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Resin glycosides from the aerial parts of *Operculina turpethum*

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ABSTRACT

Three glycosidic acids, turpethic acids A–C, and two intact resin glycosides, turpethosides A and B, all having a common pentasaccharide moiety and 12-hydroxy fatty acid aglycones of different chain lengths, were obtained from the aerial parts of *Operculina turpethum*. Their structures were elucidated by spectroscopic analyses and chemical correlations. The aglycones were characterized as 12-hydroxypentadecanoic acid in two compounds, 12-hydroxyhexadecanoic acid in two other components, and 12-hydroxyheptadecanoic acid in the fifth compound, which were all confirmed by synthesis. The absolute configurations of these aglycones were all established as *S* by Mosher's method. These compounds represent the first examples of resin glycosides with a monohydroxylated 12-hydroxy fatty acid as an aglycone, and one compound is the first described resin glycoside having a hydroxylated C_{17} fatty acid as its aglycone.

1. Introduction

Resin glycosides are unique secondary metabolites in the plant kingdom confined to the morning glory family (Convolvulaceae) (Pereda-Miranda et al., 2010; Pereda-Miranda and Bah, 2003). They are the principles responsible for the purgative action of all the important Convolvulaceous species used in traditional medicine throughout the world. They have also been reported to have other various biological activities including being haemolytic, antibacterial, antifungal, plant growth regulatory, and cytotoxic (Dembitsky, 2004; Figueroa-González et al., 2012; Fürstner, 2004; Pereda-Miranda et al., 2006a; Pereda-Miranda and Bah, 2003). To date, hundreds of resin glycosides have been isolated from the Convolvulaceae family. They are composed of differently acylated oligosaccharides glycosidically linked to hydroxylated fatty acids which are usually linked back to the sugar chain to form macrolactone rings of various sizes (Eich, 2008; Pereda-Miranda et al., 2010). The hydroxylated fatty acids are structurally quite conserved among the large number of resin glycosides reported so far. Jalapinolic acid [(*S*)-11-hydroxyhexadecanoic acid] and convolvulinic acid [(*S*)-11-hydroxytetradecanoic acid] are the most frequently found aglycones for the macrocyclic lipooligosaccharide core, although other different hydroxylated fatty acids with chain length of C_{10}, C_{12}, C_{14}, C_{15}, C_{16}, and C_{18} also being reported (Eich, 2008; Pereda-Miranda et al., 2010, 2006b). For the vast majority of resin glycosides, certain positions of the sugar units are further acylated with residues of small organic acids (C_2–C_5, volatile), straight-chain saturated fatty acids (non-volatile), and arylalkyl acids, respectively (Eich, 2008).

The genus *Operculina* (Fam. Convolvulaceae) consists of about 25 species occurring in tropical areas worldwide. *Operculina turpethum* (L.) S. Manso (syn. *Ipomoea turpethum*), a perennial herbaceous vine, is distributed in southern China (Guangdong, Guangxi, Hainan, Taiwan, and Yunnan), Southeast and South Asia (Bangladesh, Cambodia, India, Indonesia, Ryukyu Islands, Laos, Malaysia, Burma, Nepal, New Guinea, Pakistan, Philippines, Sri Lanka, Thailand, and Vietnam), the Pacific Islands, and Australia (Staples, 2007; Wu, 1979). This plant has significant ethnomedicinal values, and its roots, known as "Indian jalap", are commercially available as mild but very effective laxatives. Partly due to the introduction to the world market of jalap and other plants such as Brazilian-grown jalap (*I. operculata*, syn. *O. macrocarpa*), the demand for Mexican jalap root (*I. purga*) has declined (Pereda-Miranda et al., 2010). The roots and stems of *O. turpethum* have traditionally been used in Indian medicine to treat a wide range of ailments, such as tumors, burning diseases, jaundice, paralysis, and pain in the joints and muscles (Ahbuselvam et al., 2007). Furthermore, an *O. turpethum* extract has been used to relieve fevers and to treat anaemia, splenomegaly, raised lipid levels, obesity, gastric ulcer, and related gastrointestinal disturbances (Ahmad et al., 2009; Austin, 1982; Laakshmayya et al., 2006). Recent studies have shown that the methanolic extract of *O. turpethum* stems also has antioxidant activity and a protective effect against

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7,12-dimethylbenz(*a*)anthracene (DMBA)-induced breast cancer in female rats (Ahbuselvam et al., 2007); moreover, the aqueous extract of *O. trupethum* roots provides hepatoprotective and anticlastogenic effects against hepatic fibrosis (Ahmad et al., 2009).

Previously the isolation and hepato-protective activity of four dammarane-type triterpenoid saponins was reported from the aerial parts of this plant (Ding et al., 2011). Wagner et al. (1978) also reported the isolation from the roots of this plant of several resin glycosides which carried a number of dihydroxylated fatty acids, in addition to the common jalapinolic acid, as the aglycones. These dihydroxylated fatty acids include 3,12-dihydroxypentadecanoic acid, 3,12-dihydroxypalmitic acid, 4,12-dihydroxypentadecanoic acid and 4,12-dihydroxypalmitic acid. In continuation of our phytochemical studies on this plant, a group of new resin glycosides (**4** and **5**) and glycosidic acids (**1**–**3**) have been isolated. They are structurally interesting because they are the first examples of resin glycosides having a monohydroxylated 12-hydroxy fatty acid as aglycone and **3** is the first resin glycoside with a hydroxy C_{17} fatty acid as its aglycone.

2. Results and discussion

The EtOH extract of the powdered dry aerial parts of *O. turpethum* was suspended in water and partitioned by successive extractions with petroleum ether, EtOAc, and *n*-BuOH. The petroleum ether-soluble fraction was subjected to silica gel column chromatography (CC) and eluted with petroleum ether–acetone of increasing polarity to afford four fractions (D–G) of resin glycosides. Fraction G was further separated by ODS and Sephadex LH-20 CC followed by preparative HPLC to give two intact resin glycosides, trivially named turpethosides A (**4**) and B (**5**). Fractions D–F were investigated by the same methods, but no pure resin glycosides were obtained.

Since purification of intact resin glycosides is problematic (Pereda-Miranda et al., 2010, 2006b), our research then focused on the glycosidic acids of the resin glycosides in this plant. The unseparated resin glycoside mixtures were combined and treated with 5% KOH. The reaction mixture afforded three glycosidic acids **1**–**3** which were trivially named turpethic acids A–C, respectively.

By analysis of the HRESIMS and NMR spectroscopic data, the molecular formulae of turpethic acids A–C (**1**–**3**) were determined to be $C_{45}H_{80}O_{25}$, $C_{46}H_{82}O_{25}$, and $C_{47}H_{84}O_{25}$, respectively, each differing from the others by one or two CH_2 units. The negative ion ESIMS of **1** exhibited an $[M-H]^-$ ion peak at *m*/*z* 1019 as well as a series of fragment ions at *m*/*z* 873 [1019 – $C_6H_{10}O_4$]$^-$, 857 [1019 – $C_6H_{10}O_5$]$^-$, 711 [1019 – $C_6H_{10}O_4$ – $C_6H_{10}O_5$]$^-$, 565 [711 – $C_6H_{10}O_4$]$^-$, 419 [565 – $C_6H_{10}O_4$]$^-$, and 257 [419 – $C_6H_{10}O_5$]$^-$, obviously generated from the quasi-molecular $[M-H]^-$ ion precursor by sequential loss of five monosaccharide units including three deoxyhexose ($C_6H_{10}O_4$) units and two hexose ($C_6H_{10}O_5$) units to yield the aglycone ion (*m*/*z* 257) in accordance with a hydroxypentadecanoic acid anion. The negative ESIMS of **2** exhibited, besides an $[M-H]^-$ ion peak at *m*/*z* 1033, characteristic fragment ion peaks at *m*/*z* 887 [1033 – $C_6H_{10}O_4$]$^-$, 871 [1033 – $C_6H_{10}O_5$]$^-$, 725 [1033 – $C_6H_{10}O_4$ – $C_6H_{10}O_5$]$^-$, 579 [725 – $C_6H_{10}O_4$]$^-$, 433 [579 – $C_6H_{10}O_4$]$^-$, and 271 [433 – $C_6H_{10}O_5$], which all were 14 mass units (CH_2) more than those of **1**. For turpethic acid C (**3**), its negative ESIMS displayed an $[M-H]^-$ peak at *m*/*z* 1047 and characteristic fragment ions at *m*/*z* 901 [1047 – $C_6H_{10}O_4$]$^-$, 885 [1047 – $C_6H_{10}O_5$]$^-$, 739 [1047 – $C_6H_{10}O_4$ – $C_6H_{10}O_5$]$^-$, 593 [739 – $C_6H_{10}O_4$]$^-$, 447 [593 – $C_6H_{10}O_4$]$^-$ and 285 [447 – $C_6H_{10}O_5$]$^-$, all having 28 mass units ($2 \times CH_2$) more than those of **1**. These data suggested that **1**–**3** are pentasaccharides of monohydroxy fatty acids with chain lengths of C_{15}, C_{16}, and C_{17}, respectively.

The ^{1}H NMR spectra of turpethic acids A–C (**1**–**3**) were almost identical with each other and their ^{13}C NMR spectra were also very similar. Careful comparison of their spectra (Table 1) showed that **1**–**3** had a common oligosaccharide moiety comprised of three 6-deoxyhexose and two hexose residues. Taking turpethic acid B (**2**) as an example, its ^{1}H NMR spectrum exhibited five anomeric proton signals at δ 4.97 (1H, d, J = 7.4, Hz), 5.22 (1H, d, J = 7.8, Hz), 5.89 (1H, br s), 6.20 (1H, br s) and 6.33 (1H, br s) and three secondary methyl resonances at δ 1.56 (3H, d, J = 6.2 Hz), 1.59 (3H, d, J = 6.1 Hz) and 1.69 (3H, d, J = 6.2 Hz), assignable to the CH_3-6 of 6-deoxyhexoses. These structural features were also evident in the ^{13}C NMR spectrum of **2**, which displayed five anomeric carbon signals in the range δ 100.5–105.1. Analysis of COSY and HSQC spectra led to assignments of proton and carbon resonances for each monosaccharide residue in **1**–**3** (Table 1). The oligosaccharide moieties in **1**–**3** were found to be identical with those of operculinic acid B, a known glycosidic acid previously obtained from *Ipomoea operculata* (Ono et al., 1989). The connectivities between the hydroxyfatty acid aglycones (Ag) and sugar chains, and among monosaccharides in **1**–**3** were supported by their HMBC spectra, in which, also using **2** as an example, long range correlations were observed for δ_H 4.97 (H-1, Glc) with δ_C 77.7 (C-12, Ag), δ_H 4.20 (H-2, Glc) with δ_C 101.1 (C-1, Rha), δ_H 6.33 (H-1, Rha) with δ_C 76.9 (C-2, Glc), δ_H 4.22 (H-4, Rha) with δ_C 103.1 (C-1, Rha′), δ_H 5.89 (H-1, Rha′) with δ_C 82.0 (C-4, Rha), δ_H 4.49 (H-4, Rha′) with δ_C 102.7 (C-1, Rha″), δ_H 6.20 (H-1, Rha″) with δ_C 78.3 (C-4, Rha′) and δ_H 5.22 (H-1, Glc′) with δ_C 82.4 (C-3, Rha′). In order to confirm the monosaccharide compositions and absolute configurations, **1**–**3** were subjected to acid hydrolysis. Only two monosaccarides were detected and isolated from the hydrolysates and identified D-glucose and L-rhamnose, respectively, on the basis of their specific optical rotation and TLC comparison with authentic samples. Thus, the oligosaccharide moieties in **1**–**3** were all established to be β-D-Glc-(1→3)-[α-L-Rha-(1→4)]-α-L-Rha-(1→4)-α-L-Rha-(1→2)-β-D-Glc.

Obviously, the difference between turpethic acids A–C (**1**–**3**) lie in their aglycones, the hydroxylated fatty acids. On mild acid hydrolysis, turpethic acids A–C (**1**–**3**) afforded hydroxylated fatty acids **6**–**8**, respectively. ESIMS of **6** gave quasi-molecular ions at *m*/*z* 281 $[M+Na]^+$ and 259 $[M+H]^+$ in positive ion mode and *m*/*z* 257 $[M-H]^-$ in negative ion mode. Its ^{13}C NMR spectrum (Table 2) exhibited signals indicating the presence of 15 carbons including a carboxyl and an oxygenated methine, indicating a monohydroxylated pentadecanoic acid. In the HMBC spectrum of **6**, a long range correlation was observed from the terminal methyl proton signal at δ_H 0.95 (H-15) to the carbon resonance at δ_C 40.2, which was assignable to the methylene carbon adjacent to the oxygenated methine group, suggesting that the hydroxy group was located at C-12 of the fatty acid. Thus, aglycone **6** was identified as 12-hydroxypentadecanoic acid. In order to confirm this structure, compound **6** was converted to its methyl ester **9**-(*S*) and 12-hydroxypentadecanoic acid methyl ester in its racemic form (**9**) was synthesized starting from 12-hydroxydodecanoic acid (**12**) (Scheme 1). Indistinguishable ^{1}H and ^{13}C NMR spectra between **9**-(*S*) and **9** were observed and unambiguously confirmed that the hydroxy substituent in **6** was at C-12.

The 1D NMR spectroscopic and ESIMS data indicated that **7** and **8** were monohydroxylated hexadecanoic acid and monohydroxylated heptadecanoic acid, respectively, in accordance with deductions from the ESIMS data of **2** and **3**. By comparison of the ^{13}C NMR spectroscopic data of **7** and **8** (Table 2) with those reported for the commonly encountered jalapinolic acid (Pereda-Miranda et al., 2006b), small but evident differences in the chemical shift values of two methylene carbons adjacent to the oxymethine carbon were found between **7** (δ_C 37.4 and 37.1) and jalapinolic acid (δ_C 37.5 and 37.4), whereas the values of these two carbons (both at δ_C 37.5) in **8** were almost identical with those of jalapinolic acid. Careful analysis of the HSQC and HMBC spectra led to assignment

Table 1
^{1}H (600 MHz) and ^{13}C NMR (150 MHz) data of **1**–**3** in C_5D_5N.

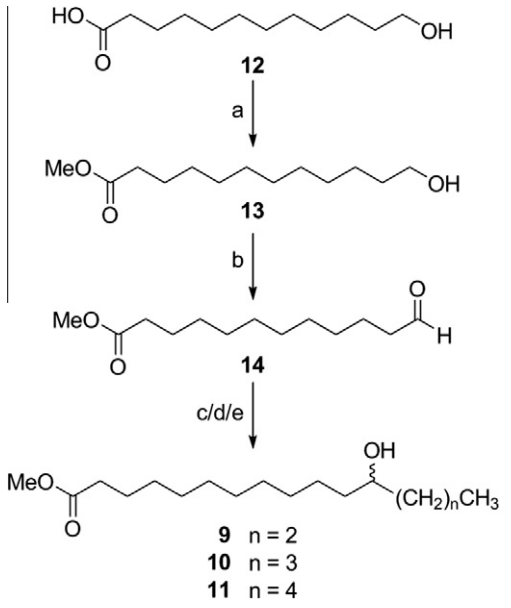
Position	**1**		**2**		**3**	
	δ_H (J in Hz)	δ_C	δ_H (J in Hz)	δ_C	δ_H (J in Hz)	δ_C
Glc-1	4.96 d (7.5)	100.5	4.97 d (7.4)	100.5	4.98 d (7.3)	100.6
2	4.20 m	77.0	4.20 dd (9.1, 7.4)	76.9	4.22 dd (9.2, 7.3)	77.0
3	4.25 m	79.2	4.23 m	79.2	4.24 m	79.2
4	4.14 t (9.1)	71.6	4.14 t (9.0)	71.4	4.14 t (9.2)	71.4
5	3.88 ddd (9.1, 5.0, 2.5)	77.7	3.89 ddd (9.0, 5.0, 2.5)	77.7	3.89 ddd (9.2, 5.0, 2.6)	77.7
6	4.34 dd (11.7, 5.0)	62.5	4.34 dd (11.7, 5.0)	62.5	4.34 dd (11.7, 5.0)	62.5
	4.52 br d (11.7)		4.52 br d (11.7)		4.53 br d (11.7)	
Glc′-1	5.21 d (7.7)	105.2	5.22 d (7.8)	105.1	5.22 d (7.8)	105.1
2	3.96 dd (9.1, 7.7)	74.7	3.96 dd (9.0, 7.8)	74.7	3.96 dd (9.0, 7.8)	74.9
3	4.18 dd (9.1, 9.1)	78.0	4.17 m	78.1	4.18 dd (9.0, 9.0)	78.1
4	4.09 t (9.1)	71.4	4.10 t (9.1)	71.6	4.11 t (9.0)	71.6
5	3.94 ddd (9.1, 5.1, 2.2)	78.1	3.94 ddd (9.1, 6.0, 2.7)	78.1	3.94 ddd (9.0, 5.0, 2.3)	78.1
6	4.25 m	62.6	4.26 m	62.6	4.27 m	62.6
	4.49 m		4.49 m		4.50 m	
Rha-1	6.32 br s	101.2	6.33 br s	101.1	6.34 br s	101.2
2	4.65 dd (3.1, 1.1)	72.3	4.66 br d (3.4)	72.2	4.66 br d (3.5)	72.3
3	4.60 dd (9.3, 3.1)	72.2	4.61 dd (9.2, 3.4)	72.3	4.61 dd (9.3, 3.4)	72.2
4	4.24 m	81.4	4.22 m	82.0	4.26 m	82.0
5	4.89 m	67.4	4.90 m	67.3	4.91 m	67.4
6	1.68 d (6.2)	18.4	1.69 d (6.2)	18.5	1.70 d (6.2)	18.5
Rha′-1	5.85 br s	103.2	5.89 br s	103.1	5.89 br s	103.1
2	5.18 br s	71.8	5.18 br s	71.8	5.18 br s	71.8
3	4.72 dd (8.9, 2.8)	82.5	4.72 dd (8.9, 2.8)	82.4	4.72 dd (8.8, 2.8)	82.4
4	4.49 m	78.3	4.49 m	78.3	4.49 m	78.3
5	4.40 m	68.2	4.40 m	68.2	4.40 m	68.2
6	1.59 d (6.1)	18.4	1.59 d (6.1)	18.6	1.60 d (6.2)	18.6
Rha″-1	6.20 br s	102.7	6.20 br s	102.7	6.20 br s	102.7
2	4.88 br s	72.2	4.87 br s	72.2	4.87 br d (3.4)	72.3
3	4.42 dd (9.0, 3.3)	72.4	4.42 dd (8.8,3.3)	72.4	4.42 dd (9.1, 3.5)	72.4
4	4.22 m	73.6	4.20 m	73.6	4.21 m	73.6
5	4.29 m	70.0	4.30 m	70.1	4.29 m	70.1
6	1.56 d (6.2)	18.0	1.56 d (6.2)	18.0	1.56 d (6.2)	18.0
Ag-1		176.6		175.9		175.9
2	2.49 t (6.7)	35.0	2.49 t (7.4)	34.8	2.49 t (7.4)	34.7
3	1.77 m	25.5	1.77 m	25.4	1.77 m	25.4
10	1.59 m	25.2	1.61 m	25.3	1.61 m	25.3
11	1.83 m	33.7	1.83 m	33.6	1.85 m	33.7
12	4.03 m	77.4	4.04 m	77.7	4.05 m	77.7
13	1.72 m, 1.68 m	37.3	1.73 m	34.7	1.75 m	35.1
14	1.58 m	18.6	1.57 m, 1.48 m	27.3	1.61 m, 1.51 m	24.9
15	0.93 t (7.2)	14.2	1.34 m	22.9	1.29 m	32.1
16			0.92 t (7.3)	14.0	1.31 m	22.7
17					0.89 t (6.9)	14.0

Table 2
^{13}C NMR spectroscopic data of **6–8** in C_5D_5N and **9**-(*S*)–**11**-(*S*) in $CDCl_3$.

Position	**6**	**7**	**8**	**9**-(*S*)	**10**-(*S*)	**11**-(*S*)
1	175.8	175.7	175.7	174.3	174.4	174.4
2	34.6	34.6	34.6	34.1	34.1	34.1
3	25.3	25.3	25.3	24.9	24.9	24.9
4–9	29.8–29.2	29.9–29.3	29.9–29.3	29.6–29.1	29.6–29.1	29.6–29.1
10	25.9	26.1	26.1	25.6	25.6	25.6
11	38.1	38.2[a]	38.1[a]	37.4	37.4[a]	37.4
12	70.3	70.6	70.3	71.7	72.0	72.0
13	40.2	37.8[a]	38.2[a]	39.6	37.1[a]	37.4
14	19.1	28.2	25.7	18.8	27.8	25.3
15	14.1	22.9	32.1	14.1	22.7	31.9
16		14.0	22.7		14.0	22.6
17			13.9			14.0
OMe				51.4	51.4	51.4

[a] Assignments interchangeable in the same column.

of the ^{13}C NMR spectroscopic data of **6**–**8** (Table 2) and indicated that the chemical shifts of C-1–C-11 for **7** and **8** were very similar to those for **6**. These findings suggested that the hydroxy substituents in both **7** and **8** were also located at the C-12 position. Similarly, the structures of **7** and **8** were finally confirmed by direct comparison of the spectroscopic data of their methyl esters **10**-(*S*) and **11**-(*S*) with those of their synthetic counterparts (**10** and **11**) which were synthesized as racemic forms (Scheme 1).



Scheme 1. Synthesis of 12-hydroxy fatty acid methyl esters **9**–**11**. *Reagents and conditions:* (a) CH_3OH, H_2SO_4; (b) IBX (5.0 equiv), DMSO, RT; (c) *n*-C_3H_7MgBr, THF, RT; (d) *n*-C_4H_9MgBr, THF, RT; (e) *n*-$C_5H_{11}MgBr$, THF, RT.

The configurations at C-12 of **6**–**8** were determined by Mosher's method (Dale and Mosher, 1973). Hydroxyfatty acid methyl esters **9**-(*S*)–**11**-(*S*) were treated with (*R*)-α-methoxy-α-phenylacetic acid (*R*-MPA) and *S*-MPA to afford three pairs of Mosher esters **16**-(*S*,*R*)/**16**-(*S*,*S*), **17**-(*S*,*R*)/**17**-(*S*,*S*), and **18**-(*S*,*R*)/**18**-(*S*,*S*), respectively. The ^{1}H NMR spectroscopic data of each ester were recorded and assigned (see Experimental) with the aid of their COSY spectra The chemical shift differences ($\Delta\delta = \delta_R - \delta_S$) of those protons around the stereogenic center of each pair of Mosher esters were then analyzed (Table 3). A positive $\Delta\delta$ value for H-10 and H-11 and a negative $\Delta\delta$ for H-13, H-14, H-15, H-16, and/or H-17 denoted an *S*-configuration at C-12 (Cao et al., 2005; Yin et al., 2008). In particular, significant chemical shift differences ($\Delta\delta$) were observed between the corresponding terminal methyl groups which were readily identifiable. A $\Delta\delta$ value of –0.16 ppm between the terminal methyl groups in both **16** and **17**, and –0.09 ppm in **18**, confirmed a 12*S*-absolute configuration according to the configurational model proposed by Kakisawa and coworkers (Ohtani et al., 1991). The $\Delta\delta$ of –0.09 ppm between the terminal methyl signals in **18** was also consistent with previously reported $\Delta\delta$ values (–0.07 to –0.035 ppm) for the corresponding methyl signals which are separated by as many as four methylene groups (Ono et al., 1993; Yin et al., 2008). Furthermore, the specific optical rotations for all free acids **6**–**8** and their methyl esters **9**-(*S*)–**11**-(*S*) had positive values, which is in accordance with the observation that monohydroxylated fatty acids with a dextrorotatory optical activity have an (*S*)-absolute configuration while their laevoisomers have the opposite (*R*)-configuration (Chérigo et al., 2008). Thus, the absolute configurations of **6**–**8** were determined to be *S* (Fig. 1).

Therefore, the structures of turpethic acids A–C were established as (*S*)-12-hydroxypentadecanoic acid 12-*O*-β-D-glucopyranosyl-(1→3)-*O*-[α-L-rhamnopyranosyl-(1→4)]-*O*-α-L-rhamnopyranosyl-(1→4)-*O*-α-L-rhamnopyranosyl-(1→2)-β-D-glucopyranoside (**1**), (*S*)-12-hydroxyhexadecanoic acid 12-*O*-β-D-glucopyranosyl-(1→3)-*O*-[α-L-rhamnopyranosyl-(1→4)]-*O*-α-L-rhamnopyranosyl-(1→4)-*O*-α-L-rhamnopyranosyl-(1→2)-β-D-glucopyranoside (**2**) and (*S*)-12-hydroxyheptadecanoic acid 12-*O*-β-D-glucopyranosyl-(1→3)-*O*-[α-L-rhamnopyranosyl-(1→4)]-*O*-α-L-rhamnopyranosyl-(1→4)-*O*-α-L-rhamnopyranosyl-(1→2)-β-D-glucopyranoside (**3**), respectively (Fig. 1).

Assignments of the NMR spectroscopic data for hydroxylated long chain fatty acids are quite challenging due to signal overlap, which makes it difficult to determine their structures based on

Table 3
^{1}H NMR $\Delta\delta$ values of *R*/*S* Mosher's esters **16**–**18**.

Compound	$\Delta\delta$ ($\delta_R - \delta_S$, in ppm)						
	H-10	H-11	H-13	H-14	H-15	H-16	H-17
16	0.32	0.13	−0.11	−0.28	−0.16		
17	0.32	0.13	−0.13	−0.39	−0.18	−0.16	
18	0.32	0.12	−0.12	−0.30	−0.24	−0.15	−0.09

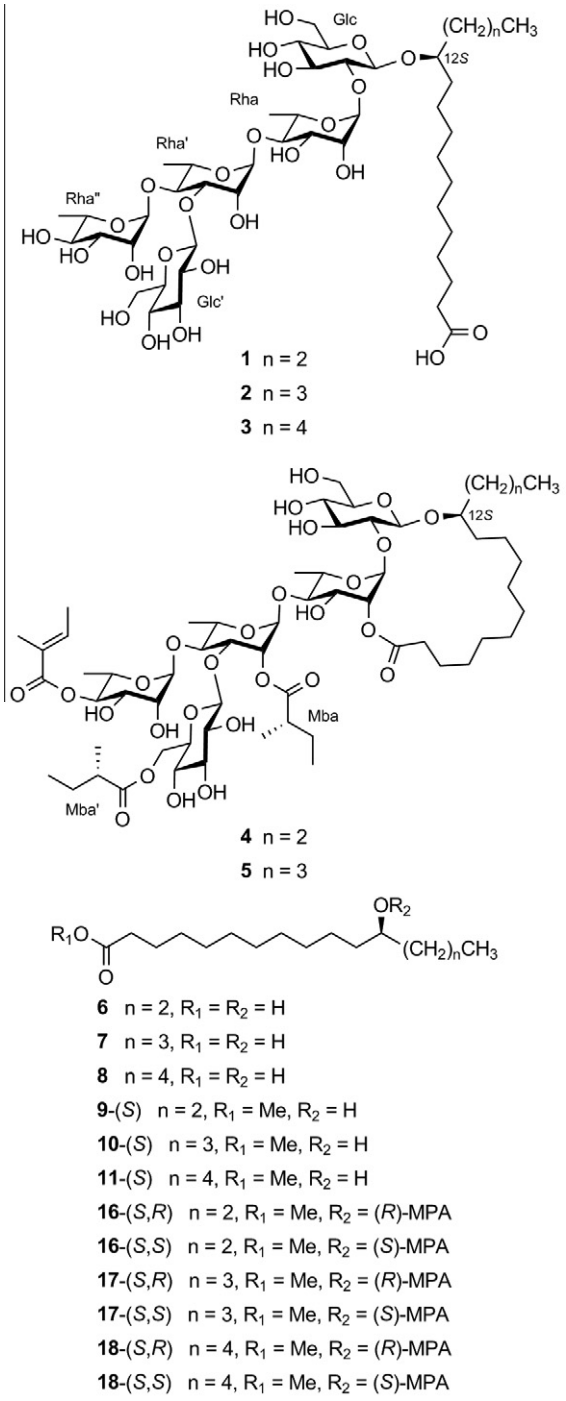


Fig. 1. Structural formulae of compounds **1**–**18**.

spectroscopic data alone. During the course of this study, with synthetic authentic samples, it was possible to make a full assignment of all carbon signals, except C-4–C-9, for the 12-hydroxy fatty acids **6**–**8** and their corresponding methyl esters **9**–**11**, based on their HSQC and HMBC data (Table 2). It was found that the chemical shifts of C-11 and C-13, adjacent to the hydroxylated methine carbon (C-12), were quite distinct in 12-hydroxypentadecanoic acid derivatives [δ_C 38.1 (C-11) and 40.2 (C-13) in **6**; δ_C 37.4 (C-11) and 39.6 (C-13) in **9**]. As the chain length increased, the absolute value of the chemical shift difference between C-11 and C-13 ($\Delta\delta = |\delta_{C\text{-}13} - \delta_{C\text{-}11}|$) became smaller [$\delta_C$ 38.2 and 37.8 (C-11, C-13) in **7**; δ_C 37.4 and 37.1 (C-11, C-13) in **10**; δ_C 38.1 and 38.2 (C-11, C-13) in **8**; δ_C 37.4 and 37.4 (C-11, C-13) in **11**] (Table 2 and Fig. 2). In fact, C-11 and C-13 collapsed into a single signal in 12-hydroxyheptadecanoic acid methyl ester **11** (Fig. 2). In order to demonstrate that such spectroscopic features also apply to 11-hydroxylated fatty acids, 11-hydroxyheptadecanoic acid methyl ester (**15**) were synthesized according to a known procedure (Jiang et al., 1995). Indeed, the two carbon atoms (C-10 and C-12) adjacent to the hydroxylated methine gave rise to only one signal in its ^{13}C NMR spectrum (Fig. 2). This trend was found to be true also for the carbon resonances reported for (*S*)-11-hydroxyhexadecanoic acid methyl ester [δ_C 37.5 (C-10 or C-12)/37.4 (C-12 or C-10), with a $\Delta\delta = 0.1$ ppm] and (*S*)-11-hydroxytetradecanoic acid methyl ester [δ_C 37.5 (C-10)/39.6 (C-12), with a $\Delta\delta = 2.1$ ppm] (Pereda-Miranda et al., 2006b). It seems that for both 11-hydroxylated and 12-hydroxylated fatty acids and their derivatives, the chemical shifts of the carbon atoms adjacent to the hydroxylated methine tend to concur as the chain length grows, and eventually become very close ($\Delta\delta < 0.1$ ppm) or identical when the hydroxylated methine is separated by at least four CH_2 units from the terminal methyl group. Such ^{13}C NMR spectroscopic characteristics may apply also to other monohydroxylated long chain fatty acids and their derivatives.

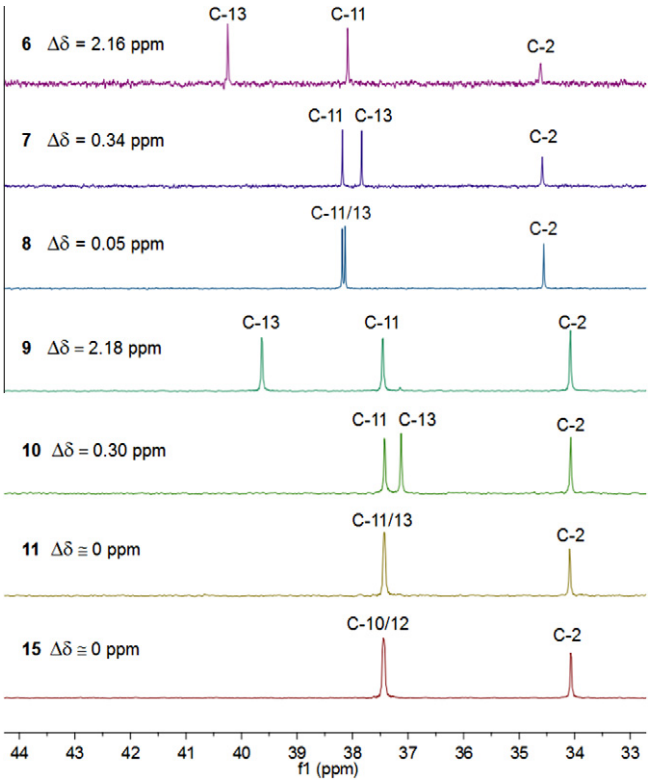


Fig. 2. ^{13}C NMR spectra of **6**–**8** in C_5D_5N and **9**–**11** and **15** in $CDCl_3$ showing chemical shifts of the carbons adjacent to the hydroxylated methine carbon.

Turpethoside A (**4**) gave a quasi-molecular ion peak at *m*/*z* 1287.6097 $[M+Cl]^-$ in its HRESIMS, corresponding to a molecular formula of $C_{60}H_{100}O_{27}$. Its ^{1}H and ^{13}C NMR spectra (Table 4) displayed characteristic signals for a glycosidic acid core closely similar to that turpethic acids **1**–**3**, as well as additional resonances indicating the presence of three short organic acid moieties. The signals readily distinguished for an olefinic methine at δ_H 6.95 (m), an olefinic tertiary methyl group at δ_H 1.80 (br s) and an olefinic secondary methyl group at δ_H 1.57 (dd, J = 7.1, 1.1 Hz) in the ^{1}H NMR spectrum, and resonances for a conjugated ester carbonyl at δ_C 167.6, an olefinic methine at δ_C 137.3, an olefinic quaternary

Table 4
^{1}H (600 MHz) and ^{13}C NMR (150 MHz) spectroscopic data of **4** and **5** in C_5D_5N.

Position	**4**		**5**	
	δ_H (J in Hz)	δ_C	δ_H (J in Hz)	δ_C
Glc-1	4.81 d (7.6)	104.2	4.82 d (7.5)	104.3
2	3.91 dd (9.1, 7.6)	81.4	3.91 dd (9.1, 7.5)	81.4
3	4.10 m	76.7	4.11 m	76.7
4	4.12 m	71.8	4.12 m	71.8
5	3.83 m	77.8	3.83 m	77.8
6	4.30 dd (11.7, 6.4)	62.7	4.30 dd (11.3, 6.2)	62.7
	4.45 br d (11.7)		4.45 br d (11.3)	
Glc′-1	5.07 d (7.7)	105.1	5.07 d (7.7)	105.1
2	3.94 dd (9.0, 7.7)	75.0	3.94 dd (9.0, 7.7)	75.0
3	4.03 t (9.0)	78.3	4.03 t (9.0)	78.3
4	3.96 m	70.7	3.96 m	70.7
5	3.71 m	75.1	3.71 m	75.1
6	4.50 dd (11.6, 4.9)	63.6	4.49 dd (11.5, 4.9)	63.6
	4.94 dd (11.6, 1.8)		4.93 br d (11.5)	
Rha-1	5.57 br s	98.5	5.57 br s	98.6
2	6.11 br s	73.8	6.11 br s	73.8
3	5.19 br d (9.6)	69.8	5.19 br d (9.6)	69.8
4	4.26 t (9.6)	79.0	4.26 t (9.6)	79.1
5	4.43 m	68.7	4.43 m	68.7
6	1.62 d (6.2)	19.6	1.63 d (6.2)	19.6
Rha′-1	6.19 br s	98.8	6.19 br s	98.8
2	5.98 dd (3.3, 1.8)	73.0	5.98 br s	73.0
3	4.72 dd (9.1, 3.3)	79.5	4.72 dd (9.1, 3.3)	79.5
4	4.33 t (9.3)	79.1	4.33 t (9.3)	79.1
5	4.40 m	68.0	4.41 m	68.0
6	1.71 d (6.1)	18.6	1.71 d (6.1)	18.7
Rha″-1	6.22 br s	103.2	6.21 br s	103.2
2	4.97 br s	72.3	4.97 br s	72.3
3	4.60 dd (8.9, 3.4)	70.3	4.60 dd (9.0, 3.3)	70.3
4	5.84 t (9.2)	75.6	5.84 t (9.0)	75.6
5	4.42 m	68.7	4.43 m	68.2
6	1.41 d (6.3)	17.9	1.41 d (6.3)	17.9
Ag-1		173.1		173.1
2	2.22 m, 2.36 m	34.6	2.22 m, 2.37 m	34.6
11	1.58 m, 1.64 m	34.3	1.62 m, 1.66 m	34.3
12	3.82 m	82.0	3.82 m	82.4
13	1.61 m, 1.88 m	37.3	1.66 m, 1.94 m	34.9
14	1.73 m	18.6	1.30 m	27.7
15	0.82 t (7.5)	14.3	1.22 m	23.1
16			0.82 t (7.4)	14.1
Mba-1		175.1		175.1
2	2.38 m	41.0	2.38 m	41.1
3	1.41 m, 1.64 m	26.8	1.41 m, 1.64 m	26.8
4	0.79 t (7.5)	11.3	0.80 t (7.5)	11.4
2-Me	1.03 d (7.0)	16.4	1.03 d (7.0)	16.4
Mba′-1		176.5		176.5
2	2.50 m	41.1	2.50 m	41.1
3	1.50 m, 1.73 m	26.8	1.50 m, 1.73 m	26.8
4	0.88 t (7.5)	11.7	0.88 t (7.4)	11.7
2-Me	1.24 d (7.0)	16.6	1.24 d (7.0)	16.6
Tga-1		167.6		167.6
2		128.9		129.0
3	6.95 m	137.3	6.95 m	137.3
4	1.57 dd (7.1, 1.1)	14.0	1.57 br d (7.1)	14.0
2-Me	1.81 br s	12.1	1.80 br s	12.2

carbon at δ_C 128.9 and two methyl carbons at δ_C 12.1 and 14.0 in the ^{13}C NMR spectrum which were characteristic of a tigloyl group (Mills et al., 2005). Additional ^1H NMR signals due to short organic acid moieties were also observed for four methyl groups including two primary methyl at δ_H 0.88 and 0.79 (each t, J = 7.5 Hz) and two secondary methyls at δ_H 1.24 and 1.03 (each d, J = 7.0 Hz). Further analysis of these methyl signals in the COSY spectrum indicated the presence of two 2-butyl groups. This, in combination with the presence of four ester carbonyl carbon resonances at δ_C 176.5–167.6 in the ^{13}C NMR spectrum, including those for tigloyl group and the hydroxylated fatty acid aglycone, indicated the presence of two 2-methylbutyric acid (Mba) moieties. Compound **4** was then subjected to saponification with 5% KOH and the products fractionated into organic and glycosidic acid fractions. The former examined by GC–MS gave two peaks corresponding to tiglic acid (Tga) and 2-methylbutyric acid by direct comparison with authentic samples. The absolute configuration of Mba was confirmed to be *S* by GC analysis using a chiral capillary column according to the previously reported method (Noda and Horiuchi, 2008; Gaspar and Barroso, 2006). The glycosidic acid fraction furnished turpethic acid A (**1**), thus confirming the structure of the glycosidic acid core of **4**.

The negative ESIMS of **4** exhibited, besides the $[M–H]^-$ ion at *m/z* 1251, fragment ions at *m/z* 1151 $[1251 – C_5H_8O_2 (Tga)]^-$, 1149 $[1251 – C_5H_{10}O_2 (Mba)]^-$, 1049 $[1251 – C_5H_8O_2 – C_5H_{10}O_2]^-$, 1047 $[1251 – 2 \times C_5H_{10}O_2]^-$ and 947 $[1251 – C_5H_8O_2 – 2 \times C_5H_{10}O_2]^-$ due to loss of the short organic acids. Furthermore, the negative ion ESIMS displayed a series of diagnostic fragment ions (Noda et al., 1988) at *m/z* 1023 $[1251 – C_{11}H_{16}O_5$ (*O*-tigloylrhamnose unit)$]^-$, 1005 $[1251 – C_{11}H_{18}O_6$ (*O*-methylbutyrylglucose unit)$]^-$, 547 $[C_{27}H_{48}O_{11}$ (hydroxypentadecanoic acid *O*-rhamnosylglucoside, intramolecular ester) – $H]^-$ and 419 $[C_{21}H_{40}O_8$ (hydroxypentadecanoic acid *O*-glucoside) – $H]^-$, arising from glycosidic cleavages, which suggested ester linkages of Tga to Rha″, Mba to Rha′, Mba′ to Glc′, and 12-hydroxypentadecanoic acid to Rha. The exact *O*-acylated positions in the monosaccharide moieties were established by detailed analysis of the 2D NMR spectra of **4**, in particular the HMBC spectrum. By analysis of the COSY and HSQC spectra, the proton signals and corresponding carbon signals for the five individual monosaccharides were assigned (Table 4). It was found that the proton resonances for H-2 of Rha (δ_H 6.11), H-2 of Rha′ (δ_H 5.98), H-4 of Rha″ (δ_H 5.84), and H_2-6 of Glc′ (δ_H 4.94 and 4.50) were shifted downfield relative to those in **1** (Table 1), indicating esterification of these positions. Subsequently, long range correlation between these protons and the ester carbonyl carbons were examined in the HMBC spectrum. As a result, correlations were observed from H-2 of Rha, H-2 of Rha′, H-4 of Rha″, and H_2-6 of Glc′ to carbonyls (C-1) of the aglycone (Ag) (δ_C 173.1), Mba (δ_C 175.1), Tga (δ_C 167.6) and Mba′ (δ_C 176.5), respectively. These observations indicated that the carboxyl group of (*S*)-12-hydroxypentadecanoic acid was linked to C-2 of Rha to form a macrolactone ring, Mba and Mba′ moieties were attached at C-2 of Rha′ and C-6 of Glc′, respectively, and a Tga moiety was located at C-4 of Rha″. Therefore, the structure of turpethoside A (**4**) was elucidated as (*S*)-12-hydroxypentadecanoic acid 12-*O*-(6-*O*-(*S*)-2-methylbutyryl)-*β*-D-glucopyranosyl-(1→3)-*O*-[4-*O*-tigloyl-α-L-rhamnopyranosyl-(1→4)]-*O*-[2-*O*-(*S*)-2-methylbutyryl]-α-L-rhamnopyranosyl-(1→4)-*O*-α-L-rhamnopyranosyl-(1→2)-*β*-D-glucopyranoside, intramolecular 1,2″-ester.

Turpethoside B (**5**) had a molecular formula of $C_{61}H_{102}O_{27}$, differing from **4** by an additional CH_2 unit, as determined from a quasi-molecular ion at *m/z* 1325.6747 $[M+AcOH–H]^-$ in the negative ion HRESIMS. Its ^1H and ^{13}C NMR spectra (Table 4) were very similar to those of **4**, except for signals due to the hydroxylated fatty acid moiety. The negative ion ESIMS of **5** showed the same fragmentation pattern as that of **4** and provided characteristic fragment ions (see Experimental) all having 14 mass units (CH_2) more than those of **4**. Alkaline hydrolysis of **5** afforded turpethic acid B (**2**), indicating that **5** has a glycosidic acid core identical to that of **2**. The presence of two *S*-Mba and a Tga moieties in **5** was also supported by GC–MS and chiral GC analyses. Therefore, the structure of turpethoside B was elucidated as (*S*)-12-hydroxyhexadecanoic acid 12-*O*-(6-*O*-(*S*)-2-methylbutyryl)-*β*-D-glucopyranosyl-(1→3)-*O*-[4-*O*-tigloyl-α-L-rhamnopyranosyl-(1→4)]-*O*-[2-*O*-(*S*)-2- methylbuty-ryl]-α-L-rhamnopyranosyl-(1→4)-*O*-α-L-rhamnopyranosyl-(1→2)-*β*-D-glucopyranoside, intramolecular 1, 2″-ester (**5**).

3. Conclusions

A group of resin glycosides with 12-hydroxy C_{15}–C_{17} fatty acid as aglycones, turpethic acids A–C (**1**–**3**) and turpethosides A and B (**4** and **5**), were obtained from the aerial parts of *O. turpethum* growing in southern China. Their structures were unambiguously assigned by spectroscopic analysis and chemical correlations. 12-Hydroxylated fatty acids are rarely found as aglycones of resin glycosides. Ricinoleic acid (12-hydroxy-9-*cis*-octadecenoic acid) was once reported to be an aglycone for ipopurpuroside, a resin glycoside from *Ipomoea purpurea* (Nikolin et al., 1978), but the structure of this resin glycoside was not established. 3,12- and 4,12-dihydroxylated fatty acids were also reported as aglycones of resin glycosides from the same plant (Wagner et al., 1978) and other species (Çaliş et al., 2007; Ono et al., 2009). However, monohydroxylated 12-hydroxy fatty acids are being reported for the first time in the present study as aglycones of resin glycosides. In addition, the aglycone of **3** is a hydroxylated C_{17} fatty acid, which is unprecedented for resin glycosides. These findings represent an important contribution to the structural diversity of these intriguing glycolipid conjugates. Furthermore, by analysis of ^{13}C NMR spectroscopic data of 12-hydroxy C_{15}–C_{17} fatty acids and their methyl esters, it has been shown that the difference ($\Delta\delta$) of ^{13}C NMR chemical shifts between two methylene carbons adjacent to the hydroxylated methine carbon, which were readily distinguished from other methylene carbons by their downfield shifts (δ_C > 37 ppm), was correlated with the position of the hydroxy group in the fatty acid chain. This finding provides an empirical ^{13}C NMR spectroscopic method for the facile location of hydroxy groups in hydroxy fatty acids.

On the other hand, a previous investigation on Indian jalap resins demonstrated that the aglycones of resin glycosides in this species were rare 3,12- and 4,12-dihydroxylated C_{15} and C_{16} fatty acids, in addition to the common jalapinolic acid (Wagner et al., 1978). However, resin glycosides with these fatty acid aglycones, including the jalapinolic acid aglycone, were not obtained in the present study. Instead, the aglycones of resin glycosides in this plant growing in southern China were found to be another group of rare hydroxy fatty acids, monohydroxylated 12-hydroxy C_{15}–C_{17} fatty acids. These suggest that the fatty acid aglycones of resin glycosides in this taxon are peculiar, diverse, and possibly habitat-specific.

4. Experimental

4.1. General experimental procedures

Optical rotations were obtained on a Perkin-Elmer 341 polarimeter with MeOH as solvent. The ^1H, ^{13}C, and 2D NMR spectra were recorded on a Bruker Avance-600 or a Bruker DRX-400 instrument using TMS as an internal standard. HRESIMS data were obtained on an API QSTAR mass spectrometer. ESIMS were collected on an MDS SCIEX API 2000 LC/MS instrument. EIMS were aquired on a

Shimadzu GCMS-QP2000Plus apparatus with ionization energy of 70 eV. Preparative HPLC was performed with a Shimadzu LC-6A pump and a Shimadzu RID-10A refractive index detector using a YMC-Pack ODS-A column (5 μm, 250 × 20 mm). For column chromatography (CC), silica gel 60 (100–200 mesh, Qingdao Marine Chemical Ltd., Qingdao, China), Develosil ODS (75 μm, Nomura Chemical Co. Ltd., Osaka, Japan), and Sephadex LH-20 (GE Healthcare, Uppsala, Sweden) were used.

4.2. Plant material

Aerial parts of *O. turpethum* were collected from Hengqin island, Zhuhai, Guangdong, China, in October 2008, and authenticated by Prof. Fuwu Xing, South China Botanical Garden, Chinese Academy of Sciences. A voucher specimen (743902) has been deposited at the Herbarium of South China Botanical Garden, Chinese Academy of Sciences.

4.3. Extraction and isolation

Powdered, air-dried, aerial parts of *O. turpethum* (8.0 kg) were extracted with EtOH–H_2O (3 × 20 L, 95:5, v/v, 48 h each) at room temperature and the EtOH solution was concentrated under reduced pressure to give a residue (1.1 kg). This latter was suspended in H_2O and sequentially extracted with petroleum ether (60–90 °C), EtOAc, and *n*-BuOH. The petroleum ether layer was concentrated in vacuo to yield a petroleum ether-soluble fraction (148.0 g). This was subjected to silica gel CC eluted with petroleum ether (60–90 °C)–acetone mixtures of increasing polarity (10:0 to 5:5), to yield ten fractions (A–J). Fraction G (6.3 g), obtained on elution with petroleum ether–acetone (6:4), was further subjected to ODS CC, using MeOH–H_2O mixtures of decreasing polarities (80:20 to 95:5) to give four subfractions (G1–G4). Subfraction G3 (276 mg) was further separated by Sephadex LH-20 CC using MeOH followed by preparative HPLC using MeOH–H_2O (85:15, v/v) with a flow-rate of 5 mL/min to afford turpethoside A (**4**) (7 mg, t_R = 50 min) and turpethoside B (**5**) (16 mg, t_R = 70 min). Compound **4** was obtained pure after a single HPLC separation while compound **5** was purified using peak shaving (Pereda-Miranda and Hernández-Carlos, 2002) by manually recycling the peak three times.

Fractions D–F were then combined and a portion (800 mg) of the combined mass (41.6 g) was treated with 5% KOH at 85 °C for 4 h. The reaction mixture was acidified to pH 4.0 and extracted with Et_2O (50 mL × 3). The aqueous layer was further extracted with *n*-BuOH (3 × 100 mL) and the *n*-BuOH layer was concentrated in vacuo to yield a residue (540 mg) which was subjected to Sephadex LH-20 CC using MeOH to afford a glycosidic acid fraction (400 mg). A portion (100 mg) of this fraction was subjected to preparative HPLC using CH_3OH–H_2O (6:1) with a flow-rate of 5 mL/min to obtain turpethic acids A (**1**) (28 mg, t_R = 25 min), B (**2**) (36 mg, t_R = 35 min), and C (**3**) (22 mg, t_R = 53 min).

4.4. Turpethic acid A (*1*)

White powder, $[\alpha]_D^{20}$ −65.0 (*c* 1.5, MeOH); for ^1H (600 MHz, C_5D_5N) and ^{13}C (150 MHz, C_5D_5N) NMR spectroscopic data, see Table 1; positive ion ESIMS *m/z*: 1,043 $[M+Na]^+$; negative ESIMS *m/z*: 1019 $[M-H]^-$, 873, 857, 711, 565, 419, and 257; negative ion HRESIMS *m/z*: 1019.4912 $[M-H]^-$ (calcd for $C_{45}H_{79}O_{25}$, 1019.4916).

4.5. Turpethic acid B (*2*)

White powder, $[\alpha]_D^{20}$ −72.9 (*c* 1.7, MeOH); for ^1H (600 MHz, C_5D_5N) and ^{13}C (150 MHz, C_5D_5N) NMR spectroscopic data, see Table 1; positive ion ESIMS *m/z*: 1057 $[M+Na]^+$; negative ion ESIMS *m/z*: 1069 $[M+Cl]^-$, 1033 $[M-H]^-$, 887, 871, 725, 579, 433, and 271; HRESIMS *m/z*: 1033.50537 $[M-H]^-$ (calcd for $C_{46}H_{81}O_{25}$, 1033.5072).

4.6. Turpethic acid C (*3*)

White powder, $[\alpha]_D^{20}$ −69.4 (*c* 1.9, MeOH); for ^1H (600 MHz, C_5D_5N) and ^{13}C (150 MHz, C_5D_5N) NMR spectroscopic data, see Table 1; positive ion ESIMS *m/z*: 1071 $[M+Na]^+$; negative ion ESIMS *m/z*: 1083 $[M+Cl]^-$, 1047 $[M-H]^-$, 901, 885, 739, 593, 447, and 285; HRESIMS *m/z*: 1047.52173 $[M-H]^-$ (calcd for $C_{47}H_{83}O_{25}$, 1047.5229).

4.7. Turpethoside A (*4*)

Colorless syrup, $[\alpha]_D^{20}$ −0.28 (*c* 0.19, MeOH); for ^1H (600 MHz, C_5D_5N) and ^{13}C (150 MHz, C_5D_5N) NMR spectroscopic data, see Table 4; positive ion ESIMS *m/z*: 1275 $[M+Na]^+$; negative ion ESIMS *m/z*: 1287 $[M+Cl]^-$, 1251 $[M-H]^-$, 1151 $[1251 - C_5H_8O_2\ (Tga)]^-$, 1149 $[1251 - C_5H_{10}O_2\ (Mba)]^-$, 1067 $[1251 + H_2O - C_5H_8O_2 - C_5H_{10}O_2]^-$, 1065 $[1251 + H_2O - 2 \times C_5H_{10}O_2]^-$, 1049 $[1251 - C_5H_8O_2 - C_5H_{10}O_2]^-$, 1047 $[1251 - 2 \times C_5H_{10}O_2]^-$, 1023, 1005, 965, 947, 921, 565, 547, 419; HRESIMS *m/z*: 1287.6097 $[M+Cl]^-$ (calcd for $C_{60}H_{100}O_{27}Cl$, 1287.6141).

4.8. Turpethoside B (*5*)

Colorless syrup, $[\alpha]_D^{20}$ −0.27 (*c* 0.39, MeOH); for ^1H (600 MHz, C_5D_5N) and ^{13}C (150 MHz, C_5D_5N) NMR spectroscopic data, see Table 4; positive ion ESIMS *m/z*: 1289 $[M+Na]^+$; negative ion ESIMS *m/z*: 1301 $[M+Cl]^-$, 1265 $[M-H]^-$, 1165 $[1265 - C_5H_8O_2]^-$, 1163 $[1265 - C_5H_{10}O_2]^-$, 1081 $[1265 + H_2O - C_5H_8O_2 - C_5H_{10}O_2]^-$, 1079 $[1265 + H_2O - 2 \times C_5H_{10}O_2]^-$, 1063 $[1265 - C_5H_8O_2 - C_5H_{10}O_2]^-$, 1061 $[1265 - 2 \times C_5H_{10}O_2]^-$, 1037, 1019, 979, 961, 935, 917, 579, 561, 433; HRESIMS *m/z* 1325.6711 $[M+AcOH - H]^-$ (calcd for $C_{63}H_{105}O_{29}$, 1325.6747).

4.9. Acid hydrolysis of *1*–*3* and preparation of (S)-12-hydroxy fatty acids *6*–*8*

The remaining portion (300 mg) of the glycosidic acid fraction was hydrolyzed by 10% H_2SO_4 at 80 °C for 4 h. The hydrolysate was cooled to room temperature and extracted with Et_2O (30 mL × 3) to afford a mixture of hydroxy fatty acids (50 mg). The mixture was separated by HPLC using MeOH–H_2O (75:25, v/v) with a flow-rate of 5 mL/min to give **6** (15.0 mg, t_R = 32 min), **7** (18 mg, t_R = 41 min), and **8** (14 mg, t_R = 56 min). The aqueous layer was neutralized with 5% NaOH and desalted by passage through a Sephadex LH-20 column using MeOH to afford a mixture of sugars (90 mg), from which glucose (R_f = 0.3) and rhamnose (R_f = 0.52) were detected by TLC ($CHCl_3$–MeOH–H_2O, 6:4:1). The mixture was subjected to silica gel CC eluted with $CHCl_3$–MeOH–H_2O (10:3:1) to obtain L-rhamnose (29 mg): $[\alpha]_D^{20}$ +8.2 (*c* 0.97, H_2O), and further eluted with $CHCl_3$–MeOH–H_2O (7:3:1) to give D-glucose (10.9 mg): $[\alpha]_D^{20}$ +69.1° (*c* 0.55, H_2O).

4.9.1. (S)-12-Hydroxypentadecanoic acid (*6*)

Colorless oil; $[\alpha]_D^{20}$ +2.2 (*c* 1.35, $CHCl_3$); ^1H NMR (600 MHz, C_5D_5N) δ: 3.84 (1H, m, H-12), 2.51 (2H, t, *J* = 7.4 Hz, H-2), 1.78 (2H, m, H-3), 1.47–1.74 (8H, m), 1.18–1.41 (12H, m), and 0.95 (3H, t, *J* = 7.1 Hz, H-15); for ^{13}C NMR (150 MHz, C_5D_5N) see Table 3; positive ion ESIMS *m/z*: 281 $[M+Na]^+$; negative ion ESIMS *m/z*: 257 $[M-H]^-$.

*4.9.2. (S)-12-Hydroxyhexadecanoic acid (**7**)*

Colorless oil; $[\alpha]_D^{20}$ +0.6 (*c* 1.42, $CHCl_3$); ^1H NMR (600 MHz, C_5D_5N) δ: 3.82 (1H, m, H-12), 2.51 (2H, t, *J* = 7.4 Hz, H-2), 1.78 (2H, m, H-3), 1.57–1.73 (6H, m), 1.44–1.57 (2H, m), 1.20–1.42 (14H, m), and 0.89 (3H, t, *J* = 7.3 Hz, H-16); for ^{13}C NMR (150 MHz, C_5D_5N) spectroscopic data, see Table 3; positive ion ESIMS *m/z*: 295 $[M+Na]^+$; negative ion ESIMS *m/z*: 271 $[M-H]^-$.

*4.9.3. (S)-12-Hydroxyheptadecanoic acid (**8**)*

Colorless oil; $[\alpha]_D^{20}$ +0.5 (*c* 0.77, $CHCl_3$); ^1H NMR (600 MHz, C_5D_5N) δ: 3.83 (1H, m, H-12), 2.51 (2H, t, *J* = 7.4 Hz, H-2), 1.78 (2H, m, H-3), 1.46–1.74 (8H, m), 1.20–1.42 (16H, m), and 0.85 (3H, t, *J* = 7.1 Hz, H-17); for ^{13}C NMR (150 MHz, C_5D_5N) spectroscopic data, see Table 3; negative ion ESIMS *m/z*: 285 $[M-H]^-$.

*4.10. Preparation of (S)-12-hydroxy fatty acid methyl esters **9**-(S)–**11**-(S) from (S)-12-hydroxy fatty acids **6**–**8***

In a typical experiment (Hecht and Kozarich, 1973), hydroxy fatty acid **6** (13 mg, 0.05 mmol) and N-nitrosomethylurea (MUN, 0.21 g, 0.2 mmol) were dissolved in 1,2-dimethoxyethane–H_2O (5:1, 10 mL). The solution was maintained at ice-bath temperature and 0.6 N KOH (10 mL added dropwise). The reaction mixture was concentrated under reduced pressure and the aqueous solution extracted with Et_2O (10 mL×3). The Et_2O layer was dried (anhydrous $MgSO_4$) and evaporated to afford the methyl ester **9**-(*S*) (11.4 mg, 83%). By the same method, hydroxy fatty acids **7** and **8** (each 13 mg) yielded **10**-(*S*) (12.0 mg, 88%) and **11**-(*S*) (11.1 mg, 81%), respectively.

*4.10.1. (S)-12-Hydroxypentadcanoic acid methyl ester [**9**-(S)]*

Colorless oil, $[\alpha]_D^{20}$ +0.8 (*c* 1.1, $CHCl_3$); ^1H NMR (400 MHz, $CDCl_3$) δ: 3.67 (3H, s, OCH_3), 3.59 (1H, m, H-12), 2.30 (2H, t, *J* = 7.6 Hz, H-2), 1.60 (2H, m, H-3), 1.23–1.50 (20H, m), 0.93 (3H, t, *J* = 6.9 Hz, H-15); for ^{13}C NMR (100 MHz, $CDCl_3$) spectroscopic data, see Table 3; EIMS *m/z* (rel. int.): 255 $[M-OH]^+$ (1), 229 $[M-C_3H_7]^+$ (9), 200 $[M-C_4H_8O]^+$ (20), 197 $[M-C_3H_7-MeOH]^+$ (55), 179 $[M-C_3H_7-MeOH-H_2O]^+$ (12), 157 (30), 143 (44), 95 (55), 87 (100).

*4.10.2. (S)-12-Hydroxyhexadecanoic acid methyl ester [**10**-(S)]*

Colorless oil, $[\alpha]_D^{20}$ +1.1 (*c* 0.9, $CHCl_3$); ^1H NMR (400 MHz, $CDCl_3$) δ: 3.67 (3H, s, OCH_3), 3.59 (1H, m, H-12), 2.30 (2H, t, *J* = 7.6 Hz, H-2), 1.62 (2H, m, H-3), 1.20–1.52 (22H, m), 0.91 (3H, t, *J* = 7.0 Hz, H-16); for ^{13}C NMR (100 MHz, $CDCl_3$) spectroscopic data, see Table 3; EIMS *m/z* (rel. int.): 269 $[M-OH]^+$ (1), 229 $[M-C_4H_9]^+$ (12), 200 $[M-C_4H_8O]^+$ (20), 197 $[M-C_4H_9-MeOH]^+$ (60), 179 $[M-C_4H_9-MeOH-H_2O]^+$ (14), 157 (31), 143 (42), 95 (47), 87 (100).

*4.10.3. (S)-12-Hydroxyheptadecanoic acid methyl ester [**11**-(S)]*

Colorless oil, $[\alpha]_D^{20}$ +0.6 (*c* 0.8, $CHCl_3$); ^1H NMR (400 MHz, $CDCl_3$) δ: 3.67 (3H, s, OCH_3), 3.59 (1H, m, H-12), 2.30 (2H, t, *J* = 7.6 Hz, H-2), 1.62 (2H, m, H-3), 1.23–1.50 (24H, m), 0.93 (3H, t, *J* = 6.8 Hz, H-17); for ^{13}C NMR (100 MHz, $CDCl_3$) spectroscopic data, see Table 3; EIMS *m/z* (rel. int.): 283 $[M-OH]^+$ (1), 229 $[M-C_5H_{11}]^+$ (17), 200 $[M-C_4H_8O]^+$ (28), 197 $[M-C_5H_{11}-MeOH]^+$ (84), 179 $[M-C_5H_{11}-MeOH-H_2O]^+$ (19), 157 (41), 143 (51), 95 (58), 87 (100).

*4.11. Synthesis of racemic 12-hydroxy fatty acid methyl esters **9**–**11***

4.11.1. Preparation of Grignard reagents

To a three-necked flask were added anhydrous THF (3 mL), Mg (1.0 g, 41.7 mmol), and an iodine crystal at room temperature. A solution of 1-bromopropane (2.0 mol/L in THF, 30 mmol) was then added dropwise to the resulting mixture under N_2 and vigorous stirring. After the addition was completed, the reaction mixture was stirred for 1 h to provide a Grignard reagent of *n*-C_3H_7MgBr (1.6 mol/L, in THF). By the same method, *n*-C_4H_9MgBr (1.6 mol/L, in THF) and *n*-$C_5H_{11}MgBr$ (1.6 mol/L, in THF) were obtained.

*4.11.2. 12-Hydroxydodecanoic acid methyl ester (**13**)*

12-Hydroxydodecanoic acid (1.10 g, 5.09 mmol) was stirred with CH_3OH (30 mL, 0.74 mol) in the presence of a catalytic amount of concentrated H_2SO_4 (20 drops) under reflux for 8 h. Volatile materials were removed via rotary evaporation, the residue was dissolved in Et_2O (200 mL), and successively washed with 10% $NaHCO_3$ (3 × 100 mL) and H_2O (3 × 100 mL). The organic layer was then dried (anhydr. Na_2SO_4) and concentrated to yield 12-hydroxydodecanoic acid methyl ester (**13**, 0.81 g, 80%): a white solid; ^1H NMR (400 MHz, $CDCl_3$) δ: 3.62 (3H, s, OCH_3), 3.58 (2H, t, *J* = 6.7 Hz, H-12), 2.25 (2H, t, *J* = 7.6 Hz, H-2), 1.47–1.58 (4H, m), 1.23–1.30 (14H, m).

*4.11.3. Methyl 12-oxododecanoate (**14**)*

1-Hydroxy-1,2-benziodoxol-3(1*H*)-one-1-oxide (IBX, 4.2 g, 15 mmol) was added to a solution of methyl ester **13** (0.8 g, 3.4 mmol) in DMSO (10 mL), and the mixture was stirred at rt for 4 h. The pale-yellow colored solution was diluted with Et_2O and successively washed with saturated NaCl and H_2O. The organic layer was dried (anhydr. Na_2SO_4) and concentrated in vacuo. The resulting residue was purified by silica gel CC to give methyl 12-oxododecanoate (**14**, 0.68 g, 85%): white waxy solid; ^1H NMR (400 MHz, $CDCl_3$) δ: 9.77 (1H, t, *J* = 1.8 Hz, H-12), 3.68 (3H, s, OCH_3), 2.42 (1H, td, *J* = 7.4, 1.8 Hz, H-11), 2.30 (2H, t, *J* = 7.5 Hz, H-2), 1.62 (4H, m), 1.24–1.29 (12H, m).

*4.11.4. Racemic12-hydroxy fatty acid methyl esters **9**–**11***

Compound **14** (200 mg, 0.88 mmol) was dissolved in anhydrous THF (3 mL). The fresh *n*-$C_5H_{11}MgBr$ in THF (1.6 mol/L, 0.9 mmol) was added under N_2 and the reaction mixture stirred for 4 h. The reaction was quenched with saturated NH_4Cl (30 mL). The resulting mixture was then extracted with Et_2O (3 × 20 mL), dried (Na_2SO_4), filtered, and evaporated under reduced pressure. The residue was purified by flash chromatography over silica gel (*n*-hexane–EtOAc, 9:1) to afford **9** (60 mg, 25%). By the same method, **10** (100 mg, 40%) and **11** (90 mg, 34%) were obtained. The ^1H and ^{13}C NMR spectroscopic data of **9**–**11** were identical with those of **9**-(*S*)–**11**-(*S*).

*4.12. Synthesis of methyl 11-hydroxyheptadecanoate (**15**)*

By a reported procedure (Jiang et al., 1995) 10-undecenoic acid (1.0 g) was first converted to methyl 11-oxoundecanoate (300 mg), which was subsequently treated with *n*-$C_6H_{13}MgBr$ to give 11-hydroxy-heptadecanoic acid methyl ester (**15**, 140 mg): colorless oil; ^1H NMR (400 MHz, $CDCl_3$) δ: 3.67 (3H, s, OCH_3), 3.58 (1H, m, H-11), 2.30 (2H, t, *J* = 7.6 Hz, H-2), 1.61–1.28 (26 H, m), 0.88 (3H, t, *J* = 6.3 Hz, H-17); ^{13}C NMR (100 MHz, $CDCl_3$) δ: 174.3 (C-1), 72.0 (C-11), 51.4 (OCH_3), 37.4 (C-10 and C-12), 34.1 (C-2), 31.8, 29.6, 29.5, 29.3, 29.3, 29.2, 29.1, 25.6 (C-9 and C-13), 24.9 (C-3), 22.6 (C-16), 14.0 (C-17); positive ion ESIMS *m/z* 301 $[M+H]^+$.

*4.13. Preparation of Mosher's esters of **9**-(S)–**11**-(S)*

A solution of (*R*)-α-methoxy-α-phenylacetic acid (MPA, 12.0 mg), 4-dimethylaminopyridine (DMAP, 10.0 mg), and *N*,*N*-dicyclohexylcarbodiimide (DCC, 10.0 mg) in CH_2Cl_2 (1.0 mL) was added to compound **9**-(*S*) (5.0 mg) in CH_2Cl_2 (1.5 mL). The solution was stirred for 20 h at 25 °C, treated with EtOAc (30.0 mL) and filtered. The filtrate was concentrated and the residue purified by

silica gel CC eluted with cyclohexane–EtOAc (95:5) to give **16**-(*S*,*R*) (5.5 mg, 71%). Similarly, treatment of **9**-(*S*) (5.0 mg) with (*S*)-MPA yielded **16**-(*S*,*S*) (6.0 mg, 78%). Using the same method, esterification of **10**-(*S*) and **11**-(*S*) with (*R*)-MPA and (*S*)-MPA gave two pairs of Mosher's esters **17**-(*S*,*R*)/**17**-(*S*,*R*) and **18**-(*S*,*R*)/**18**-(*S*,*S*), respectively.

*4.13.1. (S)-12-[(R)-α-Methoxy-α-phenylacetyloxy]pentadecanoic acid methyl ester [**16**-(S,R)]*

Colorless oil, $[\alpha]_D^{20}$ –3.5 (*c* 0.25, $CHCl_3$); ^1H NMR (400 MHz, $CDCl_3$) δ: 7.44 (2H, m, Ar-H), 7.33 (3H, m, Ar-H), 4.91 (m, H-12), 4.74 (1H, s, MPA-H-α), 3.67 (3H, s, 1-OCH_3), 3.42 (3H, s, MPA-α-OCH_3), 2.30 (2H, t, *J* = 7.6 Hz, H-2), 1.62 (2H, m, H-3), 1.50 (2H, m, H-11), 1.38 (2H, m, H-13), 1.22 (2H, m, H-10), 0.99 (2H, m, H-14), 0.71 (t, *J* = 7.3 Hz, H-15); ESIMS *m*/*z*: 421 $[M+H]^+$, 443 $[M+Na]^+$.

*4.13.2. (S)-12-[(S)-α-Methoxy-α-phenylacetyloxy]pentadecanoic acid methyl ester [**16**-(S,S)]*

Colorless oil, $[\alpha]_D^{20}$ +4.0 (*c* 0.25, $CHCl_3$); ^1H NMR (400 MHz, $CDCl_3$) δ: 7.44 (2H, m, Ar-H), 7.33 (3H, m, Ar-H), 4.92 (1H, m, H-12), 4.73 (1H, s, MPA-H-α), 3.67 (3H, s, 1-OCH_3), 3.42 (3H, s, MPA-α-OCH_3), 2.31 (2H, t, *J* = 7.5 Hz, H-2), 1.61 (2H, m, H-3), 1.49 (2H, m, H-13), 1.38 (2H, m, H-11), 1.27 (2H, m, H-14), 0.90 (2H, m, H-10), 0.88 (3H, t, *J* = 7.3 Hz, H-15); ESIMS *m*/*z*: 421 $[M+H]^+$, 443 $[M+Na]^+$.

*4.13.3. (S)-12-[(R)-α-Methoxy-α-phenylacetyloxy]hexadecanoic acid methyl ester [**17**-(S,R)]*

Colorless oil, $[\alpha]_D^{20}$ –3.6 (*c* 0.25, $CHCl_3$); ^1H NMR (400 MHz, $CDCl_3$) δ: 7.44 (2H, m, Ar-H), 7.33 (3H, m, Ar-H), 4.90 (1H, m, H-12), 4.73 (1H, s, MPA-H-α), 3.67 (3H, s, 1-OCH_3), 3.42 (3H, s, MPA-α-OCH_3), 2.30 (2H, t, *J* = 7.6 Hz, H-2), 1.61 (2H, m, H-3), 1.50 (2H, m, H-11), 1.38 (2H, m, H-13), 1.22 (2H, m, H-10), 1.07 (2H, m, H-15), 0.90 (2H, m, H-14), 0.70 (3H, t, *J* = 7.3 Hz, H-16); ESIMS *m*/*z*: 435 $[M+H]^+$, 457 $[M+Na]^+$.

*4.13.4. (S)-12-[(S)-α-Methoxy-α-phenylacetyloxy]hexadecanoic acid methyl ester [**17**-(S,S)]*

Colorless oil, $[\alpha]_D^{20}$ +3.6 (*c* 0.25, $CHCl_3$); ^1H NMR (400 MHz, $CDCl_3$) δ: 7.44 (2H, m, Ar-H), 7.33 (3H, m, Ar-H), 4.90 (1H, m, H-12), 4.74 (1H, s, MPA-H-α), 3.67 (3H, s, 1-OCH_3), 3.42 (3H, s, MPA-α-OCH_3), 2.31 (2H, t, *J* = 7.6 Hz, H-2), 1.62 (2H, m, H-3), 1.51 (2H, m, H-13), 1.37 (2H, m, H-11), 1.29 (2H, m, H-14), 1.25 (2H, m, H-15), 0.90 (2H, m, H-10), 0.86 (3H, t, *J* = 7.0 Hz, H-16); ESIMS *m*/*z*: 435 $[M+H]^+$, 457 $[M+Na]^+$.

*4.13.5. (S)-12-[(R)-α-Methoxy-α-phenylacetyloxy]heptadecanoic acid methyl ester [**18**-(S,R)]*

Colorless oil, $[\alpha]_D^{20}$ –3.6 (*c* 0.25, $CHCl_3$); ^1H NMR (400 MHz, $CDCl_3$) δ: 7.44 (2H, m, Ar-H), 7.34 (3H, m, Ar-H), 4.90 (1H, m, H-12), 4.73 (1H, s, MPA-H-α), 3.67 (3H, s, 1-OCH_3), 3.42 (3H, s, MPA-α-OCH_3), 2.30 (2H, t, *J* = 7.6 Hz, H-2), 1.62 (2H, m, H-3), 1.50 (2H, m, H-11), 1.38 (2H, m, H-13), 1.22 (2H, m, H-10), 1.10 (2H, m, H-16), 1.05 (2H, m, H-15), 0.93 (2H, m, H-14), 0.77 (3H, t, *J* = 6.9 Hz, H-17); ESIMS *m*/*z*: 449 $[M+H]^+$, 471 $[M+Na]^+$.

*4.13.6. (S)-12-[(S)-α-Methoxy-α-phenylacetyloxy]heptadecanoic acid methyl ester [**18**-(S,S)]*

Colorless oil, $[\alpha]_D^{20}$ +3.9 (*c* 0.25, $CHCl_3$); ^1H NMR (400 MHz, $CDCl_3$) δ: 7.44 (2H, m, Ar-H), 7.33 (3H, m, Ar-H), 4.90 (1H, m, H-12), 4.73 (1H, s, MPA-H-α), 3.67 (3H, s, 1-OCH_3), 3.42 (3H, s, MPA-α-OCH_3), 2.31 (2H, t, *J* = 7.6 Hz, H-2), 1.62 (2H, m, H-3), 1.50 (2H, m, H-13), 1.38 (2H, m, H-11), 1.29 (2H, m, H-15), 1.25 (2H, m, H-16), 1.23 (2H, m, H-14), 0.90 (2H, m, H-10), 0.86 (3H, t, *J* = 6.8 Hz, H-17); ESIMS *m*/*z*: 449 $[M+H]^+$, 471 $[M+Na]^+$.

*4.14. Alkaline hydrolysis of **4** and **5** and analysis of short organic acids*

Compound **4** (5 mg) was treated with 5% KOH (3 mL) at 85 °C for 4 h. The reaction mixture was acidified to pH 4.0 and extracted with Et_2O (3 × 1 mL). The Et_2O layer was dried (anhydr. $MgSO_4$) and concentrated to a small volume (about 0.2 mL) to afford a short chain organic acid fraction. The aqueous layer was further extracted with *n*-BuOH (3 × 2 mL). The *n*-BuOH solution was concentrated in vacuo to yield a glycosidic acid (3 mg) which was identified as turpethic acid A (**1**) on the basis of the ^1H NMR spectroscopic data and HPLC analysis. By the same method, compound **5** (5 mg) afforded another short chain organic acid fraction and turpethic acid B (3 mg, **2**).

Both short chain organic acid fractions obtained from **4** and **5** were analyzed by GC–MS using a Shimadzu GCMS-QP2000Plus apparatus, equipped with a Rxi®-5 ms fused silica capillary column (30 m × 0.25 mm, 0.25 μm). The carrier gas was helium. Column temperature was initially 50 °C, increased to 160 °C at 20 °C/min, then increased to 220 °C at 5 °C/min. For GC–MS detection, an electron ionization system was used with ionization energy of 70 eV. Both fractions gave two predominant peaks which were identified to be 2-methylbutyric acid (t_R 3.14 min): EIMS *m*/*z* 102 $[M]^+$ (0.8), 87 (24), 74 (100), 57 (64), 45 (16), and 41 (52), and tiglic acid (t_R 3.70 min): EIMS *m*/*z* 100 $[M]^+$ (100), 85 (29), 72 (0.8), 55 (86), 53 (16), and 39 (25), by comparison of their retention times (t_R) with those of authentic (±)-2-methylbutyric acid and tiglic acid (both 98%, Acros Organics, New Jersey).

Subsequently, 2-methylbutyric acid in fractions obtained from **4** and **5** was further analyzed by GC with an Agilent 7890A GC system using a Chir-L-Val (*N*-propionyl-L-valine *tert*-butylamide polysiloxane) chiral capillary column (Varian WCOT Fused Silica CP7495, 25 m × 0.25 mm, 0.12 μm), column temp.: 50 °C (hold 2 min)→180 °C at 3 °C/min, carrier gas H_2. The authentic sample (±)-2-methylbutyric acid gave two peaks at t_R 17.05 min and t_R 17.19 min. Each of the fractions from **4** and **5** and the authentic (*S*)-(+)-2-methylbutyric acid (98%, Acros Organics, New Jersey) gave a single peak at t_R ~17 min, and a mixture sample containing two fractions and authentic (*S*)-(+)-2-methylbutyric acid provided only one peak at t_R 17.22 min. Thus, the absolute configuration of 2-methylbutyric acid in both fractions from **4** and **5** was determined to be *S*.

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Appendix A. Supplementary data

Supplementary data associated with this article can be found, in the online version, at http://dx.doi.org/10.1016/j.phytochem.2012.05.010.

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