Chemical Constituents from the Root of *Tripterygium wilfordii*

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In this paper, the chemical constituents from the root of *Tripterygium wilfordii* were isolated and characterized. Isolation and purification were carried out on the column chromatography of silica gel and Sephadex LH-20. Fourteen compounds were isolated and identified as (2S,3R,4E,8E,2R)-2-(2'-hydroxyhexadecanoyl) amino-4,8-octadeca diene-1,3-diol (**1**), soya-cerebroside I and soya-cerebroside II (**2** mixture), adenosine (**3**), β -sitosterol-palmitate (**4**) and monopalmitin (**5**), triptohairic acid (**6**), hypolide (**7**), wilforlide B (**8**), wilforlide A (**9**), cangoronine (**10**), salaspermic acid (**11**), orthosphenic acid (**12**), 3-hydroxy-2-oxo-3-friedeien-20- α -carboxylic acid (**13**), 3 β -22a-dihydroxyolean-12-en-29-oic acid (**14**).

Keywords: *Tripterygium wilfordii*, Cerebrosides, Chemical constituents.

INTRODUCTION

Tripterygium wilfordii Hook F. (Celastraceae) as a traditional medicine in China for several hundred years, has been employed for the treatment of rheumatoid arthritis, chronic nephritis and various skin diseases¹. Aqueous chloroform extract of the root xylem of *T. wilfordii* was subjected to column chromatography to get fourteen compounds. The compounds were (2S,3R,4E,8E,2R)-2-(2'-hydroxyhexadecanoyl) amino-4,8-octadeca diene-1,3-diol (**1**), soya-cerebroside I and soya-cerebroside II (**2** mixture), adenosine (**3**), β -sitosterol-palmitate (**4**), monopalmitin (**5**), triptohairic acid (**6**), hypolide (**7**), wilforlide B (**8**), wilforlide A (**9**), cangoronine (**10**), salaspermic acid (**11**), orthosphenic acid (**12**), 3-hydroxy-2-oxo-3-friedeien-20- α -carboxylic acid (**13**), 3 β -22a-dihydroxyolean-12-en-29-oic acid (**14**). Compound **2** was mixture of cerebroside. Compounds **1-5** were obtained from the root of *T. wilfordii* for the first time. Besides, compound **1** was first isolated in plantae.

EXPERIMENTAL

¹H NMR (500 MHz) and ¹³C NMR (125 MHz) spectra were recorded with Bruker ACF-500 NMR spectrometers. Mass spectra were obtained on MS Agilent 1100 series LC/MSD Trap Mass spectrometer. All solvents used were analytical grade. Column chromatography was silica gel (100-200, 200-300, 300-400 mesh), GF₂₅₄ silica gel for TLC, Sephadex LH-20 (Pharmacia Fine Chemical Co. Ltd.) and ODS-C₁₈ (Merck).

The root xylem of *T. wilfordii* Hook F. (20 kg) were collected in Fujian province, China and were identified by Dr. Pu Sheban. A voucher specimen (CPU 2010007) was deposited in the Herbarium of China Pharmaceutical University.

Extraction and isolation: The air-dried root xylem (20 kg) of *T. wilfordii* were extracted with 75 % and 90 % EtOH (3 $\times$ 40 L) at normal temperature for one week at three times, respectively. The EtOH extracts were evaporated to dryness under reduced pressure. The residue (2000 g) was suspended in water and partitioned with petroleum ether and chloroform. The chloroform fraction (300 g) was subjected to silica gel column chromatography, eluted with CHCl₃-MeOH (100:1, 50:1, 25:1, 10:1, 5:1, 1:1). The fractions were subjected to repeated silica gel, Sephadex LH-20, ODS column chromatography and further purified by recrystallization. At last, **1** (11 mg), **2** (30 mg), **3** (40 mg), **4** (72 mg), **5** (46 mg), **6** (63 mg), **7** (125 mg), **8** (2156 mg), **9** (1052 mg), **10** (31 mg), **11** (29 mg), **12** (15 mg), **13** (21 mg), **14** (43 mg) were obtained from the root xylem of *T. wilfordii* Hook F.

RESULTS AND DISCUSSION

Compound 1: White powder (CHCl₃); ESI-MS (m/z) 552.5 [M + H]⁺, ¹H NMR (DMSO-*d*₆, 500 MHz) δ : 7.19 (1H, d, J = 9.1 Hz, NH), 5.57 (1H, J = 18.45 Hz, 5-H), 5.48 (1H, d, J = 5.05 Hz, 4-H), 5.38 (2H, m, 8,9-H), 4.86 (1H, d, J = 5.40 Hz), 4.57 (1H, t, J = 5.15 Hz), 3.62 (1H, m, 1-Ha), 3.42 (1H,

m, 1-Hb), 1.98 (4H, 6,7-H), 1.93 (2H, 10-H), 1.54 (1H, m, 3'-Hb), 1.40 (1H, m, 3'-Ha), 1.23 (36H, brs), 0.85 (6H, t, $J = 6.75$ Hz, 18,16'-H). ^{13}C NMR (DMSO- d_6 , 125 MHz) δ : 173.3 (C-1'), 131.3 (C-5), 130.3 (C-4), 130.1 (C-9), 129.3 (C-8), 60.1 (C-1), 54.2 (C-2), 34.4 (C-3'), 31.8 (C-6), 31.6 (C-7), 31.2 (C-10), 28.4-29.0 (C-11-16, 5'-14'), 24.4 (C-4'), 22.0 (C-17, 15'), 13.8 (C-18, 16'). Compound **1** was identified as (2S,3R,4E,8E,2R)-2-(2-hydroxy hexadecanoyl)amino-4,8-octadecadiene-1,3-diol by comparison of physical and spectral data with literature².

Compound 2: White powder (CHCl₃); ESI-MS (m/z) 714.4 [M + H]⁺, ^1H NMR (DMSO- d_6 , 300 MHz) δ : 7.50 (1H, d, $J = 9.3$ Hz, NH), 5.57 (1H, d, 5-H), 5.36-5.30 (3H, m, 4, 8, 9-H), 4.10-4.93 (6H, 6 groups OH peak), 3.65 (1H, m, 6''-H), 3.43 (1H, m, 6''-H), 2.92-3.14 (4H, m, 2''-5''H), 1.98 (4H, br s, 6, 7-H), 1.23 (m, 11-17, 4'-15'H), 0.85 (6H, t, $J = 6.3$ Hz, 16', 18-H). ^{13}C NMR (DMSO- d_6 , 75 MHz) δ : 173.7 (C-1'), 130.2 (C-5), 130.1 (C-4), 129.8 (C-9), 129.3 (C-8), 103.4 (C-1''), 76.8 (C-5''), 76.5 (C-3''), 73.4 (C-2''), 70.8 (C-2'), 70.5 (C-3), 69.9 (C-4''), 68.8 (C-1), 61.0 (C-6''), 49.8 (C-2), 34.3 (C-3'), 31.9 (C-6), 31.8 (C-7), 31.2 (C-10), 28.5-29.1 (C-11-16, 5'-14'), 24.3 (C-4') 22.0 (C-17, 15'), 13.8 (C-18, 16') and in ^{13}C NMR spectral data, another signal 26.6 (C-7), 26.5 (C-10) can be obtained. Compound **2** was identified as the mixture of soya-cerebroside I and soya-cerebroside II by comparison of physical and spectral data with literature³.

Compound 3: White powder; ESI-MS (m/z) 268 [M + H]⁺, ^1H NMR (py- d_5 , 500 MHz) δ : 8.66 (s, 1H, H-8), 8.58 (s, 1H, H-2), 8.23 (s, 2H, NH₂), 6.67 (d, 1H, $J = 5.58$ Hz, H-1'), 5.46 (t, 1H, $J = 5.55$ Hz, H-2'), 5.03 (m, 1H, H-3'), 4.73 (m, 1H, H-4'), 4.29 (dd, 1H, $J = 14.95$ Hz, 2.6 Hz, H-5'a), 4.12 (dd, 1H, $J = 14.95$ Hz, 2.7 Hz, H-5'b); ^{13}C NMR (py- d_5 , 125 MHz) δ : 157.68 (C-6), 153.29 (C-2), 150.22 (C-4), 140.62 (C-8), 121.51 (C-5), 90.87 (C-1'), 87.81 (C-4'), 75.50 (C-2'), 72.40 (C-3'), 63.04 (C-5'). Compound **3** was identified as adenosine by comparison of physical and spectral data with literature⁴.

Compound 4: White powder; ESI-MS (m/z) 653.1 [M + H]⁺, ^1H NMR (CDCl₃, 300 MHz) δ : 0.67 (3H, H-18), 0.82 (3H, H-27), 0.84 (3H, H-26), 0.88 (3H, H-29), 0.91 (3H, H-16'), 0.93 (3H, H-21), 1.02 (3H, H-19), 4.59 (1H, m, H-3), 5.37 (1H, d, $J = 3.84$ Hz, H-6). ^{13}C NMR (CDCl₃, 125 MHz) δ : 37.0 (C-1), 27.8 (C-2), 73.6 (C-3), 38.1 (C-), 139.7 (C-5), 122.5 (C-6), 31.9 (C-7), 31.9 (C-8), 50.0 (C-9), 36.6 (C-10), 21.0 (C-11), 39.7 (C-12), 31.9 (C-13), 56.7 (C-14), 24.3 (C-15), 28.2 (C-16), 56.0 (C-17), 11.9 (C-18), 19.3 (C-19), 36.1 (C-20), 18.7 (C-21), 33.9 (C-22), 26.1 (C-23), 45.8 (C-24), 29.1 (C-25), 19.8 (C-26), 19.0 (C-27), 23.1 (C-28), 12.0 (C-29), 173.3 (C-1'), 34.7 (C-2'), 25.0 (C-3'), 29.3 (C-4'), 29.4 (C-5'), 29.5 (C-6'), 29.6 (C-7'), 29.7 (C-8'-13'), 31.9 (C-14'), 22.6 (C-15'), 14.1 (C-16'). Compound **4** was identified as β -sitosterol-palmitate by comparison of physical and spectral data with literature⁵.

Compound 5: White powder; ESI-MS (m/z) 331.1 [M + H]⁺, ^1H NMR (CDCl₃, 300 MHz) δ : 4.20 (1H, dd, $J = 9.9, 4.7$ Hz, 1-Ha), 4.15 (1H, dd, $J = 9.9, 4.7$ Hz, 1-Hb), 3.94 (1H, m, 2-H), 3.71 (1H, dd, $J = 11.4, 4.0$ Hz, 3-Ha), 3.60 (1H, dd, $J = 11.4, 5.7$ Hz, 3-Hb), 2.35 (2H, t, $J = 7.3$ Hz, 2-H), 1.63 (2H, m, $J = 7.0$ Hz, 3-H), 1.25 (24H, 4'-15'-H), 0.85 (3H, t,

$J = 6.4$ Hz). Compound **5** was identified as monopalmitin by comparison of physical and spectral data with literature⁶.

Compound 6: White needle crystal (CHCl₃); ESI-MS m/z : 329.1 [M + H]⁺, ^1H NMR (CDCl₃, 500 MHz) δ : 1.05 (s, 3H, C20-H), 1.22 (d, 6H, $J = 6.8$ Hz, Me-16, Me-17), 1.62 (m, 1H, H β -6), 1.66 (m, 1H, H-1), 2.13 (s, 3H, Me-18), 3.30 (sept, 1H, $J = 6.9$ Hz, H-15), 7.08 (s, 2H, H-11, H-14). Compound **6** was identified as triptohairic acid by comparison of physical and spectral data with literature⁷.

Compound 7: White needle crystal (CHCl₃); ESI-MS m/z : 313.1 [M + H]⁺, ^1H NMR (CDCl₃, 300 MHz) δ : 7.05 (1H, d, $J = 8.19$ Hz), 6.93 (1H, d, $J = 8.19$ Hz), 4.80 (2H, m), 3.10 (1H, sept, $J = 6.99$ Hz), 1.27 (6H, t, $J = 5.54$ Hz), 1.03 (3H, s). Compound **7** was identified as hypolide by comparison of physical and spectral data with literature⁸.

Compound 8: White lamellae crystal (CHCl₃); ESI-MS m/z : 453.1 [M + H]⁺, ^1H NMR (CDCl₃, 500 MHz) δ : 0.88 (s, 3H, CH₃), 0.98 (s, 3H, CH₃), 1.05 (s, 6H, 2CH₃), 1.08 (s, 3H, CH₃), 1.09 (s, 3H, CH₃), 1.21 (s, 3H, CH₃), 4.16 (d, 1H, C22-H), 5.32 (t, 1H, C12-H). Compound **8** was identified as wilforlide B by comparison of physical and spectral data with literature⁹.

Compound 9: White crystal (CHCl₃); ESI-MS m/z : 437.2 [M + H]⁺, ^1H NMR (CDCl₃, 500 MHz) δ : 0.79 (s, 3H, CH₃), 0.86 (s, 3H, CH₃), 0.93 (s, 3H, CH₃), 0.94 (s, 3H, CH₃), 0.99 (s, 3H, CH₃), 1.07 (s, 3H, CH₃), 1.20 (s, 3H, CH₃), 3.21 (dd, 1H, C3-H), 4.15 (d, 1H, C22-H), 5.29 (t, 1H, C12-H). Compound **9** was identified as wilforlide A by comparison of physical and spectral data with literature⁹.

Compound 10: White crystal (CHCl₃); ESI-MS m/z : 483.3 [M-H]⁻, ^1H NMR (C5D5N, 500 MHz) δ : 0.74 (3H, s, CH₃), 0.80 (3H, s, CH₃), 1.08 (3H, s, CH₃), 1.22 (3H, s, CH₃), 1.40 (3H, s, CH₃), 1.78 (3H, s, CH₃), 3.03 (t, 1H, $J = 17.4$ Hz, H-1), 9.80 (s, 1H, -CHO). ^{13}C NMR (C5D5N, 125 MHz) δ : 10.6, 16.2, 17.1, 17.8, 18.8, 29.4, 29.5, 30.4, 30.9, 32.0, 32.3, 32.6, 33.2, 36.5, 37.1, 37.3, 39.4, 39.5, 40.6, 44.7, 49.5, 54.9, 55.5, 125.8, 148.9, 181.3, 193.3, 195.7. Compound **10** was identified as cangoronine by comparison of physical and spectral data with literature¹⁰.

Compound 11: White powder (CHCl₃-CH₃OH); ESI-MS m/z : 471.3 [M-H]⁻, ^1H NMR (DMSO- d_6 , 300 MHz) δ : 0.79 (s, 3H, CH₃), 0.82 (s, 3H, CH₃), 0.90 (s, 6H, 2CH₃), 1.03 (s, 3H, CH₃), 1.10 (s, 3H, CH₃), 3.39 (d, 1H, $J = 8.0$ Hz), 3.92 (d, 1H, $J = 8.0$ Hz), 5.59 (s, 1H). ^{13}C NMR (DMSO- d_6 , 75 MHz) δ : 18.8 (C-1), 19.5 (C-2), 104.8 (C-3), 52.6 (C-4), 46.1 (C-5), 28.7 (C-6), 35.8 (C-7), 49.2 (C-8), 38.5 (C-9), 56.2 (C-10), 36.2 (C-11), 37.9 (C-12), 38.5 (C-13), 28.6 (C-14), 29.6 (C-15), 36.9 (C-16), 39.3 (C-17), 43.9 (C-18), 31.5 (C-19), 40.2 (C-20), 29.6 (C-21), 33.9 (C-22), 7.7 (C-23), 71.8 (C-24), 16.0 (C-25), 16.4 (C-26), 31.6 (C-27), 17.2 (C-28), 33.1 (C-29), 179.5 (C-30). Compound **11** was identified as salaspemic acid by comparison of physical and spectral data with literature¹¹.

Compound 12: White powder (CHCl₃-CH₃OH); ESI-MS m/z : 489.1 [M + H]⁺, ^1H NMR (DMSO- d_6 , 300 MHz) δ : 0.74 (3H, s, H-22), 0.78 (3H, s, H-26), 0.86 (3H, s, H-25), 0.90 (3H, s, H-27), 1.03 (3H, s, H-30), 1.10 (3H, s, H-28), 3.41 (1H, d, $J = 8.07$ Hz, H-24b), 3.85 (1H, d, $J = 8.07$ Hz, H-24a), 4.45 (1H, d, $J = 3.24$ Hz), 5.49 (1H, s), 12.07 (1H, s,

COOH). Compound **12** was identified as orthosphenic acid by comparison of physical and spectral data with literature¹².

Compound 13: White needle crystal (CHCl₃-CH₃OH); ESI-MS *m/z*: 469.5 [M-H]⁻, ¹H NMR (DMSO-*d*₆, 300 MHz) δ: 0.83 (3H, s), 0.89 (6H, s), 1.02 (3H, s), 1.04 (3H, s), 1.10 (3H, s), 1.69 (3H, s), 7.64 (1H, s), 12.08 (1H, s, -COOH). ¹³C NMR (DMSO-*d*₆, 75 MHz) δ: 33.4 (C-1), 193.6 (C-2), 142.5 (C-3), 139.7 (C-4), 38.5 (C-5), 38.4 (C-6), 17.2 (C-7), 48.8 (C-8), 36.0 (C-9), 54.7 (C-10), 32.6 (C-11), 35.6 (C-12), 38.5 (C-13), 38.5 (C-14), 28.5 (C-15), 29.5 (C-16), 29.4 (C-17), 43.8 (C-18), 29.2 (C-19), 40.1 (C-20), 28.1 (C-21), 36.0 (C-22), 9.9 (C-23), 18.3 (C-24), 16.9 (C-25), 17.4 (C-26), 15.4 (C-27), 31.4 (C-28), 179.4 (C-29), 31.5 (C-30). Compound **13** was identified as 3-hydroxy-2-oxo-3-friedeinen-20- α -carboxylic acid by comparison of physical and spectral data with literature¹³.

Compound 14: white powder (CHCl₃-CH₃OH); ESI-MS *m/z*: 471.1 [M-H]⁻, ¹H NMR (DMSO-*d*₆, 500 MHz) δ: 0.68 (s, CH₃), 0.87 (s, CH₃), 0.89 (s, CH₃), 0.90 (s, CH₃), 0.92 (s, CH₃), 1.10 (s, CH₃), 1.14 (s, CH₃), 4.26 (1H, s, OH), 4.28 (1H, s, OH), 5.19 (1H, s, 12-H). ¹³C NMR (DMSO-*d*₆, 125 MHz) δ: 15.2, 15.9, 16.4, 17.9, 18.7, 20.3, 23.0, 24.4, 25.9, 26.9, 28.1, 32.0, 36.4, 37.1, 37.8, 38.1, 38.3, 39.3, 39.7, 41.6, 41.7, 45.9, 46.9, 54.6, 72.9, 76.7, 122.2, 143.3, 178.9. Compound **14** was identified as 3 β -22 α -dihydroxyolean-12-en-29-oic acid by comparison of physical and spectral data with literature¹⁴.

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