H, m, H-4″), 3.63 (1H, d, J=7.8 Hz, H-5″), 5.21 (1H, d, J=1.5 Hz, H-1‴), 4.01 (1H, dd, J=3.3, 1.5 Hz, H-2‴), 3.76 (1H, dd, J=9.5, 3.3 Hz, H-3‴), 3.31 (1H, t, J=9.5 Hz, H-4‴), 3.90 (1H, dq, J=9.5, 6.2 Hz, H-5‴), 0.91 (3H, d, J=6.2 Hz, H-6‴); ^{13}C-NMR (75 MHz, CD_3OD) aglycone moiety: δ: 158.30 (C-2), 134.32 (C-3), 179.20 (C-4), 162.88 (C-5), 100.24 (C-6), 167.17 (C-7), 94.95 (C-8), 158.40 (C-9), 105.47 (C-10), 123.35 (C-1′), 117.50 (C-2′), 145.80 (C-3′), 149.40 (C-4′), 116.01 (C-5′), 122.58 (C-6′); sugar moieties: (glucuronic acid) δ 100.23 (C-1″), 79.77 (C-2″), 77.80 (C-3″), 73.70 (C-4″), 78.67 (C-5″), 176.44 (C-6″); (rhamnose) δ: 102.50 (C-1‴), 72.25 (C-2‴), 72.17 (C-3‴), 73.95 (C-4‴), 68.89 (C-5‴), 17.32 (C-6‴).

Compound **13**: A pale yellow solid; $[\alpha]_D$ −59° (c=0.1, MeOH); EI-MS m/z (rel. int.) 286 (100), 153 (7), 121 (10); HR-FAB-MS $[M-H]^-$ m/z 769.1826, Calcd 769.1827 for $C_{33}H_{37}O_{21}$; UV (MeOH) λ_{max} (log ε): 205 (3.4), 266 (3.2), 350 (3.1). ^{1}H- and ^{13}C-NMR spectral data were listed in Table 1.

Compound **14**: A pale yellow solid; $[\alpha]_D$ −36° (c=0.1, MeOH); EI-MS m/z (rel. int): 286 (100), 153 (8), 121 (11); HR-ESI-MS $[M+Na]^+$ m/z 925.2554, Calcd 925.2556 for $C_{39}H_{50}O_{24}Na$; UV (MeOH) λ_{max} (log ε): 206 (3.5), 266 (3.3), 350 (3.2); ^{1}H- and ^{13}C-NMR spectral data were listed in Table 1.

The scavenging activities of DPPH radical were measured according to literatures.[8,9] The scavenging activity of **1** and **2** was already described in previous paper.[2] α-Tocopherol was used as a positive control (Sc_{50} 5.2 mM).

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