Bioorganic & Medicinal Chemistry Letters 20 (2010) 7435–7439

Contents lists available at ScienceDirect

Bioorganic & Medicinal Chemistry Letters

journal homepage: www.elsevier.com/locate/bmcl

28-*Nor*-oleanane-type triterpene saponins from *Camellia japonica* and their inhibitory activity on LPS-induced NO production in macrophage RAW264.7 cells

Nguyen Thi Phuong Thao [a,†], Tran Manh Hung [a,†], To Dao Cuong [a], Jin Cheol Kim [b], Eun Hee Kim [c], Seong Eun Jin [d], MinKyun Na [e], Young Mi Lee [f], Young Ho Kim [g], Jae Sue Choi [d], Byung Sun Min [a,*]

[a] College of Pharmacy, Catholic University of Daegu, Gyeongsan 712-702, Republic of Korea
[b] Korea Research Institute of Chemical Technology, Daejeon 305-600, Republic of Korea
[c] Division of Magnetic Resonance, Korea Basic Science Institute, Chungbuk 363-883, Republic of Korea
[d] Pukyung National University, Busan 608-737, Republic of Korea
[e] College of Pharmacy, Yeungnam University, Gyeongbuk 712-749, Republic of Korea
[f] College of Pharmacy, Wonkwang University, Jeonbuk 570-749, Republic of Korea
[g] College of Pharmacy, Chungnam National University, Daejeon 305-764, Republic of Korea

ARTICLE INFO

Article history:
Received 10 September 2010
Revised 4 October 2010
Accepted 5 October 2010
Available online 16 October 2010

Keywords:
Camellia japonica
Theaceae
28-*Nor*-oleanolic acid
NO inhibition

ABSTRACT

Four new 28-*nor*-oleanane-type triterpene oligoglycosides, camellenodiol 3-*O*-β-D-galactopyranosyl (1→2)[β-D-xylopyranosyl(1→2)-β-D-galactopyranosyl(1→3)]-β-D-glucuronopyranoside (**2**), camellenodiol 3-*O*-4″-*O*-acetyl-β-D-galactopyranosyl(1→2)[β-D-xylopyranosyl(1→2)-β-D-galactopyranosyl(1→3)] β-D-glucuronopyranoside (**4**), camellenodiol 3-*O*-(β-D-galactopyranosyl(1→2)[β-D-xylopyranosyl(1→2)-β-D-galactopyranosyl(1→3)]-6′-methoxy-β-D- glucuronopyranoside (**5**), and maragenin II 3-*O*-(β-D-galactopyranosyl(1→2)[β-D-xylopyranosyl(1→2)-β-D-galactopyranosyl(1→3)]-6′-methoxy-β-D-glucuronopyranoside (**6**), along with two known compounds, (**1** and **3**), were isolated from the stem bark of *Camellia japonica*. Their chemical structures were established mainly by 2D NMR techniques and mass spectrometry. The isolated compounds showed inhibitory effects on NO production in RAW264.7 macrophages.

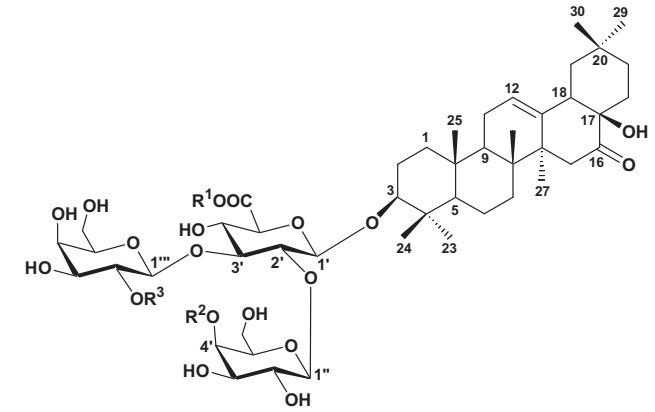
The Theaceae plant, *Camellia japonica* L. (Japanese name 'Tsubaki') has been used for treatment of hematemesis and 'Oketsu' syndrome (blood stagnation), blood vomiting and bleeding due to internal and external injury. It also has been used as an anti-inflammatory, tonic, and stomachic in Japanese and Chinese folk medicine. Previous studies have shown that the leaves are rich in flavonol glycosides and the flowers, fruits, seeds, and stem bark are rich in triterpenes[1] and saponins.[2] Regarding the bioactive constituents of *C. japonica*, flavonol glycosides having antioxidant activity,[3] triterpenes having cytotoxic activity,[1c] and saponins having antifungal, antifeedant,[4] gastroprotective and platelet aggregation activity[5] have been reported. Furthermore, some tannins from leaves, flower buds, and fruits have been shown to have an inhibitory effect on HIV-1 protease.[3,6] In the present paper, we describe isolation, structural determination of four new triterpene saponins. The NO^- inhibition study on isolated compounds was also carried out.

The stem barks of *C. japonica* were extracted with methanol under reflux. The methanolic extract was suspended in hot water and partitioned with hexane, EtOAc, and BuOH successively to afford hexane- (1.56 g), EtOAc- (41.1 g), and BuOH-soluble (113.6 g) fractions, respectively. From BuOH-soluble fraction, a combination of repeated silica gel column chromatography and high performance liquid chromatography allowed the purification of six triterpene saponins (**1**–**6**).[7] The two known compounds, camellioside A (**1**) and camellioside B (**3**) (Fig. 1), were identified by detailed NMR spectroscopic analysis and comparison with the literature data.[5]

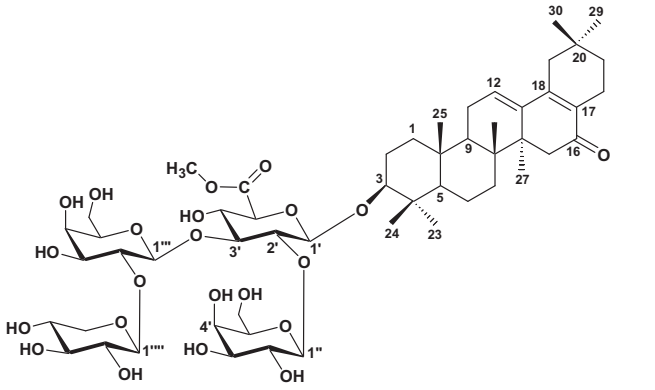
Compound **2** was isolated as a white amorphous powder of mp 225–228 °C with negative optical rotation ($[\alpha]_D^{22}$ −41.8). The IR spectrum of **2** showed absorption bands at 3454, 1078 cm^{-1} suggestive of an oligoglycosidic structure and weak bands at 1736, 1718, and 1655 cm^{-1} ascribable to carbonyl, carboxyl, and olefin functions. The molecular formula was established as $C_{52}H_{82}O_{23}$ on the basis of HRFABMS at *m/z* 1097.5149 for the $[M+Na]^+$ (calcd for $C_{52}H_{82}O_{23}Na$, 1097.5149 $[M+Na]^+$).[8a] The ^1H NMR spectrum of **2** showed signals for seven tertiary methyl groups, an axial oxymethine proton at δ 3.19 (1H, dd, *J* = 4.0, 11.6 Hz, H-3), and an olefinic proton at δ 5.40 (1H, t, *J* = 3.4 Hz, H-12). The ^{13}C NMR spectrum and DEPT spectra showed signals corresponding to 52 carbons, including, a carbonyl carbon at δ 217.2 (C-16), two olefinic carbons at δ 142.9 (C-13) and 125.7 (C-12), a tertiary oxygenated carbon at δ 77.6 (C-17), and seven tertiary methyl carbons at δ 33.1 (C-29),

* Corresponding author. Tel.: +82 53 850 3613; fax: +82 53 850 3602.
E-mail address: bsmin@cu.ac.kr (B.S. Min).
† These two authors contributed equally to this work.

0960-894X/$ - see front matter © 2010 Elsevier Ltd. All rights reserved.
doi:10.1016/j.bmcl.2010.10.013



	R^1	R^2	R^3
1	H	H	β-D-Glu
2	H	H	β-D-Xyl
3	H	Ac	β-D-Glu
4	H	Ac	β-D-Xyl
5	CH_3	H	β-D-Xyl



6

Figure 1. Structures of isolated compounds (**1**–**6**) from *C. japonica*.

28.5 (C-23), 27.4 (C-27), 24.1 (C-30), 18.1 (C-26), 17.1 (C-24), and 16.0 (C-25). The ^{1}H NMR spectrum of **2** also exhibited four anomeric protons resonated as doublets at δ 4.50 (1H, d, J = 8.0 Hz, H-1′′′′), 4.52 (1H, d, J = 7.6 Hz, H-1′), 4.97 (1H, d, J = 7.6 Hz, H-1′′′), and 5.12 (1H, d, J = 7.6 Hz, H-1′′), which corresponded to the carbon signals at δ 107.9 (C-1′′′′), 106.3 (C-1′), 102.0 (C-1′′′), and 103.1 (C-1′′) in the ^{13}C NMR spectrum, respectively. The other carbon signals were observed and assigned to twelve methylenes, eighteen methines, and five quaternary carbons. These above proton and carbon data suggested that **2** were assignable to a 28-*nor*-oleanane-type triterpene skeleton with four sugar moieties.[5] Acid hydrolysis of **2** revealed two units of galactose, one unit of glucuronic acid and one unit of xylose[9] (identified by co-TLC with the authentic samples and detailed studies of COSY, HSQC, HMBC and TOCSY spectra) along with camellenodiol, as its aglycon, which was identified by comparison of physical data ($[\alpha]_D^{26}$, IR, ^{1}H NMR, ^{13}C NMR) with reported values.[10] The β-anomeric configurations of the four sugar units were deduced from their $^3J_{H1,H2}$ coupling constants (7.6–8.0 Hz). The full NMR assignments and connectivities were determined by the ^{1}H–^{1}H COSY, TOCSY, HSQC, and HMBC spectroscopic data analyses (Tables 1 and 2). In the HMBC experiment, the long-range correlations between the protons δ 4.52 (1H, d, J = 7.6 Hz, H-1′ of GlcA), 1.10 (3H, s, H-23) and 0.90 (3H, s, H-24)

Table 1
^{1}H NMR spectroscopic data of compounds **2**, **4**–**6**

	2[a]	**4**[b]	**5**[c]	**6**[c]
1	1.86, 1.43 m	1.97, 1.60 m	1.64, 1.00 m	1.70, 1.06 m
2	1.98, 1.52 m	2.13, 1.84 m	1.81, 1.73 m	1.84, 1.75 m
3	3.19 dd (4.0, 11.6)	3.29 dd (4.0, 11.2)	3.17 dd (4.6, 11.4)	3.19 dd (4.5, 11.7)
5	0.80 d (11.2)	0.81 m	0.79 d-like (11.7)	0.83 m
6	1.46, 1.30 m	1.60, 1.40 m	1.59 br d (11.7) 1.44 d (13.5)	1.62 br d (14.4) 1.45 dd (1.8, 12.6)
7	1.57, 1.28 m	1.94, 1.24 m	1.49, 1.28 m	1.56 dd (2.7, 12.6) 1.48 m
9	1.55 m	1.58 m	1.56 m	1.59 dd (5.9, 12.2)
11	2.00, 1.95 m	1.94, 1.58 m	2.03, 1.95 m	2.21, 2.19 m
12	5.40 t (3.4)	5.45 t (3.2)	5.40 t (0.9)	6.27 t (4.5)
15	3.30, 1.64 d (13.2)	3.73, 1.96 d (13.2)	3.30, 1.64 d (13.2)	2.64, 2.11 d (14.4)
18	2.79 dd (4.0, 14.4)	3.11 dd (2.8, 14.0)	2.79 dd (3.6, 14.4)	-
19	1.99, 1.45 m	1.69, 1.32 m	1.41, 1.23 m	2.29, 2.05 d (18.0)
21	1.55, 1.04 m	1.60, 1.38 m	1.56, 1.26 m	1.42, 1.38 m
22	2.02, 1.74 m	2.53, 1.81 m	2.03, 1.41 m	2.42, 2.20 m
23	1.10 s	1.43 s	1.09 s	1.10 s
24	0.90 s	1.20 s	0.89 s	0.90 s
25	1.00 s	0.88 s	0.99 s	1.02 s
26	1.11 s	1.29 s	1.11 s	0.98 s
27	1.15 s	1.37 s	1.15 s	1.11 s
29	0.86 s	0.91 s	0.86 s	0.95 s
30	0.97 s	0.98 s	0.97 s	0.93 s
GlcA				
-1′	4.52 d (7.6)	4.87 d (8.0)	4.53 d (7.2)	4.53 d (7.2)
-2′	3.91 m	4.64 d (8.0)	3.90 d (9.9)	3.89 m
-3′	3.89 d (7.6)	4.38 m	3.88 d (9.9)	3.88 d (7.6)
-4′	3.62 m	4.47 m	3.62 m	3.61 d (4.8)
-5′	3.83 m	4.49 m	3.90 d (9.9)	3.90 m
6′-OCH_3	3.78 s	—	—	3.78 s
Gal				
-1′′	5.12 d (7.6)	6.11 d (8.0)	5.11 d (7.2)	5.11 d (7.6)
-2′′	3.55 m	4.40 m	3.54 m	3.54 m
-3′′	3.65 m	4.68 dd (3.6, 8.8)	3.64 m	3.65 m
-4′′	3.81 m	6.12 d (3.6)	3.80 m	3.80 m
-5′′	3.77 m	4.50 m	3.78 m	3.78 m
-6′′	3.75, 3.54 m	4.36, 4.30 m	3.76 m, 3.68 d (9.9)	3.76 m, 3.68 m
4′′-Ac Gal		2.20 s		
-1′′′	4.97 d (7.6)	5.67 d (7.6)	4.96 d (8.1)	4.96 d (7.2)
-2′′′	3.70 d (8.0)	4.51 m	3.70 d (7.2)	3.70 d (6.0)
-3′′′	3.75 m	3.98 dd (3.6, 9.2)	3.74 m	3.73 d (3.2)
-4′′′	3.85 br s	4.53 m	3.85 d (2.7)	3.84 d (3.2)
-5′′′	3.58 m	4.29 m	3.56 m	3.54 m
-6′′′	3.77, 3.66 m	4.40, 4.27 m	3.75, 3.63 m	3.75 d (3.2), 3.63 m
Xyl				
-1′′′′	4.50 d (8.0)	4.91 d (7.6)	4.50 d (7.2)	4.49 d (7.2)
-2′′′′	3.68 m	4.90 d (5.6)	3.67 dd (2.7, 14.4)	3.68 m
-3′′′′	3.55 m	4.12 m	3.54 m	3.54 m
-4′′′′	3.82 m	4.11 m	3.81 m	3.80 d-like (3.2)
-5′′′′	3.99 dd (2.4, 12.8) 3.57 m	4.21 br d (10.8) 3.46 br d (11.6)	3.99 dd (0.9, 10.8) 3.57 m	3.99 dd (2.0, 12.8) 3.56 m

[a] CD_3OD and [b] pyridine-d_5 at 100 MHz, and [c] CD_3OD at 225MHz. GlcA: β-D-glucopyranosiduronic acid; Gal: β-D-galactopyranosyl; Xyl: β-D-xylopyranosyl.

Table 2
^{13}C NMR spectroscopic data for compounds **2**, **4**–**6**

Position	**2**[a]	**4**[b]	**5**[c]	**6**[c]
1	39.8	38.9	39.8	40.0
2	27.2	27.1	27.2	27.2
3	92.3	90.4	92.3	92.1
4	40.6	40.2	40.6	40.6
5	57.1	56.2	57.1	57.1
6	19.4	18.9	19.4	19.3
7	34.3	33.8	34.3	34.8
8	41.2	40.7	41.2	40.1
9	48.2	47.4	48.1	47.5
10	38.0	37.4	38.0	37.9
11	24.8	24.4	24.8	25.4
12	125.7	124.3	125.5	129.9
13	142.9	143.2	142.9	140.3
14	49.5	48.8	49.5	46.2
15	44.0	43.9	44.0	45.2
16	217.2	215.8	217.2	203.0
17	77.6	76.9	77.6	129.6
18	53.6	53.3	53.6	149.7
19	49.1	48.6	49.1	41.5
20	31.8	31.5	31.8	30.2
21	37.9	37.8	37.9	35.5
22	31.8	32.2	31.8	21.7
23	28.5	27.7	28.5	28.4
24	17.1	17.1	17.1	17.1
25	16.0	15.9	15.9	16.2
26	18.1	18.3	18.1	18.5
27	27.4	28.5	27.4	23.5
29	33.1	33.2	33.2	28.8
30	24.1	24.2	24.1	28.6
GlcA				
-1′	106.3	106.2	106.3	106.3
-2′	78.7	78.1	78.7	78.8
-3′	83.6	86.4	83.5	83.5
-4′	71.8	71.7	71.8	71.8
-5′	76.5	75.8	76.4	76.4
-6′	172.3	172.5	171.1	171.1
6′-OCH$_3$			53.0	53.0
Gal				
-1″	103.1	103.0	103.1	103.1
-2″	73.9	73.8	73.9	73.9
-3″	75.0	74.7	75.0	75.0
-4″	70.4	72.8	70.7	70.7
-5″	76.4	77.8	76.5	76.5
-6″	62.3	62.3	62.8	63.1
4″-Ac		21.7		
		171.5		
Gal				
-1‴	102.0	102.3	102.0	102.0
-2‴	84.2	85.3	84.2	84.1
-3‴	76.4	74.5	75.3	75.3
-4‴	70.1	70.1	70.4	70.4
-5‴	77.2	77.6	77.2	77.1
-6‴	63.1	62.1	63.1	62.7
Xyl				
-1⁗	107.9	108.7	107.9	107.9
-2⁗	74.7	74.8	73.8	73.8
-3⁗	75.3	75.2	74.7	74.7
-4⁗	70.6	70.0	70.2	70.2
-5⁗	68.0	67.9	68.0	68.0

[a]CD_3OD and [b]pyridine-d_5 at 100 MHz, and [c]CD_3OD at 225 MHz. GlcA: β-D-glucopyranosiduronic acid; Gal: β-D-galactopyranosyl; Xyl: β-D-xylopyranosyl.

and carbon δ 92.3 (C-3) indicated the attachment of glucuronic acid at C-3 (Fig. 2). Furthermore, the long-range correlation between the proton δ 5.12 (1H, d, *J* = 7.6 Hz, H-1″ of terminal Gal) and carbon δ 78.7 (C-2′ of GlcA), δ 4.97 (1H, d, *J* = 7.6 Hz, H-1‴ of inner Gal) and δ 83.6 (C-3′ of GlcA), δ 4.50 (1H, d, *J* = 8.0 Hz, H-1⁗ of terminal Xyl) and δ 84.2 (C-2‴ of inner Gal) in the HMBC spectra, as well as the downfield shifts of the carbons C-2′ (δ 78.7) and C-3′ (δ 83.6) of GlcA and C-2‴ (δ 84.2) of inner Gal, confirmed the interglycosidic linkages of the four sugar units in **2** as terminal Gal-(1→2)[terminal Xyl-(1→2)-inner Gal-(1→3)]-GlcA-(1→3)-aglycon. On the basic of these above evidence, the structure of **2** was elucidated to be camellenodiol 3-*O*-β-D-galactopyranosyl(1→2)[β-D-xylopyranosyl(1→2)-β-D-galactopyranosyl(1→3)]-β-D-glucuronopyranoside, named camellioside E.

Compound **4** was obtained as a white amorphous powder of mp 222–225 °C with negative optical rotation ($[\alpha]_D^{22}$ −32.6). The molecular formula was determined as $C_{54}H_{84}O_{24}$ on the basis of HRFABMS (*m/z* 1139.5254 $[M+Na]^+$, calcd for $C_{54}H_{84}O_{24}Na$, 1139.5254). The IR absorption bands at 1738, 1719, and 1650 cm^{-1} ascribable to carbonyl, carboxyl, and olefin functions and broad bands at 3600 and 1075 cm^{-1} suggestive of an oligoglycoside structure. The ^{1}H NMR of **4** showed signals assignable to four sugar moieties [δ 4.87 (1H, d, *J* = 8.0 Hz, H-1′), 4.91 (1H, d, *J* = 7.6 Hz, H-1⁗), 5.67 (1H, d, *J* = 7.6 Hz, H-1‴) and 6.11 (1H, d, *J* = 8.0 Hz, H-1″)] together with an acetyl group [δ 2.20 (3H, s, –OCO*CH*$_3$)] and an aglycon moiety [δ 0.88, 0.91, 0.98, 1.20, 1.29, 1.37, 1.43 (3H each, all s, H$_3$-25, 29, 30, 24, 26, 27, 23), 3.11 (1H, dd, *J* = 2.8, 14.0 Hz, H-18), 3.29 (1H, dd, *J* = 4.0, 11.2 Hz, H-3), 5.45 (1H, t, *J* = 3.2 Hz, H-12)].[8b] The ^{13}C NMR spectrum and DEPT spectra showed signals corresponding to 54 carbons, of which 29 carbon signals were observed to a *nor*-triterpene moiety and 25 carbon signals were assigned to the oligosaccharide moieties. The proton and carbon signals in the ^{1}H and ^{13}C NMR spectra of **4** were superimposable on those of **2**, except for the appearance of an additional acetyl group. The site of linkage of the acetyl group was determined by the HMBC experiment, which showed a long-range correlation between proton at δ 6.12 (1H, br s, H-4″) and acetyl carbonyl carbon at δ 171.5 (Fig. 2). The downfield shifts observed in the HSQC spectrum for the terminal Gal H-4″/Gal C-4″ resonances at δ_H 6.12/δ_H 72.8 further confirmed that the terminal Gal residue was substituted by an acetyl group at the position C-4″.[10] On the basis of the above results, the structure of **4** was determined as camellenodiol 3-*O*-(4″-*O*-acetyl-β-D-galactopyranosyl(1→2)[β-D-xylopyranosyl(1→2)-β-D-galactopyranosyl(1→3)]-β-D-glucuronopyranoside (**4**), named camellioside F.

Compound **5** was obtained as a white powder of mp 227–229 °C with negative optical rotation ($[\alpha]_D^{22}$ −68.9). The molecular formula was found to be as $C_{53}H_{84}O_{23}$ on the basis of HRFABMS (*m/z* 1111.5305 $[M+Na]^+$, calcd for $C_{53}H_{84}O_{23}Na$, 1111.5305).[8c] Acid hydrolysis of **5** revealed two units of galatose, one unit of glucuronic acid and one unit of xylose (identified by co-TLC with the authentic samples and detailed studies of COSY, HSQC, HMBC and TOCSY spectra) along with the aglycon camellenodiol, which was identified by comparison of physical data ($[\alpha]_D^{26}$, IR, ^{1}H NMR, ^{13}C NMR) with reported values.[10] The ^{1}H NMR and ^{13}C NMR of **5** showed signal patterns similar to those of **2**, except for the signals due to an methoxy group at δ 3.78 (3H, s) in the ^{1}H NMR and at δ 53.0 in the ^{13}C NMR of **5**. In the HMBC spectra, the long-range correlation between the methoxy proton at δ 3.78 (3H, s) and carboxyl carbon at δ 171.2 confirmed the methoxy group was located at C-6′ of the glucuronopyranosyl moiety (Fig. 2). Based on the above evidence analyses, the structure of **5** was identified as camellenodiol 3-*O*-(β-D-galactopyranosyl(1→2)[β-D-xylopyranosyl(1→2)-β-D-galactopyranosyl(1→3)]-6′-methoxy-β-D-glucuronopyranoside, named camellioside G.

Compound **6** was obtained as a white powder of mp 223–225 °C with negative optical rotation ($[\alpha]_D^{26}$ −12.6). The molecular formula was established as $C_{53}H_{82}O_{22}$ on the basis of HRFABMS (*m/z* 1093.5192 $[M+Na]^+$, calcd for $C_{53}H_{82}O_{22}Na$, 1093.5200). In the UV spectra of **6**, the absorption maximum was observed at 299 (log ϵ 4.0) nm, suggestive of an α,β-unsaturated ketone group with extended conjugation.[1] The ^{1}H NMR of **6** showed signals for seven tertiary methyl groups, an axial oxymethine protons at δ 3.19 (1H, dd, *J* = 4.6, 11.4 Hz, H-3), four anomeric protons resonated as doublets at δ 4.49 (1H, d, *J* = 7.2 Hz, H-1⁗), 4.53 (1H, d, *J* = 7.2 Hz, H-1′), 4.96 (1H, d, *J* = 6.8 Hz, H-1‴), and 5.11 (1H, d, *J* = 7.6 Hz, H-1″),

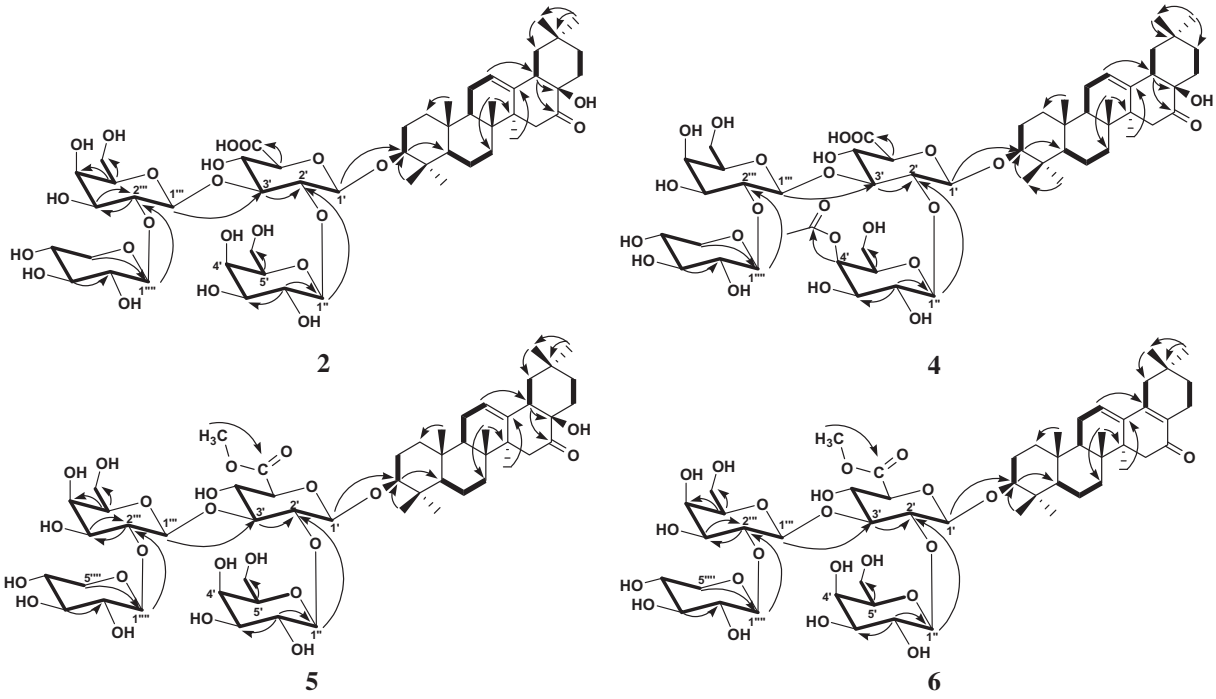


Figure 2. Key HMBC (→) and COSY (—) correlations of compounds **2**, **4**–**6**.

Table 3
Inhibition of NO production by compounds **1**–**6**

Compound	IC_{50} value (μM)[a]
1	9.59
2	18.25
3	4.96
4	11.50
5	5.16
6	15.91
AMT[b]	0.022

[a] The inhibitory effects are represented as the molar concentration (μM) giving 50% inhibition (IC_{50}) relative to the vehicle control.

[b] AMT (2-amino-5,6-dihydro-6-methyl-4*H*-1,3-thiazine hydrochloride) was used as the positive control for NO production.

and an olefinic proton at δ 6.27 (1H, t, J = 3.0 Hz, H-12).[8d] The ^{13}C NMR showed signals assignable to a carbonyl carbon at δ 203.0 (C-16), four olefinic carbons at δ 149.8 (C-18), 140.3 (C-13), and 129.6 (C-12, 17), four anomeric carbons at δ 107.9 (C-1′′′′), 106.3 (C-1′), 103.0 (C-1′′) and 102.0 (C-1′′′), and seven tertiary methyl carbons. The other carbon signals were observed and assigned to twelve methylenes, seventeen methines, and five quaternary carbons. These above proton and carbon data suggested that **6** were assignable to a *nor*-oleanane-type triterpene skeleton with four sugar units.[1,5] Analysis of the ^1H and ^{13}C NMR spectrum revealed that the oligoglycosidic structure of **6** was identical to that of **5**, but differed slightly from **5** in terms of the aglycon structure. Acid hydrolysis of **6** revealed two units of galatose, one unit of glucuronic acid and one unit of xylose (identified by co-TLC with the authentic samples and detailed studies of COSY, HSQC, HMBC and TOCSY spectra), along with maragenin II, as its aglycon, which was identified by comparison of physical data ($[\alpha]_D^{26}$, IR, ^1H NMR, ^{13}C NMR) with reported values.[10] These above evidence led us to elucidate the structure of **6** to be as maragenin II 3-*O*-(β-D-galactopyranosyl(1→2)-[β-D-xylopyranosyl(1→2)-β-D-galactopyranosyl(1→3)]-6′-methoxy-β-D-glucuronopyranoside, named camellioside H.

The cytotoxic effects of isolated compounds (**1**–**6**) were evaluated in the presence or absence of LPS using the MTT assay, and these compounds did not affect the cell viability of RAW264.7 cells in either the presence or absence of LPS even at a dose of 50 μM after a period of 24 h (data not shown). The amount of produced NO was determined by the amount of nitrite, a stable metabolite of NO. To assess the effects of these compounds on the LPS-induced NO production in RAW264.7 cells, cell culture medium was harvested and the production of nitrite was measured using the Griess reaction.[11] As shown in Table 3, all isolated compounds showed inhibitory effect with IC_{50} values ranging from 18.25 to 4.96 μM. These results are possible to demonstrate that isolated triterpenoid oligoglycosides might be important anti-inflammatory constituent of this plant.

Acknowledgements

This study was supported by the Ministry of Knowledge Economy in 2008 (Chungnam TP, Grant) and the National Research Foundation of Korea Grant funded by the Korean Government (MEST) (NRF-C1ABA001-2010-0020484).

References and notes

1. (a) Akihisa, T.; Yasukawa, K.; Kimura, Y.; Takase, S.; Yamanouchi, S.; Tamura, T. *Chem. Pharm. Bull.* **1997**, *45*, 2016; (b) Itokawa, H.; Nakajima, H.; Ikuta, A.; Iitaka, Y. *Phytochemistry* **1981**, *20*, 2539; (c) Thao, N. T. P.; Hung, T. M.; Lee, M. K.; Kim, J. C.; Min, B. S.; Bae, K. *Chem. Pharm. Bull.* **2010**, *58*, 121.
2. (a) Ito, S.; Kodama, M.; Konoike, M. *Tetrahedron Lett.* **1967**, *8*, 591; (b) Itokawa, H.; Sawada, N.; Murakami, T. *Tetrahedron Lett.* **1967**, *8*, 597; (c) Itokawa, H.; Sawada, N.; Murakami, T. *Chem. Pharm. Bull.* **1969**, *17*, 474; (d) Yoshikawa, M.; Harada, E.; Murakami, T.; Matsuda, H.; Yamahara, J.; Murakami, N. *Chem. Pharm. Bull.* **1994**, *42*, 742; (e) Yoshikawa, M.; Murakami, T.; Yoshizumi, S.; Murakami, N.; Yamahara, J.; Matsuda, H. *Chem. Pharm. Bull.* **1996**, *44*, 1899.
3. Park, J. C.; Hur, J. M.; Park, J. G.; Hatano, T.; Yoshida, T.; Miyashiro, H.; Min, B. S.; Hattori, M. *Phytother. Res.* **2002**, *16*, 422.
4. (a) Nagata, T.; Tsushida, T.; Hamaya, E.; Enoki, N.; Manabe, S.; Nishino, C. *Agric. Biol. Chem.* **1985**, *49*, 1181; (b) Nishino, C.; Manabe, S.; Enoki, N.; Nagata, T.; Tsushida, T.; Hamaya, E. *J. Chem. Soc., Chem. Commun.* **1986**, 720; (c) Numata, A.; Kitajima, A.; Katsuno, T.; Yamamoto, K.; Nagahama, N.; Takahashi, C.; Fujiki, R.; Nabae, M. *Chem. Pharm. Bull.* **1987**, *35*, 3948.

5. Yoshikawa, M.; Morikawa, T.; Asao, Y.; Fujiwara, E.; Nakamura, S.; Matsuda, H. *Chem. Pharm. Bull.* **2007**, *55*, 606.
6. (a) Hatano, T.; Shida, S.; Han, L.; Okuda, T. *Chem. Pharm. Bull.* **1991**, *39*, 876; (b) Hatano, T.; Han, L.; Taniguchi, S.; Okuda, T.; Kiso, Y.; Tanaka, T.; Yoshida, T. *Chem. Pharm. Bull.* **1995**, *43*, 2033; (c) Han, L.; Hatano, T.; Yoshida, T.; Okuda, T. *Chem. Pharm. Bull.* **1994**, *42*, 1399; (d) Hatano, T.; Han, L.; Taniguchi, S.; Shingu, T.; Okuda, T.; Yoshida, T. *Chem. Pharm. Bull.* **1995**, *43*, 1629.
7. The stem barks of *C. japonica* were collected in March, 2008 at Korea Research Institute of Chemical Technology, Daejeon, Korea, and identified by Professor Byung Sun Min. A voucher specimen (CUD-2170) is deposited in the hebarium of College of Pharmacy, Catholic University of Daegu, Korea. The air-dried stem barks of *C. japonica* (5.2 kg) were extracted three times with MeOH (15 L) at room temperature. After evaporation of the solvent under reduced pressure, the crude MeOH extract (368.0 g) was obtained. This extract was suspended in hot water and partitioned with hexane, EtOAc, and BuOH, successively. The resulting fractions were concentrated in vacuo to give the hexane (1.56 g), EtOAc (41.1 g), and BuOH (113.6 g) soluble fractions, respectively. The BuOH soluble fraction (113.6 g) was chromatographed on a silica gel (Kieselgel 60, 230–400 mesh) column eluting with a gradient of $CHCl_3$/MeOH/H_2O (7:3:0.5 to 0:1:0) to give six fractions (B1–B6). Re-chromatography of fraction B2 (12.5 g) on a silica gel (Kieselgel 230–400 mesh) column eluting with a gradient of $CHCl_3$/MeOH/H_2O (6:4:0.5 to 0:1:0) yielded five subfractions (B2.1–B2.5). Subfraction B2.1 (2.26 g) was subjected to a silica gel (Kieselgel 60, 70–230 mesh) column using a gradient of $CHCl_3$:acetone (3:1) and purified by HPLC [mobile phase: ACN/H_2O (25:75 to 60:40); UV: 210 nm; flow rate: 5 mL/min; column COSMOSIL ϕ 20 × 250 mm, 5 μm] to obtain **1** (134.6 mg; t_R: 45.8 min) and **2** (56.8 mg; t_R: 47.0 min). Subfraction B2.2 (6.26 g) was subjected to a silica gel column eluting with a gradient of $CHCl_3$/acetone (3:1) to yield five subfractions (B2.2.1–B2.2.5). Further purification of subfraction B2.2.3 (1.68 g) using HPLC [mobile phase: ACN/H_2O (35:65 to 90:10); UV: 210 nm; flow rate: 5 mL/min; column COSMOSIL ϕ 20 × 250 mm, 5 μm] resulted in the isolation of **5** (18.2 mg; t_R: 26.5 min) and **6** (16.7 mg; t_R: 34.8 min). Subfraction B2.2.5 (2.21 g) was further separated by HPLC [mobile phase: ACN/H_2O (25:75 to 60:40); UV: 210 nm; flow rate: 5 mL/min; column COSMOSIL ϕ 20 × 250 mm, 5 μm] to result in the isolation of **3** (106.6 mg; t_R: 46.9 min) and **4** (28.8 mg; t_R: 48.3 min).
8. *Physical and spectroscopic data of new compounds*: (a) *camellioside E* (**2**): white amorphous powder; mp 225–228 °C; $[\alpha]_D^{22}$ −41.8 (*c* 0.1, MeOH); IR ν_{max} cm^{-1}: 3454, 1736, 1718, 1078, 1655; UV (MeOH): 298 nm; HRFABMS *m*/*z* 1097.5149 [M+Na]$^+$ (calcd for $C_{52}H_{82}O_{23}Na$, 1097.5149); ^{1}H NMR data see Table 1 and ^{13}C NMR data see Table 2; (b) *camellioside F* (**4**): white amorphous powder; mp 222–225 °C; $[\alpha]_D^{22}$ −32.6 (*c* 0.1, MeOH); IR ν_{max} cm^{-1}: 3600, 1738, 1719, 1075, 1650; UV (MeOH): 251 nm; HRFABMS *m*/*z* 1139.5254 [M+Na]$^+$ (calcd for $C_{54}H_{84}O_{24}Na$, 1139.5254); ^{1}H NMR data see Table 1 and ^{13}C NMR data see Table 2; (c) *camellioside G* (**5**): white powder; mp 227–229 °C; $[\alpha]_D^{22}$ −68.9 (*c* 0.1, MeOH); IR ν_{max} cm^{-1}: 3540, 1736, 1719, 1660, 1080, 1050; UV (MeOH): 248 nm; HRFABMS *m*/*z* 1111.5305 [M+Na]$^+$ (calcd for $C_{53}H_{84}O_{23}Na$, 1111.5305); ^{1}H NMR data see Table 1 and ^{13}C NMR data see Table 2; (d) *camellioside H* (**6**): white powder; mp 223–225 °C; $[\alpha]_D^{26}$ −12.6 (*c* 0.1, MeOH); IR ν_{max} cm^{-1}: 3450, 1736, 1665, 1655, 1080, 1068; UV (MeOH): 298 nm; HRFABMS *m*/*z* 1093.5192 [M+Na]$^+$ (calcd for $C_{53}H_{82}O_{22}Na$, 1093.5200); ^{1}H NMR data see Table 1 and ^{13}C NMR data see Table 2.
9. *Determination of sugar components*: The individual pure saponins **2**, **4**, **5** and **6** (5 mg, each) were hydrolyzed with 10 ml of 2 N HCl in 50% aqueous methanol under reflux for 2 h at 100 °C, and thereafter sugars and glycones were separated, the aqueous solution was extracted with EtOAc (3 × 10 ml). The aglycones in the EtOAc layer were evaporated to dryness, 2.0 mg of each pure aglycone was obtained, dissolved in CD_3OD in a 3-mm tube, and analyzed by ^{1}H NMR and ^{13}C NMR. The resulting water layer was desalted with Amberlite MB-3 and dried to give a residue. The residue was dissolved in pyridine (1 mL), to which a solution of L-cysteine methyl ester hydrochloride (0.1 M) in pyridine (2 mL) was added. The mixture was kept at 60 °C for 1.5 h. After the solvent was evaporated under reduced pressure, the residue was trimethylsilylated with HMDS-TMCS (0.1 mL) at 60 °C for 1 h. The mixture was partitioned between hexane and H_2O (0.3 mL each) and the hexane layer was analyzed by GC. The sugar derivatives thus obtained showed retention time of 19.71, 15.56, 21.30, and 22.65 min, respectively, identical to those of authentic D-glactose, D-xylose, D-glucose, and D-glucuronic acid, respectively. The aqueous layer was also analyzed by silica gel TLC in comparison with standard sugars (solvent system: $CHCl_3$/MeOH/H_2O (8:5:1 v/v/v).
10. (a) Kitagawa, I.; Yoshikawa, M.; Yosioka, I. *Tetrahedron Lett.* **1974**, *15*, 469; (b) Hylands, P. I.; Salama, A. M. *Tetrahedron* **1979**, *35*, 417; (c) Yoshida, T.; Chou, T.; Haba, K.; Okano, Y.; Shingu, T.; Miyamoto, K.; Koshiura, R.; Okuda, T. *Chem. Pharm. Bull.* **1989**, *37*, 3174.
11. *Determination of NO production and the cell viability assay*: The level of NO production was determined by measuring the amount of nitrite from the cell culture supernatants as described previously. Briefly, the RAW264.7 cells (ATCC, Rockville, MD, USA, 1 × 10^5 cells/well) were stimulated with or without 1 μg/mL of LPS (Sigma Chemical Co., St. Louis, MO) for 24 h in the presence or absence of the test compounds (0.5–25 μM). The cell culture supernatant (100 μL) was then reacted with 100 μL of Griess reagent. The remaining cells after the Griess assay were used to test their viability using a MTT (Sigma Chemical Co., St. Louis, MO)-based colorimetric assay as previously described. AMT (2-amino-5,6-dihydro-6-methyl-4*H*-1,3-thiazine hydrochloride) was used as the positive control.