Asian Journal of Chemistry; Vol. 26, No. 14 (2014), 4344-4346

ASIAN JOURNAL OF CHEMISTRY

http://dx.doi.org/10.14233/ajchem.2014.16432

Chemical Constituents from the Root of *Tripterygium wilfordii*

PAN-YONG HU[1], DI ZHANG[1], SHE-BAN PU[1,*], ZHEN-LIN LI[2] and HONG-HUA ZHOU[3]

[1]State Key Laboratory of Natural Medicines, Research Department of Pharmacognosy, China Pharmaceutical University, Nanjing 211198, P.R. China
[2]Jiangsu Institute of Traditional Chinese Medicine, Nanjing 210028, P.R. China
[3]Center for Instrumental Analysis, China Pharmaceutical University, 24 Tongjia Xiang, Nanjing 210009, P.R. China

*Corresponding author: Tel: +86 25 86185139; E-mail: pusheban@126.com

Received: 26 September 2013; *Accepted*: 12 December 2013; *Published online*: 5 July 2014; AJC-15471

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'-hydroxyhexadec anoyl) amino-4,8-octadeca diene-1,3-diol (**1**), soya-cerebrosideI 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**), 3β-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 rheumatiod arthritis, chronic nephritis and various skin diseases[1]. 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**), 3β-22a-dihydroxyolean-12-en-29-oic acid (**14**). Compound **2** was mixture of cerebrosides. Compounds **1-5** were obtained from the root of *T. wilfordii* for the first time. Besides, compound **1** was first isolated in plantae.

EXPERIMENTAL

^{1}H NMR (500 MHz) and ^{13}C NMR (125 MHz) spectra were record with Brucher 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), GF_{254} silica gel for TLC, Sephadex LH-20 (Pharmacia Fine Chemical Co. Ltd.) and ODS-C_{18} (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 × 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 $CHCl_3$-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($CHCl_3$); ESI-MS (*m/z*) 552.5 $[M + H]^+$, ^{1}H 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 literature[2].

Compound 2: White powder ($CHCl_3$); ESI-MS (*m/z*) 714.4 $[M + H]^+$, ^{1}H NMR (DMSO-d_6, 300 MHz) δ:7.50 (1H, d, *J* = 9.3Hz, 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 literature[3].

Compound 3: White powder; ESI-MS (*m/z*) 268 $[M + H]^+$, ^{1}H NMR (py-d_5,500 MHz) δ:8.66 (s, 1H, H-8), 8.58 (s, 1H, H-2), 8.23 (s, 2H, NH_2), 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 literature[4].

Compound 4: White powder; ESI-MS (*m/z*) 653.1 $[M + H]^+$, ^{1}H NMR ($CDCl_3$, 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 ($CDCl_3$, 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 (C2-11), 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 literature[5].

Compound 5: White powder; ESI-MS (*m/z*) 331.1 $[M + H]^+$, ^{1}H NMR ($CDCl_3$, 300 MHz) δ: 4.20 (1H, dd, *J* = 9.9, 4.7 Hz, 1-Ha), 4.15 (1H, dd, *J* = 9.9, 4.7Hz, 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* = 6.4Hz). Compound **5** was identified as monopalmitin by comparison of physical and spectral data with literature[6].

Compound 6: White needle crystal ($CHCl_3$); ESI-MS *m/z*: 329.1 $[M + H]^+$, ^{1}H NMR ($CDCl_3$, 500 MHz) δ:1.05 (s, 3H, C20-H), 1.22(d, 6H, *J* = 6.8Hz, Me-16, Me-17), 1.62 (m, 1H, Hβ-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 literature[7].

Compound 7: White needle crystal ($CHCl_3$); ESI-MS *m/z*: 313.1 $[M + H]^+$, ^{1}H NMR ($CDCl_3$, 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 literature[8].

Compound 8: White lamellae crystal ($CHCl_3$); ESI-MS *m/z*: 453.1 $[M + H]^+$, ^{1}H NMR ($CDCl_3$, 500 MHz) δ:0.88 (s, 3H, CH_3), 0.98 (s, 3H, CH_3), 1.05(s, 6H, $2CH_3$), 1.08 (s, 3H, CH_3), 1.09(s, 3H, CH_3), 1.21 (s, 3H, CH_3), 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 literature[9].

Compound 9: White crystal ($CHCl_3$); ESI-MS *m/z*: 437.2 $[M + H]^+$, ^{1}H NMR ($CDCl_3$, 500 MHz) δ:0.79 (s, 3H, CH_3), 0.86 (s, 3H, CH_3), 0.93 (s, 3H, CH_3), 0.94 (s, 3H, CH_3), 0.99 (s, 3H, CH_3), 1.07 (s, 3H, CH_3), 1.20 (s, 3H, CH_3), 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 literature[9].

Compound 10: White crystal ($CHCl_3$); ESI-MS *m/z*: 483.3 $[M-H]^-$, ^{1}H NMR (C5D5N, 500MHz) δ:0.74 (3H, s, CH_3), 0.80 (3H, s, CH_3), 1.08 (3H, s, CH_3), 1.22 (3H, s, CH_3), 1.40 (3H, s, CH_3), 1.78 (3H, s, CH_3), 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 literature[10].

Compound 11: White powder ($CHCl_3$-CH_3OH); ESI-MS *m/z* : 471.3 $[M-H]^-$, ^{1}H NMR(DMSO-d_6, 300MHz) δ: 0.79(s, 3H, CH_3), 0.82 (s, 3H, CH_3), 0.90 (s, 6H, $2CH_3$), 1.03 (s, 3H, CH_3), 1.10 (s, 3H, CH_3), 3.39 (d, 1H, *J* = 8.0Hz), 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 salaspermic acid by comparison of physical and spectral data with literature[11].

Compound 12: White powder ($CHCl_3$-CH_3OH); ESI-MS *m/z*: 489.1 $[M + H]^+$, ^{1}H 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 literature[12].

Compound 13: White needle crystal ($CHCl_3$-CH_3OH); 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). ^{13}C 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- friedeien-20-α-carboxylic acid by comparison of physical and spectral data with literature[13].

Compound 14: white powder ($CHCl_3$-CH_3OH); ESI-MS *m/z*: 471.1 $[M-H]^-$, ^1H NMR (DMSO-d_6, 500 MHz) δ:0.68 (s, CH_3), 0.87 (s, CH_3), 0.89 (s, CH_3), 0.90 (s, CH_3), 0.92 (s, CH_3), 1.10 (s, CH_3), 1.14 (s, CH_3), 4.26 (1H, s, OH), 4.28 (1H, s, OH), 5.19 (1H, s, 12-H). ^{13}C 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 3β-22a-dihydroxyolean-12-en-29-oic acid by comparison of physical and spectral data with literature[14].

ACKNOWLEDGEMENTS

The authors thank the sponsorship of Jiangsu Overseas Research & Training Program for the University Prominent Young & Middle-aged Teachers and Presidents.

REFERENCES

1. A.M. Brinker, J. Ma, P.E. Lipsky and I. Raskin, *Photochemistry*, **68**, 732 (2007).
2. K. Mori and T. Kinsho, *Liebigs Ann. Chem*., **1988**, 807 (1988).
3. J.L. Lou, L.L. He and L.Y. Kong, *Chin. J. Nat. Med*., **4**, 382 (2006).
4. S.L. Yang and X.K. Liu, *Chin. J. Nat. Med*., **1**, 196 (2003).
5. B. Wu, Y.P. Ma, J.Z. Yuan and Q.S. Sun, *J. Shenyang Pharm. Univ*., **27**, 116 (2010).
6. Y. Sun, A.L. Lu, L. Wei and Y.H. Pei, *Chin. J. Med. Chem*., **2**, 135 (2008).
7. K. Nakano, C. Yoshida, W. Furukawa, Y. Takaishi and K. Shishido, *Phytochemistry*, **49**, 1821 (1998).
8. D.G. Wu, X.C. Sun and F. Li, *Acta Botanica Yunnannica*, **1**, 29 (1979).
9. L. Ding, Z.X. Zhang, J. Xu and H.X. Zhang, *J. Plant Res. Environ*., **1**, 50 (1992).
10. F.J. Guo, P.F. Fang and Y.C. Li, *Acta Pharmacol. Sin*., **34**, 210 (1999).
11. J.H. Sheng and B.N. Zhou, *Acta Bot. Sin*., **36**, 475 (1992).
12. K.W. Wang, X.L. Hu, L.Q. Sheng and Y.J. Pan, *China J. Chin. Mater. Med*., **32**, 1539 (2007).
13. S.L. Mao, S.M. Sang, A.N. Lao and Z.L. Cheng, *Chin. Tradit. Herbal Drugs*, **31**, 729 (2000).
14. J.P. Kutney, G.M. Hewitt, G. Lee, K. Piotrowska, M. Roberts and S.J. Rettig, *Can. J. Chem*., **70**, 1455 (1992).