Saponins and Other Constituents from the Leaves of *Aralia elata*

Ju Sun KIM,[a] Sang Hee SHIM,[a] Sungwook CHAE,[a] Sang Jun HAN,[a] Sam Sik KANG,*[,a] Kun Ho SON,[b] Hyeun Wook CHANG,[c] Hyun Pyo KIM,[d] and KiHwan BAE[e]

[a] *Natural Products Research Institute and College of Pharmacy, Seoul National University; Seoul 110–460, Korea:* [b] *Department of Food and Nutrition, Andong National University; Andong 760–749, Korea:* [c] *College of Pharmacy, Yeungnam University; Gyongsan 712–749, Korea:* [d] *College of Pharmacy, Kangwon National University; Chuncheon 200–749, Korea: and* [e] *College of Pharmacy, Chungnam National University; Taejon 305–764, Korea.*
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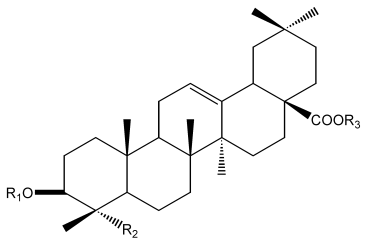
A new triterpenoid saponin, together with five known saponins, were isolated from the nonpolar *n*-hexane fraction of the leaves of *Aralia elata*. The structure of the new saponin, durupcoside C, was elucidated as hederagenin 3-*O*-β-D-glucopyranosyl(1→3)-β-D-glucopyranosyl(1→3)-α-L-arabinopyranoside on the basis of spectroscopic analysis. The known saponins were characterized as 3-*O*-α-L-rhamnopyranosyl(1→2)-α-L-arabinopyranosyl hederagenin 28-*O*-β-D-xylopyranosyl(1→6)-β-D-glucopyranosyl ester, hederagenin 3-*O*-β-D-glucopyranosyl(1→3)-α-L-rhamnopyranosyl(1→2)-α-L-arabinopyranoside, oleanolic acid 3-*O*-β-D-glucopyranosyl(1→3)-α-L-rhamnopyranosyl(1→2)-α-L-arabinopyranoside, hederagenin 3-*O*-α-L-rhamnopyranosyl(1→2)-α-L-arabinopyranoside (α-hederin), and hederagenin 3-*O*-β-D-glucopyranosyl(1→3)-α-L-arabinopyranoside (collinsonidin). In addition, two known lipids, Arisaema glyceride 3 and ceramide mixtures were also isolated and characterized. Collinsonidin and two known lipids were isolated for the first time from this plant.

Key words *Aralia elata*; Araliaceae; triterpenoid saponin; durupcoside C; lipid

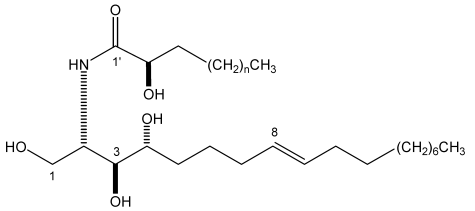
Aralia elata SEEM. (Araliaceae) is widely distributed in the northeast of China and Korea. Its root bark has been used as a folk medicine for tonic, anti-arthritic, and anti-diabetic effect.[1)] The young shoot of *A. elata* is a popular edible plant, especially in the spring in Korea and Japan. Many saponins have been isolated from this plant,[2—16)] and were reported to show anti-diabetic activity,[17)] cytoprotective effect on CCl_4-induced hepatic injury,[7)] ethanol absorption inhibitory effect,[6,14)] and hypoglycemic activity.[10—12)] Recently, we reported the isolation and identification of various triterpenoid saponins including two new saponins, durupcosides A and B,[2,3)] and a cerebroside, aralia cerebroside[18)] from the root bark of this plant. As part of our continuing search for anti-inflammatory agents from plants,[19)] a nonpolar *n*-hexane extract of the leaves of *A. elata* was subjected to a chemical investigation leading to the isolation of eight compounds, among which one was new. This paper deals with the isolation and characterization of the new triterpenoid saponin named durupcoside C (**5**), together with five known saponins (**1**—**4**, **6**) and two known lipids (**7**, **8**) from the *n*-hexane fraction of the leaves of *A. elata*.

The dried leaves of *A. elata* was extracted with MeOH and partitioned as described in the Experimental section. Six saponins (**1**—**6**) and two lipids (**7**, **8**) were isolated by successive chromatography using silica and reverse-phase C_{18} from the *n*-hexane fraction. Five known saponins and two lipids were identified as α-hederin (**1**),[20)] collinsonidin (**2**),[21)] oleanolic acid 3-*O*-β-D-glucopyranosyl(1→3)-α-L-rhamnopyranosyl(1→2)-α-L-arabinopyranoside (**3**)[22)] and hederagenin 3-*O*-β-D-glucopyranosyl(1→3)-α-L-rhamnopyranosyl(1→2)-α-L-arabinopyranoside (**4**),[4)] 3-*O*-α-L-rhamnopyranosyl(1→2)-α-L-arabinopyranosyl hederagenin 28-*O*-β-D-xylopyranosyl(1→6)-β-D-glucopyranosyl ester (**6**),[4)] urtica ceramides (**7**)[23)] and arisaema glyceride 3 (**8**),[24)] respectively, by direct comparison of the spectral data with the published values.

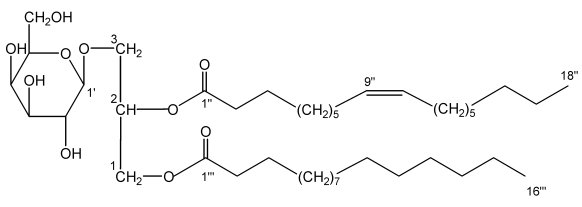
Compound **5** was obtained as a white amorphous powder. Negative high-resolution FAB-MS gave the composition $C_{47}H_{76}O_{18}$. An inspection of the ^{1}H- and ^{13}C-NMR spectra of the compound readily indicated the presence of three mono-



	R_1	R_2	R_3
1	−Ara^2 — Rha	CH_2OH	H
2	−Ara^3 — Glc	CH_2OH	H
3	−Ara^2 — Rha^3 — Glc	CH_3	H
4	−Ara^2 — Rha^3 — Glc	CH_2OH	H
5	−Ara^3 — Glc^3 — Glc	CH_2OH	H
6	−Ara^2 — Rha	CH_2OH	−Glc^6 — Xyl



7 n = 20 (major), n = 18, 19, 21, 22 (minor)



8

* To whom correspondence should be addressed. e-mail: sskang@snu.ac.kr

Table 1. NMR Data of Hederagenin 3-*O*-*β*-D-Glucopyranosyl(1→3)-*β*-D-glucopyranosyl(1→3)-*α*-L-arabinopyranoside (**5**) in Pyridine-d_5 (500 MHz)

Carbon No.	δ_H (*J*)	δ_C (DEPT)	HMBC (H→C)
C-1		38.8 (CH_2)	
C-2		26.2 (CH_2)	
C-3	4.27 (dd, 4.8, 10.5)	81.9 (CH)	C-23, C-24
C-4	—	43.6 (C)	
C-5		47.6 (CH)	
C-6		18.2 (CH_2)	
C-7		33.2 (CH_2)	
C-8	—	39.8 (C)	
C-9		48.2 (CH)	
C-10	—	36.9 (C)	
C-11		23.7 (CH_2)	
C-12	5.46 (br s)	122.6 (CH)	C-9, C-14, C-18
C-13	—	144.8 (C)	
C-14	—	42.2 (C)	
C-15		28.3 (CH_2)	
C-16		23.9 (CH_2)	
C-17	—	46.9 (C)	
C-18	3.28 (br dd, 3.9, 13.8)	42.0 (CH)	C-12, C-13, C-14, C-19
C-19		46.4 (CH_2)	
C-20	—	30.9 (C)	
C-21		34.2 (CH_2)	
C-22		32.9 (CH_2)	
C-23	3.73 (br d, 11.7)	64.2 (CH_2)	C-3, C-4, C-5
	4.34 (br d, 11.7)		
C-24	0.93 (s)	13.7 (CH_3)	C-3, C-4, C-23
C-25	0.92 (s)	16.1 (CH_3)	C-1
C-26	1.01 (s)	17.5 (CH_3)	C-7
C-27	1.23 (s)	26.1 (CH_3)	C-8, C-13, C-15
C-28	—	180.2 (C)	
C-29	0.92 (s)	33.2 (CH_3)	C-20, C-30
C-30	0.99 (s)	23.9 (CH_3)	C-29
Arabinose			
C-1	4.93 (d, 7.5)	106.7 (CH)	C-3
C-2	4.54 (dd, 7.5, 9.0)	72.1 (CH)	Ara C-1, Ara C-3
C-3	4.03 (dd, 3.6, 9.0)	83.9 (CH)	Ara C-2
C-4	4.30 (overlapped)	69.4 (CH)	
C-5	3.58 (br d, 11.5)	67.2 (CH_2)	Ara C-1, Ara C-3, Ara C-4
	4.12 (br d, 11.5)		
Glucose I (inner)			
C-1	5.33 (d, 8.0)	105.8 (CH)	Ara C-3
C-2	4.05 (t, 9.0)	74.4 (CH)	Glc I C-1, Glc I C-3
C-3	4.21 (t, 8.4)	88.3 (CH)	Glc I C-2
C-4	4.09 (overlapped)	69.7 (CH)	Glc I C-3, Glc I C-5, Glc I C-6
C-5	3.93 (m)	78.3 (CH)	
C-6	4.46 (dd, 1.8, 12.0)	62.4 (CH_2)	Glc I C-4
	4.26 (overlapped)		
Glucose II (terminal)			
C-1	5.30 (d, 7.5)	105.9 (CH)	Glc I C-3
C-2	4.06 (t, 9.0)	75.7 (CH)	Glc II C-1, Glc II C-3
C-3	4.24 (t, 8.5)	78.3 (CH)	Glc II C-1, Glc II C-2
C-4	4.18 (t, 9.0)	71.7 (CH)	Glc II C-3, Glc II C-6
C-5	4.05 (m)	78.7 (CH)	
C-6	4.30 (overlapped)	62.5 (CH_2)	Glc II C-4
	4.54 (dd, 2.7, 11.7)		

saccharide units through easily identifiable signals for anomeric protons and carbons. Acid hydrolysis of **5** afforded hederagenin as an aglycon and arabinose and glucose as the sugar components identified on GLC analysis by comparison with authentic samples. Absolute configurations for sugars were determined to be the L-form for arabinose and D-form for glucose, respectively, which were identified by GLC analysis of the thiazolidine derivatives.[25] The negative-ion mode FAB mass spectrum of **5** exhibited an $[M-H]^-$ ion at *m*/*z* 927 which is consistent with a trisaccharide glycoside carrying one arabinose, two glucose, and an aglycon with a molecular mass of 472. The fragments at *m*/*z* 765 $[M-H-162]^-$, 603 $[M-H-162-162]^-$ and 471 $[M-H-162-162-132]^-$ showed that the presence of the linear sugar chain, and the sugar sequence would seem to be that of glucose–glucose–arabinose. Comparison of the ^{13}C-NMR data of the aglycon moiety of **5** with hederagenin[26] showed that the signal for C-3 of **5** was significantly shifted downfield by +8.0 ppm due to a glycosidation shift.[27] The ^1H–^1H correlation spectroscopy experiment with **5** allowed the sequential assignments of three monosaccharides. Their multiplet patterns and coupling constants allowed the identi-

Table 2. NMR Data of Sugar Moiety of 3-*O*-α-L-Rhamnopyranosyl(1→2)-α-L-arabinopyranosyl Hederagenin 28-*O*-β-D-Xylopyranosyl(1→6)-β-D-glucopyranosyl Ester (**6**) in Pyridine-d_5 (500 MHz)

Carbon No.	δ_C	δ_C [a)]	δ_H (J)	HMBC (H→C)
3-*O*-Arabinose				
C-1	104.3	105.6	5.12 (d, 6.3)	C-3, Ara C-5, Ara C-3
C-2	75.9	75.9	4.58 (dd, 6.3, 7.5)	Ara C-1, Ara C-3, Rha C-1
C-3	74.7	74.7	4.13 (overlapped)	
C-4	69.4	67.0	(overlapped)	
C-5	65.6	64.0	3.71 (br d, 11.0)	Ara C-1
			4.26 (br d, 11.0)	
3-*O*-Rhamnose				
C-1	101.7	101.7	6.24 (d, 1.2)	Ara C-2, Rha C-5, Rha C-2, Rha C-3
C-2	72.4	72.3	4.74 (dd, 1.2, 4.0)	
C-3	72.6	72.5	4.65 (dd, 4.0, 10.5)	Rha C-4
C-4	74.2	74.5	(overlapped)	
C-5	69.8	69.7	(overlapped)	
C-6	18.6	18.5	1.65 (d, 6.0)	Ara C-5, Ara C-4
28-*O*-Glucose				
C-1	95.7	95.6	6.28 (d, 8.5)	COOH, Glc C-2
C-2	73.9	73.9	4.13 (overlapped)	Glc C-1, Glc C-3
C-3	78.8	78.7	4.24 (t, 9.0)	Glc C-2, Glc C-4
C-4	71.1	71.0	(overlapped)	
C-5	78.2	77.3	(overlapped)	
C-6	69.2	69.2	4.36 (br d, 4.2, 13.5)	Xyl C-1
			4.72 (dd, 3.0, 13.5)	
28-*O*-Xylose				
C-1	105.7	104.2	4.94 (d, 7.5)	Glc C-6, Xyl C-2, Xyl C-5
C-2	74.8	74.1	3.19 (t, 7.5)	Xyl C-1, Xyl C-3
C-3	77.9	74.7	(overlapped)	Xyl C-4
C-4	71.0	70.9	4.37 (overlapped)	Xyl C-3, Xyl C-5
C-5	67.1	65.5	3.65 (dd, 11.1, 12.6)	Xyl C-3, Xyl C-4
			4.32 (overlapped)	

a) Data from ref. 4.

fication of an α-L-arabinopyranosyl ($^4C_1$) and β-D-glucopyranosyl ($^4C_1$) unit. The arabinosyl unit was shown to be directly attached at C-3 of the aglycon by an heteronuclear multiple-bond correlation between the signals of the anomeric proton of the arabinosyl unit at δ 4.93 and C-3 of the aglycon at δ 81.9. The anomeric proton of the terminal glucosyl moiety at δ 5.30 showed a $^3J_{C,H}$ correlation with C-3 of the inner glucosyl residue at δ 88.3, the anomeric proton of which at δ 5.33 in turn showed a long-range correlation with C-3 of the arabinosyl moiety at δ 83.9. In light of the above observations, the structure of the durupcoside C (**5**) was determined to be hederagenin 3-*O*-β-D-glucopyranosyl(1→3)-β-D-glucopyranosyl(1→3)-α-L-arabinopyranoside. Very recently, Dong *et al.* reported the isolation of ixeris saponin A from *Ixeris sonchifolia*[28] whose structure was determined as echinocystic acid 3-*O*-β-D-glucopyranosyl(1→3)-β-D-glucopyranosyl(1→3)-α-L-arabinopyranoside. Comparison of the ^{13}C-NMR spectral data between **5** and ixeris saponin A showed that signals for sugar moieties were superimposable.

The ^{1}H- and ^{13}C-NMR spectra of **6** were assigned unambiguously by two-dimensional NMR spectra, which led to the revision of chemical shifts of arabinose C-1, C-4, C-5, xylose C-1, C-3 and C-5 as indicated in Table 2. The structures of **7** were characterized as a mixture of six phytosphingosine-type ceramides, which comprised a common long-chain base, (2*S*,3*S*,4*R*,8*E*)-2-amino-8(*E*)-octadecene-1,3,4-triol[18] with either five 2-hydroxy fatty acids of varying chain lengths (C_{22}—C_{26}) or palmitic acid linked to the amino group. The absolute configuration at C-2 of 2-hydroxy fatty acids was determined to be *R* from the ^{1}H-NMR chemical shift for the methoxy group at δ 3.65 in the *R*-(+)-α-methoxy-α-trifluoromethylphenylacetic acid (MTPA) ester[29,30] of the mixture. Of two minor ceramide components containing palmitic acid and 2-hydroxy hexacosanoic acid are new to the literature while others have been separated from the roots of *Urtica dioica*.[23] Other lipid component (**8**) was identified as arisaema glyceride 3 by comparison of its NMR, MS spectra, and optical rotation data with an authentic sample from *Arisaema amurense*.[24] Collinsonidin (**2**) and two known lipids (**7**, **8**) were isolated for the first time from this plant.

Experimental

Melting points were measured on a Mitamura–Riken apparatus and are uncorrected. Optical rotations were determined on a JASCO P-1020 polarimeter. The IR spectra were obtained on a JASCO FT/IR-5300 spectrometer. The EI-MS was performed on a Hewlett Packard 5989B mass spectrometer. The FAB mass spectrum was obtained in a 3-nitrobenzyl alcohol matrix in positive/negative ion mode on a JEOL-700 spectrometer. The NMR spectra were measured on a Varian Gemini 2000 instrument (300 MHz) or a Bruker AM-500 (500 MHz), and the chemical shifts were referenced to TMS. GC analysis was performed with a Hewlett Packard 5890 Series II gas chromatograph equipped with an H_2 flame ionization detector. The column was HP-5 capillary column (30 m×0.32 mm×0.25 μm): column temperature, 200 °C; injector and detector temperature, 290 °C; and He flow rate, 30 ml/min. GC-MS analysis was performed as previously described[18] using a Hewlett Packard 5989B mass spectrometer equipped with a 5890 series II plus gas chromatograph. TLC was performed on silica gel 60F_{254} (Merck) and cellulose plates (Art No. 5716, Merck). The spots on TLC were visualized by spraying 10% H_2SO_4 for silica gel and aniline phthalate for cellulose plates followed by heating, respectively.

Plant Material The leaves of *Aralia elata* were collected in August 2003, in Chungcheong Namdo Province, Korea, and authenticated by Dr. K.-H. Bae (CNN). A voucher specimen (2003-1) was deposited in the herbarium of the Natural Products Research Institute, Seoul National University.

Extraction and Isolation Air-dried leaves of *A. elata* (6.3 kg) were chopped into small pieces and extracted four times with MeOH at room temperature. The MeOH extract was evaporated under reduced pressure to dryness and then partitioned in succession between H_2O and *n*-hexane, CH_2Cl_2, EtOAc, and then *n*-BuOH. The *n*-hexane fraction (170 g) was chromatographed on a silica gel column eluted with *n*-hexane, CH_2Cl_2, CH_2Cl_2–MeOH mixtures of increasing polarity to give 48 fractions (H-01—48). Fraction H-42 (6.1 g) was further purified on a silica gel column [$CHCl_3$–MeOH–H_2O (7 : 1 : 0.5, 7 : 1.2 : 0.5, 7 : 1.5 : 0.5, 7 : 2 : 0.5, 7 : 3 : 1)] to yield thirty fractions (H-42-01—30), among which fraction H-42-11 was crystallized from MeOH–EtOAc to yield **1** (192 mg). Fraction H-42-15 (0.3 g) was further purified by silica gel column [(EtOAc, EtOAc–H_2O (100 : 1, 100 : 2), EtOAc saturated with H_2O–MeOH (100 : 1—10)] to yield nine fractions (H-42-15-01—09). Fraction H-42-15-06 was crystallized from EtOAc–H_2O (100 : 1) to yield **2** (20 mg). Fractions H-42-20 (100 mg) was applied to an RP_{18} column [MeOH–H_2O (9 : 1)] to give **3** (19 mg) from fraction H-42-20-10. Fractions H-42-23 (50 mg) and H-42-25 (180 mg) were treated in the same way as H-42-20, yielding nine (H-42-23-01—09) and ten (H-42-25-01—10) fractions, respectively. Fraction H-42-23-06 (10 mg) was further purified on a silica gel column [EtOAc–MeOH–H_2O (100 : 8 : 6)] to yield eleven fractions (H-42-23-06-01—11). Fraction H-42-23-06-07 was crystallized from EtOAc–MeOH–H_2O (100 : 8 : 6) to yield **4** (4 mg). Fraction H-42-25-05 (17 mg) was further purified on a silica gel [$CHCl_3$–MeOH–H_2O (7 : 1.5 : 0.5)] to yield twenty fractions (H-42-25-05-01—20). Fraction H-42-25-05-09 was crystallized from $CHCl_3$–MeOH–H_2O (7 : 1.5 : 0.5) to yield **5** (6 mg). Fraction H-42-28 (150 mg) was rechromatographed on an RP_{18} column [MeOH–H_2O (8 : 2)] and repeated silica gel column chromatography using $CHCl_3$–MeOH–H_2O (7 : 3 : 1) to afford **6** (5 mg). Fraction H-39 (4.1 g) was further purified on a silica gel column [hexane, hexane–EtOAc gradient, EtOAc] to yield twenty fractions (H-39-01—20), among which fraction H-39-16 was crystallized from MeOH–CH_2Cl_2 to yield **7** (220 mg). Fraction H-39-17 (0.8 g) was further purified by silica gel column [$CHCl_3$–MeOH–H_2O (7 : 1 : 0.5)] to yield ten fractions (H-39-17-01—10). Fraction H-39-17-05 (0.3 g) was further purified by RP_{18} column (MeOH) to give **8** (8 mg) from fraction H-39-17-05-30.

Compound **5** was obtained as an amorphous powder (MeOH), $[\alpha]_D^{27}$ +35.7° (c=0.28, MeOH), ^{1}H-NMR (pyridine-d_5, 500 MHz): see Table 1, ^{13}C-NMR (pyridine-d_5, 125.8 MHz): see Table 1, (−)-FAB-MS *m/z*: 927 $[M-H]^-$, 765 $[M-H-162]^-$, 603 $[M-H-(162\times2)]^-$, 471 $[M-H-(162\times2)-132]^-$. (−)-HR-FAB-MS *m/z*: 927.4957. Calcd for $C_{47}H_{75}O_{18}$: 927.4953.

Compound **6** was obtained as an amorphous powder (MeOH), $[\alpha]_D^{27}$ −6.23° (c=1.3, MeOH), ^{1}H-NMR (pyridine-d_5, 500 MHz): see Table 2, ^{13}C-NMR (pyridine-d_5, 125.8 MHz): see Table 2, (−)-FAB-MS *m/z*: 1043 $[M-H]^-$, 749 $[(M-H)-(132+162)]^-$, 603 $[(M-H)-(132+162)-146]^-$, 471 $[(M-H)-(132+162)-(146+132)]^-$. (−)-HR-FAB-MS *m/z*: 1043.5453. Calcd for $C_{52}H_{83}O_{21}$: 1043.5427.

Compound **7** was obtained as an amorphous powder (MeOH), ^{1}H-NMR (pyridine-d_5, 300 MHz) δ: 0.85 (6H, t-like, J=6.6 Hz, 2×Me), 1.24—1.30 $[(CH_2)_n]$, 4.28 (1H, m, H-4), 4.33 (1H, dd, J=6.3, 5.1 Hz, H-3), 4.42 (1H, dd, J=11.1, 5.1 Hz, H-1a), 4.51 (1H, dd, J=11.1, 4.5 Hz, H-1b), 4.62 (1H, m, H-2′), 5.11 (1H, dt, J=13.5, 4.5 Hz, H-2), 5.46 (1H, dd, J=15.3, 5.7 Hz, H-8), 5.56 (1H, dd, J=15.3, 5.7 Hz, H-9), 8.58 (1H, d, J=8.7 Hz, NH), ^{13}C-NMR (pyridine-d_5, 75.5 MHz) δ: 62.0 (C-1), 53.0 (C-2), 76.8 (C-3), 72.9 (C-4), 33.8 (C-5), 25.8 (C-6), 33.0 (C-7), 130.7 (C-8), 130.8 (C-9), 33.3 (C-10), 175.3 (C-1′), 72.5 (C-2′), 35.7 (C-3′), 26.7 (C-4′), 22.9—35.7 $[(CH_2)_n]$, 22.9 (C-17, 23′), 14.3 (C-18, 24′), (+)-HR-FAB-MS *m/z*: 704.6624. Calcd for $C_{42}H_{83}O_5N$+Na: 704.6169, *m/z*: 690.6045. Calcd for $C_{41}H_{81}O_5N$+Na: 690.6013, *m/z*: 676.5859. Calcd for $C_{40}H_{79}O_5N$+Na: 676.5856, *m/z*: 338 $[C_{18}H_{35}O_3N+Na]^+$.

Compound **8** was obtained as an amorphous powder (MeOH), ^{1}H-NMR (CD_3OD, 300 MHz) δ: 0.89 (6H, t-like, J=6.6 Hz, H-18″, H-16‴), 1.28—1.32 $[(CH_2)_n]$, 1.60 (4H, m, H-3″, 3‴), 2.03 (4H, m, H-8″, 11″), 2.31 (2H, t, J=7.5 Hz, H-2″*), 2.32 (2H, t, J=7.2 Hz, H-2‴*), 3.44 (1H, dd, J=9.6, 3.0 Hz, H-3′), 3.70 (1H, dd, J=11.1, 5.4 Hz, H-6′b), 3.73 (1H, dd, J=11.1, 6.0 Hz, H-3b), 3.76 (1H, dd, J=11.1, 6.9 Hz, H-6′a), 3.82 (1H, dd, J=3.0, 0.9 Hz, H-4′), 3.98 (1H, dd, J=11.1, 5.7 Hz, H-3a), 4.21 (1H, dd, J=12.0, 6.9 Hz, H-1b), 4.22 (1H, d, J=7.2 Hz, H-1′), 4.44 (1H, dd, J=12.0, 3.0 Hz, H-1a), 5.26 (1H, m, H-2), 5.34 (2H, m, H-9″, 10″), ^{13}C-NMR (CD_3OD, 75.5 MHz) δ: 64.0 (C-1), 71.8 (C-2), 68.7 (C-3), 105.4 (C-1′), 72.4 (C-2″), 74.9 (C-3′), 70.2 (C-4′), 76.8 (C-5′), 62.5 (C-6′), 175.0, 174.7 (C-1″, 1‴), 35.2, 35.0 (C-2″, 2‴), 26.0 (C-3″, 3‴), 28.2 (C-8″, 11″), 130.9, 130.8 (C-9″, 10″), 14.5, 14.6 (C-18″, 16‴), (+)-FAB-MS *m/z*: 779 $[M+Na]^+$, 577 $[M-179]^+$, 523 $[M+Na-256]^+$, 497 $[M+Na-282]^+$, 339 $[(M+H)-179-239]^+$, 313 $[(M+H)+179-265]^+$, 265 $[C_{17}H_{33}CO$ (oleoyl)$]^+$, 239 $[C_{15}H_{31}CO$ (palmitoyl)$]^+$.

Acid Hydrolysis of 5 and Determination of the Absolute Configuration of Sugars A solution of **5** (2 mg) in 2 N HCl–dioxane (1 : 1, 2 ml) was heated at 100 °C for 1 h. The reaction mixture was neutralized with Ag_2CO_3, filtered and then concentrated to dryness *in vacuo* to give a residue. The residue was treated with L-cysteine methyl ester hydrochloride (1 mg) in pyridine (0.2 ml) at 60 °C for 1 h. The solution was treated with *N*,*O*-bis(trimethylsilyl)trifluoroacetamide (0.05 ml) at 60 °C for 1 h. The supernatant was applied to GC as described previously.[31] The retention times (t_R) of the peaks were 18.24 min (L-arabinose) and 37.87 min (D-glucose).

Acid Hydrolysis of 7 Compound **7** (30 mg) was refluxed with 0.9 N HCl in 82% aqueous MeOH (10 ml) for 18 h. The resulting solution was extracted with *n*-hexane, and the combined organic phase was dried over Na_2SO_4. Evaporation of the hexane yielded a fatty acid methyl ester. The H_2O layer was neutralized with NH_4OH, filtered, and then concentrated to yield a long-chain base, which was identical with (2*S*,3*S*,4*R*,8*E*)-2-amino-8(*E*)-octadecene-1,3,4-triol by direct comparison with an authentic sample.[18] The fatty acid methyl ester was recrystallized from MeOH to give an amorphous white powder and then analyzed by GC-MS. Peak 1 (t_R 19.62 min, 6%): methyl palmitate; Peak 2 (t_R 27.78 min, 9%): methyl 2-hydroxybehenate; Peak 3 (t_R 29.85 min, 10%): methyl 2-hydroxytricosanoate; Peak 4 (t_R 32.66 min, 57%): methyl 2-hydroxylignocerate; Peak 5 (t_R 35.91 min, 9%): methyl 2-hydroxypentacosanoate; Peak 6 (t_R 40.24 min, 4%): methyl 2-hydroxyhexacosanoate. The MTPA ester of 2-hydroxy fatty acids was prepared as previously reported,[30] which was measured ^{1}H-NMR in CCl_4. The signal for the methoxy group was appeared at δ 3.65 as a singlet.

Alkaline Hydrolysis of 8 A solution of **8** (5 mg) in 3% dry NaOMe–MeOH (2 ml) was stirred at 40 °C for 2 h. The reaction mixture was neutralized with 2 N-HCl in MeOH and partitioned between MeOH and *n*-hexane. The *n*-hexane layer was concentrated under reduced pressure to yield fatty acid methyl esters, which were analysed as palmitic and oleic acid methyl esters by GC-MS. The MeOH layer was concentrated under reduced pressure and purified by C-18 reverse-phase column chromatography [MeOH–H_2O (10 : 3, 5 : 1)] and Sephadex LH-20 column chromatography (MeOH) to afford (2*R*)-1-*O*-β-D-galactopyranosyl glycerol, which was identified by direct comparison with an authentic sample.[24]

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