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Full Length Research Paper

Isolation and chemotaxonomic significance of megastigmane-type sesquiterpenoids from *Sarcandra glabra*

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In this study, twelve known compounds including six megastigmane-type sesquiterpenoids, two coumarins and four phenols were isolated for the first time from *Sarcandra glabra* (Thunb.) Nakai, a traditional medicinal plant with a variety of pharmaceutical activities. Their structures were determined using nuclear magnetic resonance (NMR) and mass spectrometry (MS) techniques and the chemotaxonomic significance of megastigmane-type sesquiterpenoids was briefly discussed.

Key words: *Sarcandra glabra*, Chloranthaceae, sesquiterpenoid, chemotaxonomy.

INTRODUCTION

The genus *Sarcandra* (Chloranthaceae) consists of three species distributed in Southeastern Asia to Indomalesia. *Sarcandra glabra* (Thunb.) Nakai [*syn. Chloranthus glaber* (Thunb.) Makino] is an evergreen shrub growing in Southern China and has been traditionally used for the treatment of bruises, bone fractures and arthritis. The whole plant of *S. glabra* is specified in the Chinese Pharmacopoeia (2010 edition) as a traditional medicine used for its anticancer, antibacterial and antivirus activities (State Pharmacopeia Committee of China, 2010). The chemistry of *S. glabra* has attracted considerable interest. So far, many sesquiterpenoids (including dimeric sesquiterpenes), flavonoids, coumarins and phenolic acids have been isolated from this species (Uchida et al., 1978; Wang et al., 1988; Takeda et al., 1993; Okamura et al., 1994; Okamura et al., 1995; Tsui et al., 1996; Luo et al., 2005a, b; Li et al., 2006a, b; Huang et al., 2007, 2008; Yuan et al., 2008; Zhu et al., 2008; He et al., 2010; Wang et al., 2010). Our previous phytochemical studies on the species resulted in the isolation of a new coumarin and three novel sesquiterpene glycosides (Xu et al., 2008; Hu et al., 2009). In the continuation of our efforts to investigate bioactive constituents of *S. glabra*, twelve known compounds including six megastigmane-type sesquiterpenes, two coumarins and four phenols were isolated and structurally characterized using mass spectrometry (MS) and nuclear magnetic resonance (NMR) techniques for the first time. Besides, the chemotaxonomic significance of megastigmane-type sesquiterpenes is also briefly discussed herein.

MATERIALS AND METHODS

Collection and preparation of plant material

The medicinal material was collected at Jiujiang in Jiangxi province, China, in May, 2003, and authenticated by Prof. Ceming Tan at Jiujiang Institute of Forest Plants, Jiangxi, China. A voucher specimen (No. CSH2003058018) was deposited at the Herbarium of the Institute of Medicinal Plant Development, Peking Union Medical College and Chinese Academy of Medical Sciences, Beijing, China.

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Extraction and isolation

In our experiments, the whole air-dried and powered plant material (5 kg) was exhaustively extracted three times with 70% aqueous acetone at room temperature. The acetone extract was evaporated to dryness under reduced pressure and followed by extraction with ethyl acetate after suspension in water. The ethyl acetate fraction was separated on (MCI) gel column using water and 75 and 90% methanol – water (MeOH – H_2O) and MeOH, respectively in sequence to afford four fractions (F1 to F4). On the basis of thin layer chromatography (TLC) investigations, combined fraction F2 (60 g) and F3 (2.3 g) was subjected to silica gel column chromatography eluted with a gradient of petroleum ether: ethyl acetate (1:0 to 0:1, v/v) to afford eight fractions (Fractions A to H). Fraction D was separated on a Sephadex LH-20 column eluted with MeOH and preparative high performance liquid chromatography (HPLC) with MeOH–H_2O to afford Compounds 1 to 6. Fraction F was further chromatographed on silica gel column eluted with petroleum ether: ethyl acetate: methanol (20:1:0.1, v/v/v) and followed by LH-20 (MeOH) to yield Compounds 7 to 12. These compounds including six megastigmane-type sesquiterpenes (1 to 6), two coumarins (7 to 8), and four phenols (9 to 12) were identified as dihydrovomifoliol (1) (Andersson et al., 1988), dihydrovomifoliol-O-β-D-glucopyranoside (2) (Andersson et al., 1988), drovomifoliol-O-β-D-glucopyranoside (3) (Andersson et al., 1988), asicariside B_1 (4) (Miyase et al., 1987), (*S*)-abscisic acid (5) (Constantino et al., 1986), β-D-glucopyranosyl abscisate (6) (Boyer et al., 1982), fraxin (7) (Ma and Zhao, 2008), scopolin (8) (Dawa et al., 2008), vanilloloside (9) (Ida et al., 1994), 3,5-dimethoxyl-4-hydroxybenzyl alcohol 4-O-β-D-glucoside (10) (Yao et al., 2008), *β*-hydroxypropiovanillone (11) (Wu et al., 2008), glucosyringic acid (12) (Li et al., 2006) by comparing their physical and spectroscopic data with those reported in the literatures, respectively (Figure 1). Compounds 1-12 were isolated from *S. glabra* for the first time.

Compound 1 $C_{13}H_{20}O_3$. MS data: ESI-MS *m/z*: 247 $[M + Na]^+$. NMR data: ^{1}HNMR (CD_3OD, 600 MHz) *δ*: 5.86 (1H, s, H-2), 3.77 (1H, m, H-9), 2.50 (1H, d, *J* = 18.0 Hz, H-6α), 2.24 (1H, d, *J* = 18.0 Hz, H-6β), 2.01 (1H, m, H-7α), 1.83 (1H, m, H-7β), 1.64 (1H, m, H-8α), 1.51 (1H, m, H-8β), 1.23 (3H, d, *J* = 6.0 Hz, H-10), 1.06 (3H, s, H-11), 1.10 (3H, s, H-12), 2.06 (3H, s, H-13); ^{13}CNMR (CD_3OD, 150 MHz) *δ*: 201.6 (C-1), 127.2 (C-2), 172.2 (C-3), 79.8 (C-4), 43.3 (C-5), 51.6 (C-6), 35.4 (C-7), 34.1 (C-8), 76.7 (C-9), 20.6 (C-10), 24.6 (C-11), 25.1 (C-12), 22.2 (C-13).

Compound 2 $C_{19}H_{32}O_8$. MS data: ESI-MS *m/z*: 411 $[M + Na]^+$, 799 $[2M + Na]^+$; NMR data: ^{1}HNMR (CD_3OD, 600 MHz) *δ*: 5.84 (1H, s, H-2), 3.81 (1H, q, *J* = 6.0 Hz, H-9), 2.60 (1H, d, *J* = 18.0 Hz, H-6α), 2.14 (1H, d, *J* = 18.0 Hz, H-6β), 2.01 (1H, m, H -7α), 1.79 (1H, ddd, *J* = 13.5, 12.0, 4.8 Hz, H-7β), 1.79 (1H, dddd, *J* = 15.0, 9.6, 6.5, 2.4 Hz, H-8β), 1.17 (3H, d, *J* = 6.0 Hz, H-10), 1.10 (3H, s, H-11), 1.02 (3H, s, H-12), 2.04 (3H, s, H-13), 4.31 (1H, d, *J* = 7.8 Hz, H-1′), 3.13 (1H, dd, *J* = 9.0, 7.8 Hz, H-2′), 3.31 (1H, m, H-3′), 3.34 (1H, m, H-4′), 3.35 (1H, t, *J* = 9.0, 8.4 Hz, H-5′), 3.85 (1H, *J* = 12.0, 1.8 Hz, H-6′a), 3.65 (1H, dd, *J* = 12.0, 5.4 Hz, H-6′b); ^{13}CNMR (CD_3OD, 150 MHz) *δ*: 201.3 (C-1), 126.8 (C-2), 171.9 (C-3), 79.4 (C-4), 43.0 (C-5), 51.2 (C-6), 35.0 (C-7), 33.7 (C-8), 76.3 (C-9), 20.2 (C-10), 24.3 (C-11), 24.8 (C-12), 21.9 (C-13), 102.4 (C-1′), 75.2 (C-2′), 78.2 (C-3′), 71.9 (C-4′), 78.2 (C-5′), 63.0 (C-6′).

Compound 3 $C_{19}H_{30}O_8$. MS data: ESI-MS *m/z*: 409 $[M + Na]^+$. NMR data: ^{1}HNMR (CD_3OD, 600 MHz) *δ*: 5.84 (1H, s, H-2), 5.85 (2H, br s, H-7, 8), 4.40 (1H, dd, *J* = 10.5, 1.0 Hz, H-9), 2.51 (1H, d, *J* = 17.0 Hz, H-6α), 2.14 (1H, d, *J* = 17.0 Hz, H-6β), 1.28 (3H, d, *J* = 6.5 Hz, H-10), 1.03 (3H, s, H-11), 1.02 (3H, s, H-12), 2.04 (3H, s, H-13), 4.33 (1H, d, *J* = 9.0 Hz, H-1′), 3.13 (1H, dd, *J* = 9.0, 7.8 Hz, H-2′), 3.31 (1H, m, H-3′), 3.34 (1H, m, H-4′), 3.35 (1H, t, *J* = 9.0, 8.4 Hz, H-5′), 3.85 (1H, *J* = 12.0, 1.8 Hz, H-6′a), 3.65 (1H, dd, *J* = 12.0, 5.4 Hz, H-6′b); ^{13}CNMR (CD_3OD, 150 MHz) *δ*: 201.6 (C-1), 127.2 (C-2), 172.2 (C-3), 79.4 (C-4), 43.0 (C-5), 51.2 (C-6), 132.2 (C-7), 128.3 (C-8), 76.3 (C-9), 20.2 (C-10), 24.3 (C-11), 25.1 (C-12), 22.2 (C-13), 102.8 (C-1′), 75.6 (C-2′), 78.2 (C-3′), 71.9 (C-4′), 78.2 (C-5′), 63.4 (C-6′).

Compound 4 $C_{19}H_{30}O_8$. MS data: ESI-MS *m/z*: 409 $[M + Na]^+$.NMR data: ^{1}HNMR (CD_3OD, 600 MHz) *δ*: 5.84 (1H, s, H-8), 4.44 (1H, d, *J* = 7.8 Hz, H-1′), 4.33 (1H, m, H-3), 2.19 (3H, s, H-10), 2.36 (1H, br d, *J* = 11.4 Hz, H-4a), 2.08 (1H, br d, *J* = 10.8 Hz, H-4b), 1.49 (1H, dd, *J* = 12, 4.2 Hz, H-2a), 1.49 (1H, dd, *J* = 12, 4.2 Hz, H-2b), 1.39 (3H, s, H-12), 1.16 (3H, s, H-13); ^{13}CNMR (CD_3OD, 150 MHz) *δ*: 37.2 (C-1), 46.8 (C-2), 72.8 (C-3), 48.3 (C-4), 72.6 (C-5), 120.3 (C-6), 201.1 (C-7), 101.4 (C-8), 211.7 (C-9), 26.7 (C-10), 29.6 (C-11), 31.0 (C-12), 32.4 (C-13), 102.9 (C-1′), 75.3 (C-2′), 78.3 (C-3′), 71.8 (C-4′), 78.1 (C-5′), 62.9 (C-6′).

Compound 5 $C_{15}H_{20}O_4$. MS data: ESI-MS *m/z*: 265 $[M + H]^+$, 287 $[M + Na]^+$, 551 $[2M + Na]^+$, 263 $[M – H]^–$, 527 $[2M – H]^–$. NMR data: ^{1}HNMR (CD_3OD, 600 MHz) *δ*: 7.75 (1H, d, *J* = 16.2 Hz, H-7), 6.11 (d, 1H, *J* = 16.2 Hz, H-8), 5.92 (1H, br s, H-10), 5.76 (1H, br s, H-4), 2.52 (1H, d, *J* = 16.8 Hz, H-2a), 2.17 (1H, d, *J* = 16.8 Hz, H-2b), 2.03 (3H, br s), 1.93 (3H, d, *J* = 1.2 Hz), 1.07 (3H, s), 1.03 (3H, s); ^{13}CNMR (CD_3OD, 150 MHz) *δ*: 199.3, 164.6, 149.3, 136.0, 127.7, 126.0, 118.1, 78.9, 49.2, 41.2, 23.6, 22.5, 20.6, 18.6.

Compound 6 $C_{21}H_{30}O_9$. MS data: ESI-MS *m/z*: 449 $[M + Na]^+$, 425 $[M – H]^–$, 461 $[2M + Cl]^–$. NMR data: ^{1}HNMR (CD_3OD, 600 MHz) *δ*: 7.80 (1H, d, *J* = 15.6 Hz, H-8), 6.32 (d, 1H, *J* = 16.2 Hz, H-7), 5.94 (1H, br s, H-4), 5.82 (1H, br s, H-10), 2.52 (1H, d, *J* = 16.8 Hz, H-2a), 2.18 (1H, d, *J* = 16.8 Hz, H-2b), 2.08 (3H, br s), 1.94 (3H, br s), 1.07 (3H, s), 1.03 (3H, s); ^{13}CNMR (CD_3OD, 150 MHz) *δ*: 199.6, 164.9, 164.5, 152.2, 137.9, 127.9, 126.3, 116.7, 78.9, 49.2, 41.5, 23.3, 22.2, 20.0, 18.2, 94.0, 77.4, 76.7, 72.6, 69.7, 61.0.

Compound 7 $C_{16}H_{18}O_{10}$. MS data: ESI-MS *m/z*: 371 $[M + H]^+$, 393 $[M + Na]^+$. NMR data: ^{1}HNMR (CD_3OD, 600 MHz) *δ*: 7.87 (1H, d, *J* = 9.6 Hz, H-4), 6.99 (1H, s, H-5), 6.25 (1H, d, *J* = 9.0 Hz, H-3), 4.98 (1H, d, *J* = 7.8 Hz, H-1′), 3.91(3H, s, 6-OCH_3), 3.76 (1H, dd, *J* = 12, 2.4 Hz, H-6′a), 3.64 (1H, dd, *J* = 12, 5.4 Hz, H-6′b), 3.51(1H, dd, *J* = 9.0, 7.8 Hz, H-2′), 3.45 (1H, t, *J* = 9.0 Hz, H-3′), 3.39 (1H, t, *J* = 9.0 Hz, H-4′), 3.24 (1H, ddd, *J* = 9.6, 5.4, 2.4 Hz, H-5′).

Compound 8 $C_{16}H_{18}O_9$. ESI-MS *m/z*: 377 $[M + Na]^+$. NMR data: ^{1}HNMR (CD_3OD, 600 MHz) *δ*: 6.32 (1H, d, *J* = 9.6 Hz, H-3), 7.90 (1H, d, *J* = 9.0 Hz, H-4), 7.22 (1H, s, H-8), 7.18 (1H, s, H-5), 3.92 (3H, s, OCH_3), 5.08 (1H, d, *J* = 7.8 Hz, H-1′).

Compound 9 $C_{14}H_{20}O_8$. NMR data: ^{1}HNMR (CD_3OD, 600 MHz) *δ*: 7.13 (1H, d , *J* = 8.4 Hz, H-6), 7.03 (1H, d , *J* = 1.2 Hz, H-5), 6.88 (1H, dd, *J* = 8.4, 1.2 Hz ,H-3), 4.87 (1H, d, *J* = 7.8 Hz, H-1′), 4.54 (2H, s, H-7), 3.87 (3H, s, H-8), 3.86～3.40 (6H, m, H-2′, 3′, 4′, 5′, 6′); ^{13} CNMR (CD_3OD, 150 MHz) *δ*: 138.0 (C-1), 112.0 (C-2), 151.0 (C-3),147.5 (C-4), 118.2 (C-5), 120.9 (C-6), 65.2 (C-7), 56.9 (C-8), 103.2 (C-1′), 75.1 (C-2′), 78.1 (C-3′), 71.6 (C-4′), 78.4 (C-5′), 62.7 (C-6′).

Compound 10 $C_{15}H_{22}O_9$. MS data: ESI-MS *m/z*: 369 $[M + Na]^+$, 714 $[2M + Na]^+$. NMR data: ^{1}HNMR (CD_3OD, 600 MHz) *δ*: 6.71 (2H, br s, H-2, 6), 4. 84 (lH, d, *J* = 7. 2 Hz, Glc H-l), 4.55 (2H,br s, H-7), 3.76 (lH, dd, *J* = 12.0, 2.4 Hz, H-6′a), 3.65 (1H, dd, *J* = 12.0, 4.8 Hz, H-6′b), 3.85 (6H, s, 2×OCH_3); ^{13}CNMR (CD_3OD, 150 MHz) *δ*: 135.5 (C-l), 105.9 (C-2, 4), 154.4 (C-3, 5), 139.9 (C-4), 65.3 (C-7), 105.7 (C-l′), 75.9 (C-2′), 78.4 (C-3′), 71.5 (C-4′), 78.5 (C-5′), 62.8 (C-6′).

Compound 11 $C_{10}H_{12}O_4$. NMR data: ^{1}HNMR(CD_3OD, 600 MHz) *δ*: 7.54(2H, br s, H-2), 6.96 (1H, d, *J* = 8.0 Hz, H-5), 7.55 (1H, dd, *J* = 8.0, 2.0 Hz, H-6), 3.17 (2H, t, *J* = 5.5 Hz， H-8), 4.01 (2H, t, *J* = 5.5 Hz, H-9); ^{13}CNMR (CD_3OD, 125MHz) *δ*: 129.8 (C-l), 123.7 (C-2), 146.7(C-3), 150.8 (C-4), 114.0 (C-5), 109.6 (C-6), 199.0 (C-7), 39.8(C-8), 58.4 (C-9).

Compound 12 $C_{16}H_{22}O_9$. NMR data: ^{1}HNMR (CD_3OD, 600 MHz) *δ*: 7.36 (2H, s, H-2, 6), 5.06 (1H, d, *J* = 7.2 Hz, H-1′), 3.89 (6H, s, OCH_3×2), 3.40 (1H, m, H-2′), 3.22 (1H, m, H-3′), 3.49 (1H, m, H-4′), 3.40 (1H, m, H-5′), 3.64 (1H, dd, *J* = 12.0, 5.4 Hz, H-6′a), 3.77 (1H, dd, *J* = 12.0, 2.4 Hz, H-6′b); ^{13}CNMR (CD_3OD, 150 MHz) *δ*: 128.4 (C-1), 108.8 (C-2), 154.3 (C-3), 140.2 (C-4), 154.3 (C-5), 108.8 (C-6), 169.6 (C-7), 104.7 (C-1′), 75.9 (C-2′), 78.1 (C-3′), 71.6 (C-4′),

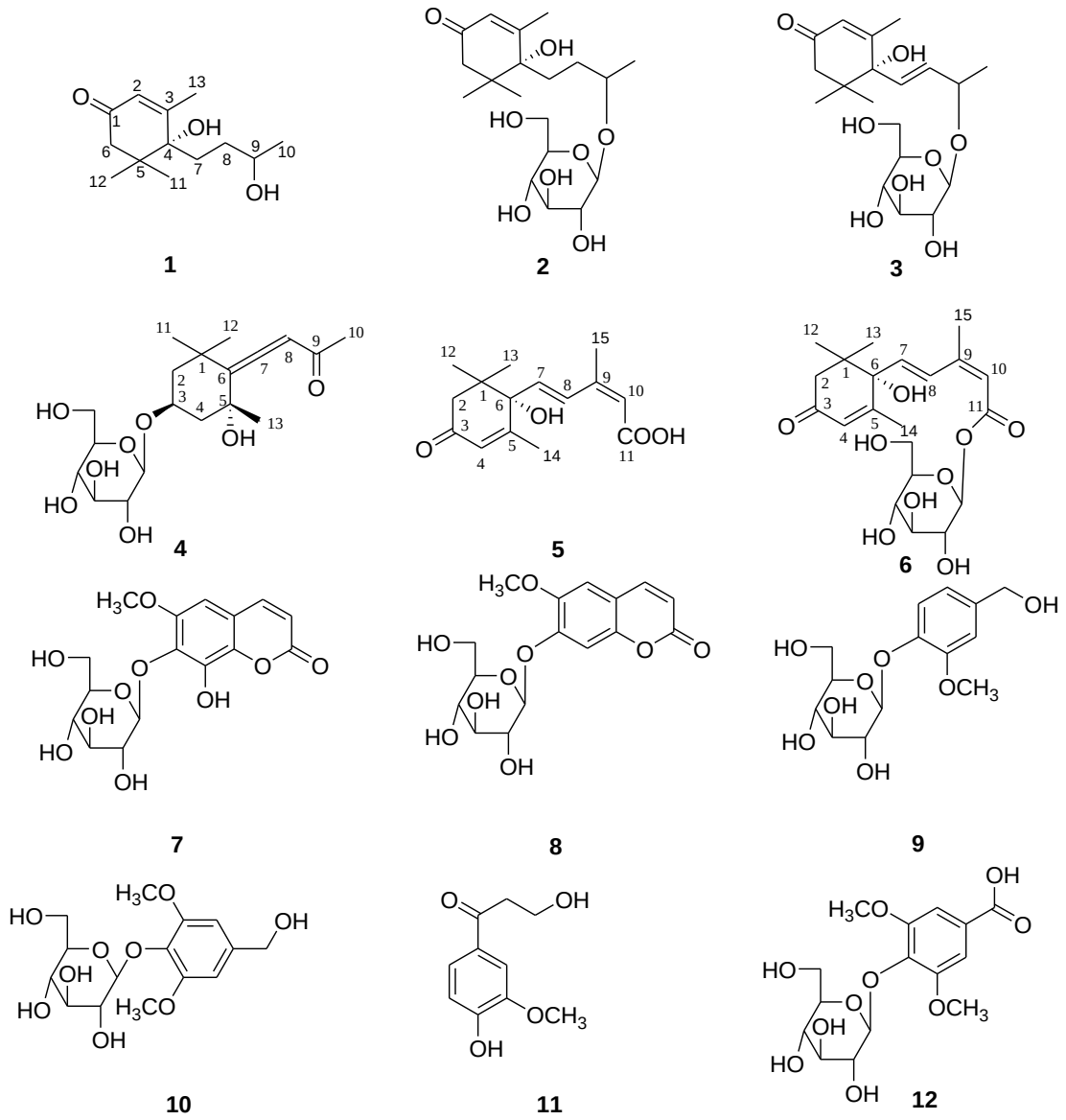

Figure 1. Structures of compounds 1 to 12.

78.6 (C-5′), 62.7 (C-6′), 57.2 (2×OCH_3).

RESULTS AND DISCUSSION

Chloranthaceae comprises of four genera, totalling about seventy species around the world. With various biological activities, sesquiterpenoids are the principal components in evaluating the quality as well as taxonomy of various taxa of the family. According to different structural types, sequiterpenes isolated are classified into lindenane, eudesmane, elemene, eremophilane, germacrane and aromadendrane, as well as dimeric sequiterpene. So far, there is no report on megastigmane-type sesquiterpenes from *S. glabra* available. The isolation and identification of megastigmane-type sesquiterpenes facilitates further chemotaxonomic studies on the family Chloranthaceae.

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