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Chemical Constituents of *Ainsliaea yunnanensis* Franch

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Chemical investigation of the constituents of the whole herb of *Ainsliaea yunnanensis* Franch has led to isolation of hentriacontane (**1**), (6*Z*,9*Z*)-henicosa-6, 9-diene (**2**), methyl linoleate (**3**), dodecyl (*Z*)-9-hexadecenoate (**4**), heptadecan-8-ol (**5**), α-linolenic acid (**6**), (*Z*)-6-hexadecenoic acid (**7**), (*Z*)-10-eicosenoic acid (**8**), stearic acid (**9**), phytenoic acid (**10**), tripalmitolein (**11**), trilinolenin (**12**), phthalic acid *bis*-(2-ethylhexyl) ester (**13**), diisobutyl phthalate (**14**), 2-hydroxy-butanedioic acid-4-methyl ester (**15**), diacylgalactolipids I (**16**), β-D-galactopyranoside-1,2-*bis*[(1-oxo-9,12,15-octadecatrienyl)oxy]propyl (**17**), (2S)-1,2-O-(9*Z*,12*Z*-octadecadienoyl)-3-O-[α-D-galactopyranosyl-(1‴→6‴)-O-β-D-galactopyranosyl]glycerol (**18**). The structures of the compounds were identified by comparison of their NMR data with literature data.

Keywords: *Ainsliaea yunnanensis* Franch, Chemical constituents, Structure identification.

INTRODUCTION

Ainsliaea yunnanensis Franch, a plant of Ainsliaea, is mainly distributed in Yunnan, Sichuan and Guazhou provinces of China, which has been used in Chinese folk medicine to treat traumatic injury and rheumatic pain [1,2]. The mainly chemical constituents of *A. yunnanensis* are triterpenes, sesquiterpenes, phenolic acids and flavonoids, which have been reported previously [3-8]. In present chemical study on this plant, 18 compounds were isolated from the whole plants of *A. yunnanensis*. They were identified as hentriacontane (**1**) [9], (6Z,9Z)-henicosa-6,9-diene (**2**) [10], methyl linoleate (**3**) [11], dodecyl (*Z*)-9-hexadecenoate (**4**) [12], heptadecan-8-ol (**5**) [13], α-linolenic acid (**6**) [14], (*Z*)-6-hexadecenoic acid (**7**) [15], (*Z*)-10-eicosen-oic acid (**8**) [15], stearic acid (**9**) [16], phytenoic acid (**10**) [17], tripalmitolein (**11**) [18], trilinolenin (**12**) [19], phthalic acid *bis*-(2-ethylhexyl) ester (**13**) [20], diisobutyl phthalate (**14**) [21], 2-hydroxy-butanedioic acid-4-methyl ester (**15**) [22], diacylgalactolipids I (**16**) [23], β-D-galactopyranoside-1,2-*bis*[(1-oxo-9,12,15-octadecatrienyl)oxy]propyl (**17**) [24], (2*S*)-1,2-O-(9*Z*,12*Z*-octadecadienoyl)-3-O-[α-D-galactopyranosyl-(1‴→6‴)-O-β-D-galactopyranosyl]glycerol (**18**) [25], To our best of knowledge, this is the first report on the isolation of these compounds from *A. yunnanensis*.

EXPERIMENTAL

The plant materials of *A. yunnanensis* were collected from Chuxiong, Yunnan province, P.R. China during June 2013 and authenticated by Prof. Ceming Tan (Jiujiang Forest Herbarium, Jiangxi, P.R. China). A voucher specimen (No. 20130629) was deposited at the Department of Pharmacognosy of the Second Military Medical University.

NMR spectra were obtained on a Bruker Avance 600 NMR spectrometer (Bruker Co., Germany). MS were acquired on a Mass spectrometer HPMS5973 (HP Co., USA). Column chromatography was performed by using silica gel (100-200 mesh; Yantai Jiangyou Silica Gel Development Co. Ltd., Yantai, China), Sephadex LH-20 (40-70 μm; Pharmacia Company, Uppsala, Sweden). Semi-preparative HPLC isolation was achieved with an Agilent 1200 instrument (Agilent, Santa Clara, USA) equipped with a refractive index detector (RID), using

a C18 column (250 mm × 10 mm × 5 μm, YMC) and eluting with MeOH-H_2O (35 % – 50 %) at 2.0 mL/min. Precoated silica gel GF_{254} and HF_{254} plates were used for TLC and zones were visualized under UV light (254 and 365 nm) or by spray with 10 % H_2SO_4-EtOH followed by heating.

Extraction and isolation: The air-dried herb of *A. yunnanensis* (5 kg) were extracted three times with 80 % EtOH each for 1 h, and then the EtOH extract was concentrated *in vacuo* to an aqueous residue. The residue was suspended in H_2O (5 L) and then partitioned successively with petroleum ether (3 × 5 L), EtOAc (3 × 5 L), and *n*-BuOH (3 × 5 L).

The petroleum ether fraction (150 g) was chromatographed over silica gel column (100-200 mesh, 60 × 800 mm) and eluted with a solvent system of petroleum ether-EtOAc (100:1 →70:1→50:1→20:1→10:1) to afford fractions A1-A4. Fr.A1 (20 g) was repeatedly purified by silica gel column using a step gradient of petroleum ether-EtOAc (100:1 to 10:1, 10 % increase in each gradient) and Sephadex LH-20 CH_2Cl_2-MeOH (1:1) to give compound **1** (25 mg), **2** (35 mg), **3** (40 mg), **4** (70 mg). Meanwhile, further purification on the Fr.A2 (32 g), A3 (45 g), A4 (24 g) using the same method gave compound **5** (55 mg), **6** (31 mg), **7** (22 mg), **8** (88 mg), **9** (560 mg), **10** (310 mg), **11** (120 mg), **12** (402 mg), **13** (98 mg), **14** (668 mg).

The *n*-BuOH fraction (120 g) was separated by silica gel column using a step gradient of CH_2Cl_2-MeOH (50:1→30:1→10:1→5:1) to afford 5 fractions (B1-B5). Fr.B1 (12 g) was first purified by Sephadex LH-20 CH_2Cl_2-MeOH (1:1), then was further separated by HPLC on a semi-preparative C18 column (250 mm × 10 mm, 2.0 mL/min) with 50 % MeOH-H_2O as mobile phase to give compound **15** (23 mg), **16** (15 mg). Purification on Fr.B2 (30 g) using the same method [Sephadex LH-20 CH_2Cl_2-MeOH (1:1), HPLC with 35 % MeOH-H_2O] gave compound **17** (26 mg), **18** (15 mg).

Spectral data

Hentriacontane (1): EI-MS *m/z*: 436 $[M]^+$; ^{1}H NMR (600 MHz, $CDCl_3$) δ: 1.26-1.29 (58H, m, 29 × CH_2), 0.87 (6H, t, *J* = 6.0 Hz, 2 × CH_3). ^{13}C NMR (150 MHz, $CDCl_3$) δ: 14.1 (C-1, 31), 22.8 (C-2, 30), 31.8 (C-3, 29), 29.6 (C-5-27), 29.5 (C-4, 28).

(6*Z*,9*Z*)-Henicosa-6,9-diene (2): EI-MS *m/z*: 292 $[M]^+$, ^{1}H NMR (600 MHz, $CDCl_3$) δ: 5.39-5.31 (4H, m, CH=CH, H-6, 7, 9, 10), 2.76 (2H, t, *J* = 6.6 Hz, H-8), 2.03 (4H, m, H-5, 11), 1.38-1.18 (24H, m, CH_2), 0.87 (3H, t, *J* =7.2 Hz, H-1), 0.85 (3H, t, *J* = 7.4 Hz, H-21). ^{13}C NMR (150 MHz, $CDCl_3$) δ: 130.0, 128.1 (C-6, 7, 9, 10), 31.9, 31.4, 29.7, 29.6, 29.4, 29.3, 29.2, 29.1, 29.0, 27.2, 25.7, 22.7, 22.4 (CH_2), 14.0, 13.9 (CH_3).

Methyl linoleate (3): ESI-MS *m/z*: 294 $[M]^+$, ^{1}H NMR (600 MHz, $CDCl_3$) δ: 5.35-5.31 (4H, m, CH=CH, H-9, 10, 12, 13), 3.71 (3H, s, OCH_3), 2.74 (2H, t, *J* = 6.6 Hz, CH_2, H-11), 2.33-1.25 (22H, m, CH_2), 0.88 (3H, t, *J* = 6.6 Hz, H-18). ^{13}C NMR (150 MHz, $CDCl_3$) δ: 174.9 (C=O), 130.3, 130.2, 128.2, 128.0 (CH=CH), 52.2 (OCH_3), 34.2, 31.6, 29.7, 29.6, 29.3, 29.2, 27.0, 25.5, 25.0, 22.7 (CH_2), 14.2 (CH_3).

Dodecyl (*Z*)-9-hexadecenoate (4): ESI-MS *m/z*: 422 $[M]^+$, ^{1}H NMR (600 MHz, $CDCl_3$) δ: 5.45-5.30 (2H, m, CH=CH), 4.15 (2H, t, *J* = 7.1 Hz, OCH_2), 1.32-1.22 (44H, m, CH_2), 0.95 (3H, t, *J* = 6.4 Hz, CH_3), 0.88 (3H, t, *J* = 6.4 Hz, CH_3). ^{13}C NMR (150 MHz, $CDCl_3$) δ: 174.0 (C=O), 130.1, 130.0 (CH=CH), 64.3 (OCH_2), 34.6, 32.1, 30.9, 29.9, 29.8, 29.7, 29.6, 29.4, 27.5, 25.3, 25.2, 22.9 (CH_2), 14.3, 14.2 (CH_3).

Heptadecan-8-ol (5): EI-MS *m/z*: 256 $[M]^+$, ^{1}H NMR (600 MHz, $CDCl_3$) δ: 3.41 (1H, m, CHOH), 1.50–1.37 (4H, brs, H-7, 9), 1.25-1.28 (24H, m, CH_2), 0.87 (6H, t, *J* = 7.0 Hz, 2 × CH_3). ^{13}C NMR (150 MHz, $CDCl_3$) δ: 72.0 (C-8), 37.5 (C-7, 9), 31.9, 29.7, 29.6, 29.3, 25.7, 22.7 (CH_2), 14.1, 13.9 (CH_3).

α-Linolenic acid (6): EI-MS *m/z*: 278 $[M]^+$, ^{1}H NMR (600 MHz, $CDCl_3$) δ: 5.44-5.26 (6H, m, CH=CH, H-9, 10, 12, 13, 15, 16), 2.76 (4H, m, H-11, 14), 1.30-1.23 (16H, m, CH_2), 0.94 (3H, t, *J* = 7.4 Hz, CH_3). ^{13}C NMR (150 MHz, $CDCl_3$) δ: 180.6 (COOH), 132.2, 130.0, 128.3, 128.2, 128.0, 127.9 (CH=CH), 34.1, 29.7, 29.2, 29.1, 27.3, 25.4, 24.8, 24.6, 20.7 (CH_2), 14.1 (CH_3).

(*Z*)-6-Hexadecenoic acid (7): EI-MS *m/z*: 254 $[M]^+$, ^{1}H NMR (600 MHz, $CDCl_3$) δ: 5.38-5.25 (2H, m, H-6, 7), 2.30 (2H, t, *J* = 6.8 Hz, H-2), 2.00 (4H, m, H-5, 8), 1.61 (2H, m, H-3), 1.39 (2H, m, H-4), 1.33-1.19 (14H, m, H-9-15), 0.89 (3H, t, *J* = 6.6 Hz, H-16). ^{13}C NMR (150 MHz, $CDCl_3$) δ: 180.3 (COOH), 130.0, 129.2 (CH=CH), 34.1, 31.6, 29.7, 29.6, 29.4, 29.3, 29.2, 29.2, 27.2, 27.1, 24.7, 22.7 (CH_2), 14.1 (CH_3).

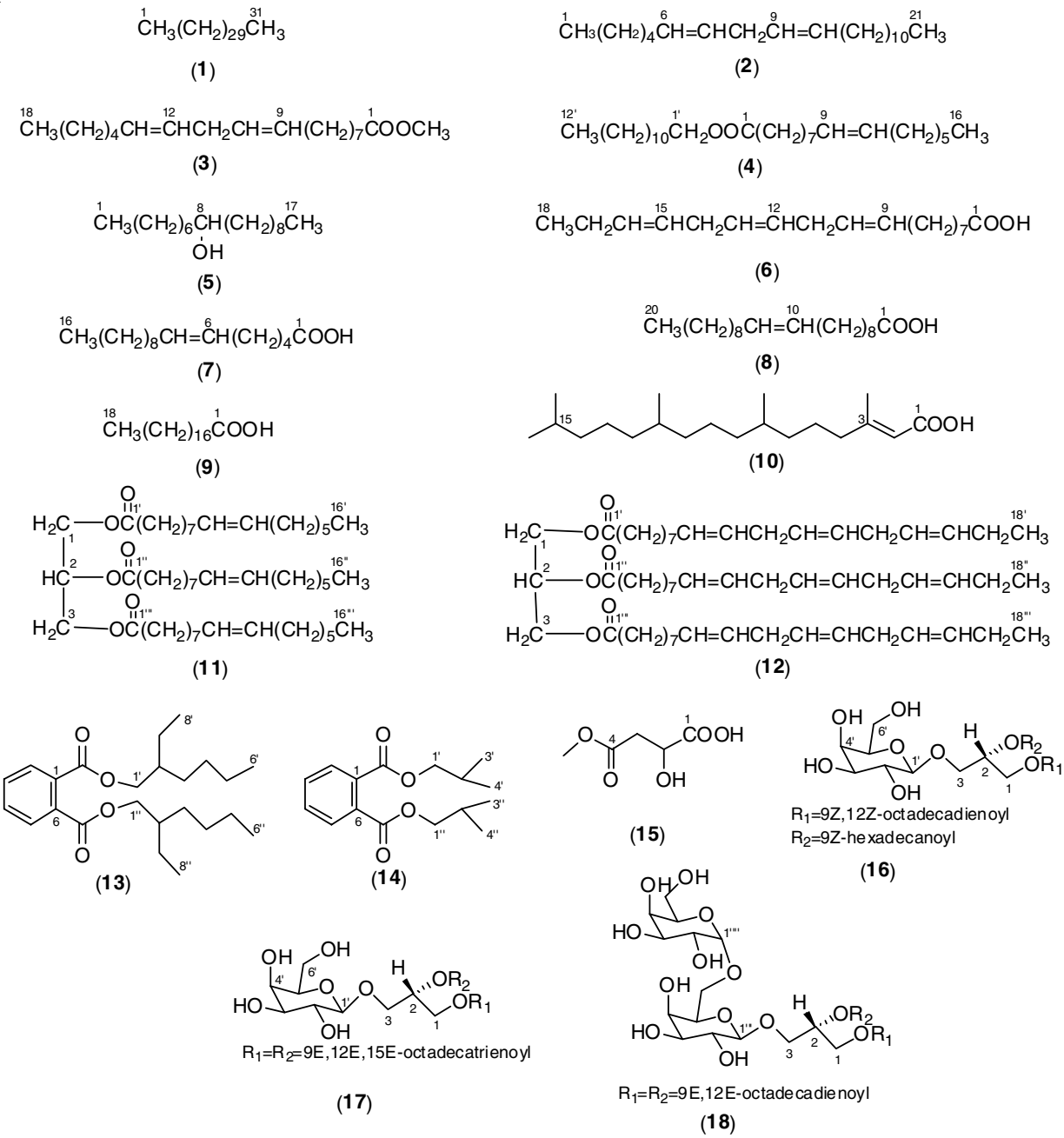
(*Z*)-10-Eicosenoic acid (8): EI-MS *m/z*: 310 $[M]^+$, ^{1}H NMR (600 MHz, $CDCl_3$) δ: 5.43- 5.32 (2H, m, H-10, 11), 2.31 (2H, t, *J* = 7.5 Hz, H-2), 1.98 (4H, m, H-9, 12), 1.62-1.22 (24H, m, H-3-8, 13-19), 0.89 (3H, t, *J* = 6.6 Hz, H-20). ^{13}C NMR (600 MHz, $CDCl_3$) δ: 180.1 (COOH), 130.0, 129.7 (CH=CH), 34.0, 31.9, 29.7, 29.6, 29.4, 29.3, 29.2, 29.1, 29.0, 28.9, 27.7, 27.2, 24.7, 22.7 (CH_2), 14.1 (CH_3).

Stearic acid (9): EI-MS *m/z*: 284 $[M]^{+\cdot}$, ^{1}H NMR (600 MHz, $CDCl_3$) δ: 2.33 (2H, t, *J* = 9.8 Hz, H-2), 1.65 (2H, m, H-3), 1.32-1.27 (28H, m, H-4-17), 0.87 (3H, t, *J* = 8.8 Hz, H-18). ^{13}C NMR (150 MHz, $CDCl_3$) δ: 179.1 (COOH), 33.9, 31.7, 29.8, 29.7, 29.6, 29.2, 29.1, 28.9, 28.7, 24.5, 22.5 (CH_2), 14.0 (CH_3).

Phytenoic acid (10): EI-MS *m/z*: 310 $[M]^+$, ^{1}H NMR (600 MHz, $CDCl_3$) δ: 5.70 (1H, s, C=CH), 2.16 (3H, s, CH_3), 2.11 (2H, t, *J* = 7.6 Hz, CH_2), 1.31-1.26 (16H, m, CH_2), 0.88 (3H, d, *J* = 6.6 Hz, CH_3), 0.85 (6H, d, *J* = 6.4 Hz, 2 × CH_3), 0.83 (3H, d, *J* = 6.4 Hz, CH_3) . ^{13}C NMR (150 MHz, $CDCl_3$) δ: 172.0 (C-1), 163.4 (C-2), 114.8 (C-3), 41.3 (C-4), 39.2 (C-5), 37.5 (C-6), 37.3 (C-7, 8), 36.6, 32.6, 32.6, 28.2, 25.0, 24.8, 24.3 (CH_2), 22.5, 22.4, 19.7, 19.6, 19.0 (CH_3).

Tripalmitolein (11): EI-MS *m/z*: 800 $[M]^+$, ^{1}H NMR (600 MHz, $CDCl_3$) δ: 5.37-5.32 (6H, m, CH=CH), 5.26 (1H, m, H-2), 4.27 (2H, m, H-1a,3a), 4.16 (2H, m, H-1b,3b), 2.32 (6H, m, O=CCH_2), 2.01 (6H, m, CH=CHCH_2), 1.63 (6H, m, O=C-CH_2-CH_2), 1.31-1.27 (54H, m, CH_2), 0.90 (9H, d, *J* = 7.0 Hz, CH_3). ^{13}C NMR (150 MHz, $CDCl_3$) δ: 173.1, 172.9 (C=O), 130.1, 129.8 (CH=CH), 68.7 (C-2), 62.0 (C-1,3), 34.1, 33.9 ($COCH_2$), 31.8, 29.8, 29.7, 29.6, 29.4, 29.3, 29.0, 27.9, 27.1, 27.0, 24.5, 24.2, 22.5 (CH_2), 14.0 (CH_3).

Trilinolenin (12): EI-MS *m/z*: 872 $[M]^+$, ^{1}H NMR (600 MHz, $CDCl_3$) δ: 5.36-5.31 (18H, m, CH=CH), 5.29 (1H, m, H-2), 4.29 (2H, m, H-1a,3a), 4.17 (2H, m, H-1b,3b), 2.81 (12H, m, CH=CH-CH_2-CH=CH), 2.30 (6H, m, O=CCH_2), 2.06 (12H, m, CH=CH-CH_2), 1.60 (6H, m, O=C-CH_2-CH_2), 1.31-1.26 (24H, m, CH_2), 0.97 (9H, d, *J* = 7.5 Hz, CH_3). ^{13}C NMR (150



Structures of compounds **1**-**18**

MHz, $CDCl_3$) δ: 173.2, 172.7 (C=O), 131.7, 130.1, 129.9, 128.3, 128.2, 128.0, 127.8, 127.7 (CH=CH), 69.0 (C-2), 62.2 (C-1, 3), 34.3, 34.2, 29.8, 29.7, 29.3, 29.2, 29.1, 29.0, 27.1, 25.4, 24.8, 24.7, 20.4 (CH_2), 14.1 (CH_3).

Phthalic acid *bis*-(2-ethylhexyl) ester (13): EI-MS *m/z*: 390 $[M]^+$, ^1H NMR (600 MHz, $CDCl_3$) δ: 7.70 (2H, dd, *J* = 5.8, 3.4 Hz, H-2,5), 7.51 (2H, dd, *J* = 5.8, 3.4 Hz, H-3,4), 4.30 (4H, m, H-1′,1″), 1.68 (2H, m, H-2′,2″), 1.41 (4H, m, H-7′,7″), 1.30 (4H, m, H-3′,3″), 1.29 (8H, m, H-4′,4″,5′,5″), 0.91 (6H, t, *J* = 7.6 Hz, H-8′,8″), 0.89 (6H, t, *J* = 7.8 Hz, H-6′, 6″). ^{13}C NMR (150 MHz, $CDCl_3$) δ: 167.2 (C=O), 131.9 (C-1, 6), 130.3, 128.3 (CH=CH), 67.6 (C-1′,1″), 38.2 (C-2′,2″), 29.8, 28.4, 23.2, 22.5 (CH_2), 13.5, 10.4 (CH_3).

Diisobutyl phthalate (14): EI-MS *m/z*: 278 $[M]^+$, ^1H NMR (600 MHz, $CDCl_3$) δ: 7.77 (2H, m, H-2,5), 7.68 (2H, m, H-3,4), 4.10 (4H, m, H-1′,1″), 2.02 (2H, m, H-2′,2″), 1.00-0.88 (12H, m, H-3′,3″,4′,4″). ^{13}C NMR (150 MHz, $CDCl_3$) δ: 167.2 (C=O), 132.3 (C-1, 6), 130.8, 128.8 (CH=CH), 71.7 (C-1′,1″), 27.7 (C-2′,2″), 19.1 (C-3′,3″,4′,4″).

2-Hydroxy-butanedioic acid-4-methyl ester (15): ESI-MS *m/z*: 149 $[M+H]^+$, ^1H NMR (600 MHz, C_5D_6N) δ: 5.12 (1H, dd, *J* = 4.6, 2.4 Hz, H-2), 3.72 (3H, s, OCH_3), 3.27 (2H, m, H-3). ^{13}C NMR (150 MHz, C_5D_6N) δ: 175.0, 173.2 (C=O), 68.7 (C-2), 51.6 (OCH_3), 40.4 (C-3).

Diacylgalactolipids I (16): ESI-MS *m/z*: 776 $[M+Na]^+$, ^1H NMR (600 MHz, CD_3OD) δ: 5.42-5.28 (6H, m, CH=CH), 5.26 (1H, m, H-2), 4.44 (2H, m, H-1), 4.21 (1H, d, *J* = 7.6 Hz, H-1′), 4.00 (1H, m, H-3a), 3.83 (1H, d, *J* = 2.4 Hz, H-4′), 3.74 (1H, m, H-3b), 3.72 (1H, m, H-6′), 3.52 (2H, m, H-2′,5′), 3.43 (1H, m, H-3′), 2.75-1.29 (CH_2), 0.89 (6H, m, 2 × CH_3). ^{13}C NMR (150 MHz, CD_3OD) δ: 174.8, 173.9 (C=O), 132.2, 130.9, 129.2, 129.1, 128.9, 128.7 (CH=CH), 105.6 (C-1′), 77.0, 75.1,

72.2, 72.0, 70.6, 68.9, 63.8, 62.4 (C-1, 2, 3, 2′, 3′, 4′, 5′, 6′), 35.4, 35.1, 33.2, 32.5, 30.9, 30.8, 30.7, 30.5, 30.3, 30.1, 30.0, 26.8, 26.2, 23.7, 23.6 (CH_2), 14.3 (CH_3).

β-D-Galactopyranoside-1,2-*bis*[(1-oxo-9,12,15-octadecatrienyl)oxy]propyl (17): ESI-MS *m/z*: 796 $[M+Na]^+$, ^{1}H NMR (600 MHz, CD_3OD) δ: 5.37-5.32 (12H, m, CH=CH), 5.27 (1H, m, H-2), 4.44 (1H, m, H-1a), 4.23 (1H, d, *J* = 7.2 Hz, H-1′), 4.21 (1H, m, H-1b), 3.98 (1H, m, H-3a), 3.83 (1H, m, H-4′), 3.76 (1H, m, H-6′a), 3.73 (1H, m, H-3b), 3.72 (1H, m, H-6′b), 3.51 (2H, m, H-2′, 5′), 3.44 (1H, m, H-3′), 2.81 (8H, m, H-11″,14″,11‴,14‴), 2.31-1.32 (CH_2), 0.98 (6H, t, *J* = 7.2 Hz, H-18″, 18‴). ^{13}C NMR (150 MHz, CD_3OD) δ: 174.8, 174.4 (C=O), 132.4, 130.8, 129.0, 128.8, 128.4, 128.2 (CH=CH), 105.6 (C-1′), 76.5, 74.6, 72.2, 71.6, 70.0, 68.5, 63.8, 62.2 (C-1, 2, 3, 2′, 3′, 4′, 5′, 6′), 35.0, 30.6, 30.4, 30.2, 30.0, 28.1, 26.4, 26.3, 25.9, 21.3 (CH_2), 14.5 (C-18″, 18‴)

(2S)-1,2-*O*-(9*Z*,12*Z*-octadecadienoyl)-3-*O*-[α-D-galactopyranosyl-(1‴→6‴)-*O*-β-D-galactopyranosyl]-glycerol (18): ESI-MS *m/z*: 964 $[M+Na]^+$, ^{1}H NMR (600 MHz, CD_3OD) δ: 5.36-5.32 (8H, m, CH=CH), 5.23 (1H, m, H-2), 4.90 (1H, d, *J* = 3.6 Hz, H-1⁗), 4.44 (1H, m, H-1a), 4.30 (1H, d, *J* = 7.2Hz, H-1″), 4.21 (1H, m, H-1b), 3.92 (1H, m, H-3a), 3.91 (1H, m, H-6a‴), 3.89 (1H, m, H-4⁗), 3.86 (1H, m, H-4‴), 3.81 (1H, m, H-5⁗), 3.77 (1H, m, H-2⁗), 3.73 (1H, m, H-3⁗), 3.72 (1H, m, H-6a⁗), 3.71 (1H, m, H-3b), 3.70 (1H, m, H-5‴), 3.69 (1H, m, H-6b‴), 3.68 (1H, m, H-6b⁗), 3.49 (1H, m, H-2‴), 3.48 (1H, m, H-3‴), 2.75 (4H, m, H-11′, 11″), 2.33 (1H, t, *J* = 7.8 Hz, H-2′), 2.31 (1H, t, *J* = 7.8 Hz, H-2″), 2.05-1.29 (CH_2), 0.87 (6H, m, H-18′, 18″). ^{13}C NMR (150 MHz, CD_3OD) δ: 174.8, 174.4 (C=O), 131.3, 130.7, 129.3, 129.0 (CH=CH), 105.6 (C-1‴), 100.4 (C-1⁗), 74.8, 74.5, 72.7, 72.3, 71.7, 71.4, 70.2, 69.9 (C-2, 2‴, 2⁗, 3‴, 3⁗, 4‴, 4⁗, 5‴, 5⁗), 68.9 (C-3), 67.6 (C-6‴), 64.2 (C-1), 63.0 (C-6⁗), 35.2 (C-2′), 34.9 (C-2″), 33.3 (C-16″), 32.9 (C-16′), 30.9, 30.6, 30.4, 30.3, 30.2, 28.3, 26.5, 26.1, 24.0, 23.6 (CH_2), 14.5 (C-18′, 18″).

RESULTS AND DISCUSSION

The genus Ainsliaea of Asteraceae comprises about 70 species, which is mainly distributed in the southeast of Asia. A large number of Ainsliaea species have been long used for the treatment of various diseases. Present continuation of research on this plant provides a scientific basis for further understanding of its chemical constituents.The compounds were analyzed by spectroscopic methods, including NMR and mass spectrometry. All the isolated 18 compounds were well-agreed with the reported data.

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CONFLICT OF INTEREST

The authors declare that there is no conflict of interests regarding the publication of this article.

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