Phytochemistry Letters 4 (2011) 38–42

Contents lists available at ScienceDirect

Phytochemistry Letters

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

2-Acety-1-(3-glycosyloxyoctadecanoyl)glycerol and dammarane triterpenes in the exudates from glandular trichome-like secretory organs on the stipules and leaves of *Cerasus yedoensis*

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ARTICLE INFO

Article history:
Received 9 July 2010
Received in revised form 28 October 2010
Accepted 1 November 2010
Available online 18 November 2010

Keywords:
Cerasus yedoensis
Rosaceae
Glycolipid
Acylglycerol
Dammarane triterpene
Secretory organ

ABSTRACT

A glycolipid, 2-acetyl-1-{3-[3,4-di-*O*-acetyl-β-D-glucopyranosyl-(1 → 3)-2-*O*-acetyl-α-L-rhamnopyranosyloxy]octadecanoyl}-*sn*-glycerol (**1**) and a dammarane triterpene, (2α,20*S*)-2,20-dihydroxydammar-24-en-3-one (**2**), along with known (20*S*)-20-hydroxydammar-24-en-3-one (**3**), were isolated from the exudates of the glandular trichome-like secretory organs in the young stipules and leaves of *Cerasus yedoensis* (Rosaceae).

1. Introduction

Various secretory structures develop on the surface of leaves, stems, and roots of many plants. For example, glandular trichomes often produce and accumulate oily secondary metabolites. Extrafloral nectarines and glands of many insectivorous plants secrete sugars and a viscous substance containing digestive enzymes. Solid secretions are referred to as food bodies (Bell and Bryan, 2010). We have been interested in phytochemical studies aimed at further understanding of the biological roles of the secondary metabolites in the secretions from such secretory organs of plants, and reported the characterization of oxygenated fatty acylglycerols from the glandular trichome exudate on the leaves of *Paulownia tomentosa* (Asai et al., 2009) and geranylated flavanones from the oily secretions on the surface of the plant's immature fruits (Asai et al., 2008). More recently we reported the identification of glycosylated fatty acids and dammarane triterpenes from the glandular trichome exudates on the leaves of *Ibicella lutea* and *Proboscidea louisiana* (Asai et al., 2010) and cyclic fatty acyl glycosides in the glandular trichome exudates on the calyxes of *Silene gallica* (Asai and Fujimoto, 2010).

Cerasus yedoensis (=*Prunus yedoensis* Matsumura; Someiyoshino in Japanese) of the family of Rosaceae, is the most commonly cultivated species of cherry tree in Japan and believed to be an interspecific hybrid between *P. spachiana f. ascendens* and *P. speciosa* (Innan et al., 1995; Roh et al., 2007). We recently noticed that *C. yedoensis* has glandular trichome-like secretory organs on the edge of young leaves and stipules, and they exude oily substances (Fig. 1). The secretion can be observed only during their young stage, e.g., in April–May in Japan. The same secretary organs are also found in other *Cerasus* species such as *C. jamasakura*.

Previous investigation on the constituents of leaves and barks of *C. yedoensis* resulted in the isolation of several flavonoids, including a flavonoid glucoside, sakuranetin 5-glucoside (Hasegawa and Shirota, 1952). As part of our continuing study on the secondary metabolites in plant exudates, we have now carried out phytochemical investigation of the exudates from glandular trichome-like secretory organs of *C. yedoensis*. The study led to the isolation of 2-acetyl-1-{3-[3,4-di-*O*-acetyl-β-D-glucopyranosyl-(1 → 3)-2-*O*-acetyl-α-L-rhamnopyranosyloxy]octadecanoyl}-*sn*-glycerol (**1**) and (2α,20*S*)-2,20-dihydroxydammar-24-en-3-one (**2**), along with known (20*S*)-20-hydroxydammar-24-en-3-one (**3**) (see Fig. 2).

2. Results and discussion

In a preliminary study the oily material that was obtained by briefly dipping the dissected glandular trichome-like organs from young leaves into Et_2O was compared with the sample similarly obtained from stipules by TLC. The two samples exhibited one

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doi:10.1016/j.phytol.2010.11.001

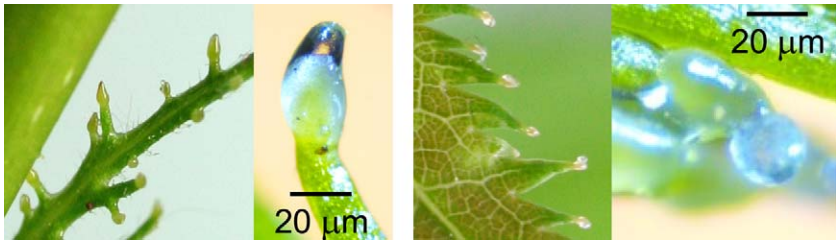

Fig. 1. Pictures of glandular trichome-like secretory organs at the edge of young stipules (left) and leaves (right) of *Cerasus yedoensis*.

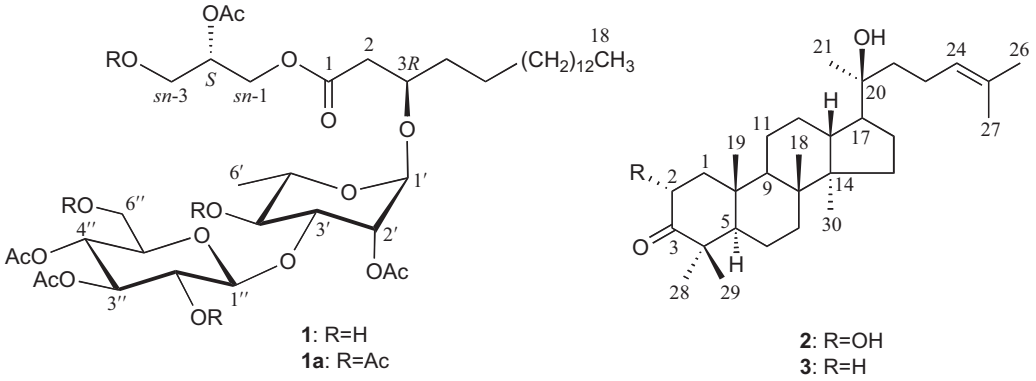

Fig. 2. Structures of compounds **1**–**3**.

intense spot and two weak ones and were not differentiated from each other. A large-scale extraction was conveniently carried out by briefly dipping the whole stipules into Et_2O to give the oily extract, which showed the same three spots on TLC. Silica gel column chromatography of the hexane soluble part of the extract yielded dammarane triterpenes (**2** and **3**), while the hexane insoluble part of the extract furnished the glycolipid (**1**).

Compound **1**, obtained as colorless oil, showed a pseudo-molecular ion at *m*/*z* 849.4509 $[M-H]^-$ in the negative HRFABMS, which suggested the molecular formula $C_{41}H_{70}O_{18}$. Analysis of the ^{1}H NMR spectrum of **1**, assisted with the H-H COSY spectrum, revealed the presence of three sub-structures in the molecule (Table 1). Proton signals with 5H units [δ 4.38 (*dd*, *J* = 12.2, 3.5 Hz, *sn*-1-Ha), 3.99 (*dd*, *J* = 12.2, 7.8 Hz, *sn*-1-Hb), 5.05 (*m*, *sn*-2-H) and 3.73 (*m*, *sn*-3-H_2)] were ascribable to the glycerol moiety of a 1,2-diacylglycerol. Three protons with an ABX pattern [δ 2.50 (*dd*, *J* = 14.4, 4.7 Hz, Ha-2), 2.47 (*dd*, *J* = 14.4, 7.4 Hz, Hb-2), 4.13 (*m*, H-3)] and intense methylene protons (δ 1.25) terminated by a methyl triplet [δ 0.88 (*t*, *J* = 6.8 Hz)] were due to a C-3 oxygenated fatty acyl moiety. Proton signals with 15H units, including two anomeric protons at δ 4.74 (*brs*) and 4.53 (*d*, *J* = 7.8 Hz) and one methyl doublet at δ 1.31 (*d*, *J* = 5.2 Hz), could be assigned to those of a disaccharide moiety composed of a hexose and a 6-deoxy hexose (Table 1). In addition, four acetyl methyl signals were discerned at δ 2.14, 2.13, 2.12 and 2.04. The ^{13}C NMR spectrum of **1** (Table 1) showed characteristic signals for five ester carbonyls, two anomeric, 12 oxymethine and three oxymethylene carbons among others, which corroborated the three sub-structures. H–H COSY correlations and coupling constants of the proton resonances ascribable to the two sugar moieties allowed the sequential assignments from H-1″ to H_2-6″ of β-linked 3,4-di-*O*-acetylglucopyranosyl and H-1′ to H_3-6′ of α-linked 2-*O*-acetylrhamnopyranosyl moieties (Table 1). The acetylated positions in the two sugars were predicted from the chemical shifts of the oxymethine protons and unambiguously established by HMBC correlations from the oxymethine protons to the ester carbonyl carbons (Fig. 3). A β-linkage at the anomeric center of the glucose moiety was obvious from a large coupling constant (*J* = 7.8 Hz) of H-1″, whereas an α-linkage at the anomeric center of the rhamnose moiety was deduced from the results of NOE experiments in which no NOE enhancement was observed between H-1′ and H-3′, nor between H-1′ and H-5′ in spite of the presence of a clear NOE between H-3′ and H-5′. HMBC correlations from H-1″ (δ 4.53) of the glucose moiety to C-3′ (δ 80.6) of the rhamnose moiety and from H-1′ (δ 4.74) of the rhamnose moiety to C-3 (δ 74.0) of the fatty acyl moiety provided evidence for the connectivity of the three sub-structures. HMBC correlations further established that the glycerol moiety was acetylated at the C-2 position (δ_C 170.9 of acetyl carbonyl) and fatty acylated at the C-1 position (δ_C 171.9 of fatty acyl carbonyl) (Fig. 3).

The configuration at *sn*-2 of glycerol moiety was determined to be *S* by converting compound **1** to a known stereo-defined compound, 1,2-di-*O*-(*S*)-MTPA-3-*O*-TBDPS-*sn*-glycerol as reported in our previous paper (Asai et al., 2009). The configuration at C-3 of the fatty acyl moiety was determined to be *R* by applying Mosher's ester method (Ohtani et al., 1991) to the methyl 3-hydroxyoctadecanoate which was obtained by alkaline hydrolysis of **1**, acidic treatment of the intermediate glycosylated fatty acid, and

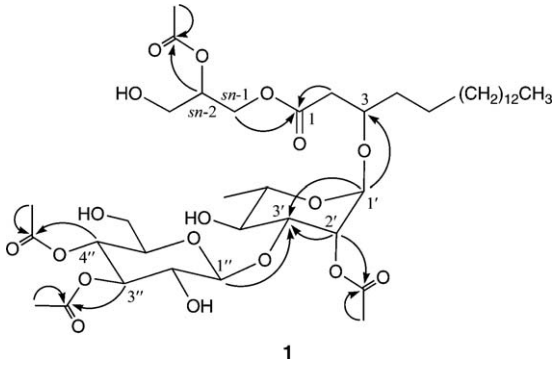

Fig. 3. Pertinent HMBC correlations (H → C) of **1**.

Table 1
^{1}H (500 MHz) and ^{13}C NMR (125 MHz) spectroscopic data for **1** and **1a**.[a,b]

Position	**1**		**1a**
	δ_H	δ_C	δ_H
sn-1	4.38 (*dd*, 12.2, 3.5)	63.2	4.32 (*dd*, 12.1, 4.2)
	3.99 (*dd*, 12.2, 7.8)		4.15 (*dd*, 12.1, 6.4)
sn-2	5.05 (*m*)	72.7	5.27 (*m*)
sn-3	3.73 (*m*)	60.4	4.31 (*dd*, 12.1, 4.2)
	3.73 (*m*)		4.15 (*dd*, 12.1, 6.4)
1	–	171.9	
2	2.50 (*dd*, 14.4, 4.7)	39.4	2.56(*dd*, 15.4, 7.4)
	2.47 (*dd*, 14.4, 7.4)		2.52 (*dd*, 15.4, 4.9)
3	4.13 (*m*)	74.0	4.05 (*m*)
4	1.61 (*m*)	31.6	1.61 (*m*)
5	1.46 (*m*)	24.6	1.47 (*m*)
6–15	1.25 (*m*)	29.7–29.3	1.25 (*m*)
16	1.25 (*m*)	31.9	1.25 (*m*)
17	1.25 (*m*)	22.6	1.25 (*m*)
18	0.88 (*t*, 7.2)	14.1	0.88 (*t*, 6.9)
1′	4.74 (*brs*)	93.8	4.83 (*d*, 1.2)
2′	5.24 (*brd*, 3.2)	71.5	5.06 (*dd*, 3.6, 1.2)
3′	3.68 (*dd*, 9.1, 3.2)	80.6	4.00 (*dd*, 9.9, 3.6)
4′	3.63 (*t*, 9.1)	71.2	5.03 (*t*, 9.9)
5′	3.61 (*m*)	68.1	3.76 (*m*)
6′	1.31 (*d*, 5.2)	17.4	1.15 (*d*, 6.3)
1″	4.53 (*d*, 7.8)	103.5	4.65 (*d*, 8.0)
2″	3.46 (*dd*, 9.6, 7.8)	72.5	4.94 (*dd*, 9.6, 8.0)
3″	5.08 (*t*, 9.6)	74.0	5.15 (*t*, 9.6)
4″	4.80 (*t*, 9.6)	68.6	5.06 (*t*, 9.6)
5″	3.61 (*m*)	74.9	3.71 (*m*)
6″	3.59 (*dd*, 12.1, 2.6)	61.8	4.23 (*dd*, 12.3, 4.6)
	3.46 (*dd*, 12.1, 6.2)		4.11 (*dd*, 12.3, 2.4)
Ac	2.14 (*s*)	20.7	2.11 (*s*)
		170.9	2.10 (*s*)
	2.13 (*s*)	21.2	2.10 (*s*)
		171.8	2.09 (*s*)
	2.12 (*s*)	21.1	2.09 (*s*)
		170.6	2.03 (*s*)
	2.04 (*s*)	20.6	2.01 (*s*)
		170.0	1.99 (*s*)

[a] Multiplicity and coupling constants (*J* in Hz) are in parentheses.
[b] Recorded in $CDCl_3$.

diazomethane treatment of the resulting 3-hydroxyfatty acid. The structure and the homogeneity of the methyl ester were further established by ^{1}H and ^{13}C NMR spectroscopic data, as well as GLC and EIMS (as trimethylsilyl ether) analyses in direct comparison with a reference material (Asai et al., 2009). Glucose and rhamnose were identified in the aqueous layer of the above acidic treatment by TLC and GLC (as their trimethylsilyl derivatives) analysis. Acetylation of **1** in a usual manner (Ac_2O in pyridine) yielded the tetra-acetate derivative **1a** of the original material, which was characterized by ^{1}H NMR (Table 1) and negative FABMS (*m*/*z* 1017 $[M-H]^-$). The structure of **1** was thus determined to be 2-acetyl-1-{3-[3,4-di-*O*-diacetyl-β-D-glucopyransyl-(1 → 3)-2-*O*-acetyl-α-L-rhamnopyransyloxy]octadecanoyl}-*sn*-glycerol.

In addition to the glycolipid **1**, two triterpenes **2** and **3** were isolated as minor components of the exudates. Compound **3** was identified as known (20*S*)-20-hydroxydammar-24-en-3-one (hydroxydammarenone II) by comparison of the ^{1}H and ^{13}C NMR spectroscopic data with those reported values (Asakawa et al., 1977; Yamashita et al., 1998) and based on EIMS data.

Compound **2** was obtained as white amorphous and its positive HREIMS showed a molecular ion peak at *m*/*z* 458.3639 $[M]^+$, corresponding to the molecular formula $C_{30}H_{50}O_3$. The ^{1}H NMR spectrum of **2** showed signals of eight tertiary methyl groups including two olefinic methyls at δ 1.69 and 1.62, an olefinic proton at δ 5.12 (*t*, *J* = 7.0 Hz) and an oxymethine proton at δ 4.55 (*ddd*, *J* = 12.4, 7.0, 3.2 Hz, the last coupling was due to the OH proton). The ^{13}C NMR spectrum showed 30 signals, among which two olefinic carbons at δ 131.7 and 124.6, an oxymethine carbon at δ 69.4 and an oxygen-bearing quaternary carbon at δ 75.3 were characteristic. Comparison of the NMR data with those of **3** revealed that compound **2** is a mono-hydroxy derivative of **3** with an extra hydroxy group being located presumably in the A ring of a dammarane skeleton (Table 1). Further inspection of the H–H COSY spectrum revealed that the oxymethine proton (δ 4.55, H-2) was correlated with Hα-1 at δ 1.14 (*dd*, *J* = 12.6, 12.4 Hz), assigned by HMBC and HSQC spectra). The hydroxy group was therefore located at C-2. A large coupling constant ($J_{H\alpha\text{-}1,H\text{-}2}$ = 12.4 Hz) established an α-orientation of the C-2 hydroxy group. The structure of **2** was thus determined to be (2α,20*S*)-2,20-dihydroxydammar-24-en-3-one. The H_3-19 signal (δ 1.19) of **2** was shifted downfield compared to that (δ 0.95) of **3**. This characteristic deshielding caused by the introduction of the 2α-hydroxy group was previously observed in triterpenes having the same 2α-hydroxy-3-one functionality (Ohsaki et al., 2004). The chemical shift of H_3-19 of **2** therefore further supported the assigned structure. Complete assignments of ^{13}C signals for **2**, based on 2D NMR including H–H COSY, HMBC and HSQC experiments, are listed in Table 2.

In the present study we demonstrated that the oily substances from the secretary glands distributed on the stipules and leaves of *C. yedoensis* contained a unique glycolipid (**1**) and (2α,20*S*)-2,20-dihydroxydammar-24-en-3-one (**2**), along with known (20*S*)-20-hydroxydammar-24-en-3-one (**3**). Compound **1** was a major constituent (70%) of the exudate and the two terpenes were minor ones (**2**: 8% and **3**: 5%). The same results were obtained when the samples collected in May 2007 and from other *C. yedoensis* trees in Tokyo were analyzed. The co-occurrence of relatively polar fatty acid derivatives and triterpenes, particularly dammarane triterpenes, as components of glandular trichome exudates was previously demonstrated in *I. lutea* and *P. louisiana* (Asai et al., 2010). Similarity in the chemical structures of the constituents secreted from glandular trichome-like organs from the stipules

Table 2
^{13}C NMR (125 MHz, in $CDCl_3$) spectroscopic data for **2** and **3**.[a]

Position	**2**	**3**
1	50.2	39.9
2	69.4	34.1
3	216.6	218.2
4	47.8	47.4
5	58.3	55.3
6	19.1	19.6
7	34.9	34.5
8	40.5	40.2
9	50.3	50.0
10	37.9	36.8
11	21.8	22.0
12	27.3	27.5
13	42.2	42.3
14	50.3	50.2
15	31.1	31.1
16	24.8	24.8
17	49.8	49.8
18	15.7	15.2
19	16.7	16.0
20	75.3	75.4[b]
21	25.4	25.4
22	40.5	40.4
23	22.5	22.5
24	124.6	124.6
25	131.7	131.7
26	25.7	25.7
27	17.7	17.7
28	24.6	26.7
29	21.3	21.0
30	16.4	16.3

[a] Assignments were based on COSY, HSQC and HMBC experiments.
[b] The value (δ 73.36) reported in Yamashita et al., 1988 should be read as δ 75.36 (personal communication from Prof. K. Matsuda).

and leaves of the *Cerasus* species suggests that they may play related physiological roles, although their roles remain to be clarified. We often observe ants being attracted by sugars secreted from a pair of extrafloral nectarines locating at the boundary between the petiole and leaf of *C. yedoensis* like other *Cerasus* species (Stephenson, 1982; Heil and Mckey, 2003). However, the exudates from the trichome-like organs do not appear to have such attracting effect on ants.

3. Experimental

3.1. General experimental procedures

^{1}H and ^{13}C NMR spectra were recorded on a Bruker DRX500 (500 MHz for ^{1}H and 125 MHz for ^{13}C) spectrometer in $CDCl_3$ or CD_3OD solution. Tetramethylsilane (δ 0.00) and CD_2HOD (δ 3.30) signals were used as an internal standard for ^{1}H shifts, and $CDCl_3$ (δ 77.00) and CD_3OD (δ 49.00) signals were used as a reference for ^{13}C shifts. EIMS, HREIMS (70 eV) and FABMS spectra were obtained on a JEOL JMS-700 spectrometer. IR spectra were recorded on a JASCO-FT/IR-5300 spectrometer. Optical rotations were measured on a JASCO P-2200 polarimeter. Silica gel 60 N (spherical neutral, 40–100 μm, Kanto Chemical, Japan). TLC analysis was performed using Merck precoated Si gel 60 F254 glass plates (0.25 mm thickness) and the spots were detected by treating the plates with a 5% ethanolic solution of phosphomolybdic acid followed by heating at 120 °C. PTLC was carried out using the same Si gel plates. GLC was carried out on a Shimadzu GC-14B apparatus equipped with a J&W Scientific DB-5 capillary column (15 m × 0.25 mm, 0.25 μm film thickness) under the following conditions for the analysis of fatty acid derivatives: injection temperature 270 °C, column temperature 208 °C, detection temperature 270 °C, He carrier gas flow rate of P1, 50 kPa and P2, 125 kPa, H_2 flow rate, 50 kPa, air flow rate, 50 kPa, and split (40:1) injection. GLC analysis of trimethylsilylated sugars was similarly performed under the conditions of injection temperature 260 °C, column temperature 175 °C and detection temperature 280 °C.

3.2. Plant material

Stipules and young leaves of the *Prunus yedoensis* (Rosaceae) were collected in May in 2008 on the campus of Tokyo Institute of Technology. The plant was identified by Prof. S. Kohshima, Department of Biological Sciences, Graduate School of Bioscience and Biotechnology, Tokyo Institute of Technology. A voucher specimen (CMS20-04) was deposited in the Department of Chemistry and Materials Science, Tokyo Institute of Technology.

3.3. Extraction and isolation

Fresh young stipules (fresh wt. 7.2 g, ca. 1.0 cm wide and 2.5 cm long, 800 stipules) were briefly (ca. 2 s) rinsed twice in a beaker containing Et_2O (total volume 100 mL), the Et_2O solution being concentrated to dryness (230 mg) under reduced pressure. The extract was treated with hexane (10 mL × 2) and the hexane soluble part (40 mg) was subjected to silica gel (10 g) CC. Elution with a gradient of hexane–AcOEt (10:1 → 1:1, total volume 50 mL) to yield **2** (18 mg, eluted with hexane–AcOEt 6:1) and **3** (12 mg, eluted with hexane–AcOEt 4:1). The hexane insoluble part (190 mg) was applied to silica gel (20 g) CC. Elution with a gradient of $CHCl_3$–MeOH (30:1 → 10:1, total volume 60 mL) gave **1** (160 mg, eluted with $CHCl_3$–MeOH 20:1).

Fresh young leaves (fresh wt. 700 g, ca. 3.0 cm wide and 5.0 cm long, 400 leaves) were similarly processed to give the Et_2O extract (30 mg), from which compounds **1** (20 mg), **2** (1.5 mg) and **3** (0.8 mg) were isolated.

3.4. 2-Acetyl-1-{3-[3,4-di-O-acetyl-β-D-glucopyranosyl-(1 → 3)-2-O-acetyl-α-L-rhamnopyranosyloxy]octadecanoyl}-sn-glycerol (1)

Colorless oil; negative HRFABMS *m/z*: 849.4509 $[M-H]^-$ (calcd for $C_{41}H_{69}O_{18}$, 849.4484); $[\alpha]_D^{24}$ +3.0 (*c* = 1.3, MeOH); for ^{1}H and ^{13}C NMR ($CDCl_3$) spectroscopic data, see Table 1.

A mixture of **1** (5 mg) and TBDPSCl (8 μL) in pyridine (50 μL) was stirred at room temperature for 12 h. Et_2O (0.3 mL) was added, and Et_2O-soluble portion was washed with saturated aqueous NH_4Cl, and then brine, dried (Na_2SO_4) and concentrated. The resulting di-TBDPS ether (12 mg) was reduced with DIBAL in the same manner as reported previously (Asai et al., 2009) to yield 3-*O*-TBDPS-*sn*-glycerol (1.5 mg, 77% from **1**). The TBDPS ether (0.5 mg) was reacted with (*R*)-MTPACl (1.0 μL) in the same manner as reported previously (Asai et al., 2009) and a crude product was purified by PTLC (hexane-AcOEt, 7:1) to give 1,2-di-*O*-(*S*)-MTPA-3-*O*-TBDPS-*sn*-glycerol (90% yield). The ^{1}H NMR ($CDCl_3$) spectroscopic data [δ: 7.58–7.27 (20H, *m*), 5.36 (1H, *m*), 4.75 (1H, *dd*, *J* = 12.4, 3.2 Hz), 4.50 (1H, *dd*, *J* = 12.4, 6.4 Hz), 3.72 (2H, *m*), 3.44 (3H, *s*, MeO), 3.38 (3H, *s*, MeO), 1.01 (9H, *s*, ^{t}Bu)] were identical to those of authentic sample, but different from those of 2,3-di-*O*-(*S*)-MTPA-1-*O*-TBDPS-*sn*-glycerol (Asai et al., 2009).

To a solution of LiOH (10 mg) in water (200 μL) was added a solution of **1** (15 mg) in DME (800 μL) and the mixture was stirred at room temperature for 48 h. Saturated aqueous NH_4Cl was added and the mixture was partitioned between $CHCl_3$ and H_2O. The $CHCl_3$ layer was concentrated to give a glycosylated fatty acid (6.5 mg, 90%). Colorless oil; $[\alpha]_D^{24}$–23.1 (*c* = 8.7, MeOH); negative HRFABMS *m/z* 607.3664 $[M-H]^-$ (calcd for $C_{30}H_{55}O_{12}$, 607.3694); ^{1}H NMR (CD_3OD) δ: 4.81 (*d*, *J* = 1.5 Hz, H-1′), 4.50 (*d*, *J* = 7.7 Hz, H-1″), 4.08 (*m*, H-3), 4.04 (*dd*, *J* = 3.0, 1.8 Hz, H-2′), 3.80 (*dd*, *J* = 11.9, 2.4 Hz, Ha-6″), 3.72 (*m*, Hb-6″), 3.72 (*m*, H-5′), 3.70 (*dd*, *J* = 9.5, 2.4 Hz, H-3′), 3.53 (*t*, *J* = 9.5 Hz, H-4′), 3.37 (*m*, H-3″), 3.36 (*m*, H-4″), 3.28 (*m*, H-2″), 3.28 (*m*, H-5″), 2.49 (*d*, *J* = 6.4 Hz, H_2-2), 1.61–1.25 (CH_2), 1.22 (*d*, *J* = 7.4 Hz, H_3-6′), 0.89 (*t*, *J* = 6.7 Hz, CH_3). ^{13}C NMR ($CDCl_3$) δ: 175.2 (C-1), 105.8 (C-1″), 99.9 (C-1′), 83.2 (C-3′), 77.7 (C-3″), 77.7 (C-5″), 75.5 (C-3), 75.4 (C-2″), 72.6 (C-4′), 71.9 (C-2′), 70.9 (C-4″), 69.9 (C-5′), 62.1 (C-6″), 41.2 (C-2), 34.4 (C-4), 33.1 (C-16), 30.8–30.5 (CH_2), 25.9 (C-5), 23.7 (C-17), 18.0 (C-6′), 14.4 (C-18).

A mixture of the glycosylated fatty acid (5 mg) and 1.5 N HCl (200 μL) was heated at 80 °C for 6 h and cooled down to room temperature. The solution was partitioned between ether and water, and the ether layer was concentrated to dryness in vacuo. The residue was treated with excess ethereal diazomethane, and the product was separated by PTLC (hexane-AcOEt, 6:1) to give methyl 3-hydroxyoctadecanoate (2.2 mg) as a white powder, (+)-FABMS *m/z*: 315 $[M+H]^+$; ^{1}H and ^{13}C NMR spectroscopic data was identical with those reported in our previous paper (Asai et al., 2009). The water layer was concentrated and analyzed by TLC (R_f values 0.55 and 0.85, developed with CH_3CN–H_2O 6:1, three times) in comparison with authentic glucose and rhamnose. A part (100 μg) of the residue was treated with TMS-HT (HMDS and TMSCl in anhydrous pyridine) (50 μL) at 70 °C for 1.5 h and cooled to room temperature. Hexane (200 μL) and H_2O (100 μL) were added and a portion of the hexane layer was analyzed by GLC. The chromatogram showed two intense peaks at 3.1 and 9.1 min, corresponding to rhamnose tetra-TMS ether (α-anomer) and glucose penta-TMS ether (α-anomer) in ca. 1:1 ratio.

Methyl 3-hydroxyoctadecanoate (0.5 mg) was treated with (*R*)-MTPACl (1.2 μL) in pyridine (30 μL) at room temperature. After 30 min, MeOH (100 μL) was added to the reaction mixture and the solution was purified by PTLC (hexane/AcOEt, 6:1) to give the (*S*)-MTPA ester (0.7 mg, 93% yield). Colorless oil; FABMS *m/z* 531 $[M+H]^+$; ^{1}H NMR ($CDCl_3$) δ: 7.58–7.54 (*m*, 5H), 5.47 (*m*, H-3), 3.59 (*s*, CO_2Me), 3.47 (*d*, *J* = 1.0 Hz, OMe), 2.65 (*dd*, *J* = 16.1, 8.0 Hz, Ha-2), 2.57 (*dd*, *J* = 16.1, 5.1 Hz, Hb-2), 1.25 (CH_2), 0.88 (*t*, *J* = 6.7 Hz, H_3-

18). Reaction of methyl 3-hydroxyoctadecanoate (0.5 mg) with (*S*)-MTPACl (1.2 μL) gave the (*R*)-MTPA ester (0.6 mg, 86% yield). Colorless oil; FABMS *m*/*z* 531 $[M+H]^+$; ^{1}H NMR ($CDCl_3$) δ: 7.58–7.54 (*m*, 5H), 5.47 (*m*, H-3), 3.66 (*s*, CO_2Me), 3.54 (*d*, *J* = 1.2 Hz, OMe), 2.70 (*dd*, *J* = 15.9, 8.3 Hz, Ha-2), 2.60 (*dd*, *J* = 15.9, 4.6 Hz, Hb-2), 1.25 (CH_2), 0.88 (*t*, *J* = 6.7 Hz, H_3-18).

3.5. Per-acetyl derivative of 1 (1a)

Compound **1** (5.0 mg) was treated with Ac_2O and pyridine at room temperature overnight. Extractive workup gave a crude product which was purified by PTLC (hexane-AcOEt, 1:1) to afford **1** acetate (**1a**) (5.6 mg, 93% yield): Colorless oil; negative FABMS *m*/*z*: 1017 $[M-H]^-$; for ^{1}H NMR ($CDCl_3$) spectroscopic data, see Table 1.

3.6. (2α,20S)-2,20-Dihydroxydammar-24-en-3-one (2)

White amorphous powder; $[\alpha]_D^{24}$ +29.7° (*c* = 0.15, $CHCl_3$); ν_{max} ($CHCl_3$) 3600, 3500, 2920, 1700, 1460, 1390, 1380 cm^{-1}; HREIMS *m*/*z*: 458.3639 $[M]^+$ (458.3631 calcd for $C_{30}H_{49}O_4$). ^{1}H NMR ($CDCl_3$) δ: 5.12 (*t*, *J* = 7.0 Hz, H-24), 4.53 (*ddd*, *J* = 12.4, 7.0, 3.2 Hz, Hβ-2), 3.61 (*d*, *J* = 3.2 Hz, OH), 2.49 (*dd*, *J* = 12.6, 7.0 Hz, Hβ-1), 2.05 (*m*, H_2-23), 1.69 (*s*, H_3-26), 1.62 (*s*, H_3-27), 1.19 (*s*, H_3-19), 1.15 (*s*, H_3-28), 1.15 (*s*, H_3-21), 1.14, (*dd*, *J* = 12.6, 12.4 Hz, Hα-1), 1.11 (*s*, H_3-29), 1.01 (*s*, H_3-18), 0.85 (*s*, H_3-30); for ^{13}C NMR ($CDCl_3$) spectroscopic data, see Table 2.

Acknowledgements

The authors thank Prof. Shiro Kohshima, Department of Biological Science, Graduate School of Bioscience and Biotechnology, Tokyo Institute of Technology, for botanical identification. We acknowledge Prof. Kazuo Matsuda, Showa College of Pharmaceutical Sciences, through whom we became aware that the ^{13}C shift of C-20 for compound **3** reported in their paper needed a revision.

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