Acta Pharmaceutica Sinica B 2013;3(1):51–54

Institute of Materia Medica, Chinese Academy of Medical Sciences
Chinese Pharmaceutical Association

Acta Pharmaceutica Sinica B

www.elsevier.com/locate/apsb
www.sciencedirect.com

ORIGINAL ARTICLE

Two new steroidal tetraglycosides from the rhizomes of *Smilacina atropurpurea*

Ying Zhang[a,b], Haizhou Li[a], Chongren Yang[a,c], Yingjun Zhang[a,*]

[a]*State Key Laboratory of Phytochemistry and Plant Resources in West China, Kunming Institute of Botany, Chinese Academy of Sciences, Kunming 650201, China*
[b]*College of Chinese Medicine, Jilin Agricultural Science and Technology College, Jilin 132101, China*
[c]*Weihe Biotech Research and Development Center, Yuxi 653101, China*

Received 28 August 2012; revised 23 October 2012; accepted 13 November 2012

KEY WORDS

Smilacina atropurpurea;
Convallariaceae;
Tetraglycosides;
Atropuroside H;
Atropuroside I

Abstract *Smilacina atropurpurea* (family Convallariaceae) is a perennial herb used medicinally for the treatment of lung ailments, rheumatism, menstrual disorders, and cuts and bruises, by the local people of its growing areas in southwest China. The tender aerial part has also been used as a wild vegetable by Lisu, Naxi and Tibetan people. Chemical analysis of the rhizomes of this plant has allowed us to isolate two new minor steroidal tetraglycosides, specifically atropurosides H (**1**) and I (**2**), by repeated column chromatography over silica gel and reversed phase silica. The structures were determined to be (25*R*,*S*)-12*β*-hydroxy-spirost-5-ene-3*β*-yl-*O*-*β*-*D*-glucopyranosyl-(1→2)-[*β*-*D*-xylopyranosyl-(1→3)]-*β*-*D*-glucopyranosyl-(1→4)-*β*-*D*-galactopyranoside (**1**) and (25*S*)-5*α*-spirost-17*α*-hydroxy-3*β*-yl-*O*-*β*-*D*-glucopyranosyl-(1→2)-[*β*-*D*-xylopyranosyl-(1→3)]-*β*-*D*-glucopyranosyl-(1→4)-*β*-*D*-galacto pyranoside (**2**), respectively, on the basis of detailed spectroscopic analysis including 1D and 2D NMR techniques.

*Corresponding author. Tel.: +86 871 5223235; fax: +86 871 5150124.
E-mail address: zhangyj@mail.kib.ac.cn (Yingjun Zhang).

Peer review under responsibility of Institute of Materia Medica, Chinese Academy of Medical Sciences and Chinese Pharmaceutical Association.

ELSEVIER Production and hosting by Elsevier

2211-3835 © 2013 Institute of Materia Medica, Chinese Academy of Medical Sciences and Chinese Pharmaceutical Association. Production and hosting by Elsevier B.V. All rights reserved.
http://dx.doi.org/10.1016/j.apsb.2012.12.002

1. Introduction

Smilacina atropurpurea (Franch) Wang et Tang, a convallariaceous perennial herb, is distributed mainly in the southwest region of the People's Republic of China[1]. The tender aerial part has been used as a wild vegetable by Lisu, Naxi and Tibetan people. The rhizome, as a traditional folk medicine, has been used for the treatment of lung ailments, rheumatism, menstrual disorders, cuts and bruises[2]. Our previous study on the fresh rhizomes of this herb led to the isolation of seven new steroidal saponins, atropurosides A–G, with polyhydroxylated aglycones[3]. This paper presents the continuing study on the minor constituents of this plant, from which two new spirostanol saponins possessing the same tetrasaccharide sugar chain are obtained (Fig. 1). Their structures were determined from detailed spectroscopic analysis.

2. Results and discussion

Further chemical study on the *n*-butanol fraction from the MeOH extract of the rhizomes of *S. atropurpurea* led to the isolation of two new saponins (**1** and **2**) by repeated CC over silica gel and reversed phase silica gel (RP-8).

The new compounds **1** and **2** showed positive reaction (green color) to the anisaldehyde reagent, but negative reaction to Ehrlich reagent, indicating the presence of a spirostanol skeleton[4].

Atropuroside H (**1**) was obtained as a white amorphous powder. Its molecular formula was assigned to be $C_{50}H_{80}O_{23}$ on the basis of the negative ion HR-ESI-MS (*m/z* 1047.5003 $[M–H]^-$; calcd. for 1047.5012) and ^{13}C NMR (DEPT) spectra. The ^1H and ^{13}C NMR spectra of **1** displayed four methyl proton signals at δ_H 0.65 (s), 0.87 (s), 1.04 (s), 1.41 (d, $J=6.4$ Hz), as well as two quaternary carbon signals at δ_C 109.6 and 110.0 (Table 1), typical of a steroidal skeleton. An olefinic proton at δ_H 5.27 (Br, s) and two olefinic carbon signals at δ_C 141.1 (C) and 121.7 (CH) indicated the presence of a double bond between C-5 and C-6 in the aglycone part of **1**[5]. Moreover, the down-field shifted methylene signal at δ_C 30.1 (C-11) and the up-field Me-18 signal at δ_C 11.1 suggested the existence of one β-oriented hydroxyl group at C-12 (δ_C 79.0), which was further confirmed by ROESY correlations between H-12 and H-14, H-17 and H-9. The ^{13}C NMR data displayed two sets of carbon signals from C-20 to C-27 at δ_C 43.1/43.5, 14.2/14.3, 109.6/110.0, 32.0/26.5, 29.4/26.3, 30.7/27.6, 66.9/65.2, and 17.4/16.3, belonging to the 25*R* and 25*S* epimers in F-ring. These signals were coincident with those of isochiapagenin [(25*R*)-3β,12β-diol-spirost-5-ene][6] and chiapagenin [(25*S*)-3β,12β-diol-spirost-5-ene][7]. Thus, the aglycone of **1** was determined as (25*R,S*)-3β,12β-diol-spirost-5-ene. The ^1H NMR spectrum displayed four anomeric proton signals at δ_H 4.85 (d, $J=7.5$ Hz), 5.16 (d, $J=7.7$ Hz), 5.21 (d, $J=7.6$ Hz), 5.55 (d, $J=7.1$ Hz), indicating a configuration at the anomeric centers. The presence of three hexosyls and one xylosyl was indicated according to the ^{13}C NMR and HMQC-TOCSY spectral data. The characteristic fragment ion peaks at *m/z* 885 $[M–162–H]^-$, 915 $[M–132–H]^-$, 753 $[M–162–132–H]^-$, 591 $[M–162–131–162–H]^-$ in the negative ion FAB-MS revealed the presence of two terminal saccharide units, including one xylosyl and one hexosyl unit. In the HMBC spectrum of **1**, long-range interactions were observed between the first hexosyl anomeric proton at δ_H 4.85 with C-3 (δ_C 78.3) of the aglycone, the second hexosyl anomeric proton at δ_H 5.16 with C-4 (δ_C 79.9) of the first hexosyl unit, and the xylosyl anomeric proton at δ_H 5.21 with C-3 (δ_C 86.9) of the second hexosyl unit, whose C-2 (δ_C 81.3) was correlated with the terminal third hexosyl anomeric proton (δ_H 5.55). The aforementioned data revealed the sugar linkage at C-3 of **1** as glucopyranosyl-(1→2)-[xylopyranosyl-(1→3)]-glucopyranosyl-(1→4)-galactopyranoyl, which was confirmed by the comparison of their NMR data with those reported in reference[8]. Therefore, the

Table 1 ^{13}C NMR spectral data of compounds **1** and **2** (in pyridine-d_5).

Position	1	2	Position	1	2
1	37.5	37.4	Gal-1	102.8	102.5
2	30.9	29.9	2	73.2	73.2
3	78.3	78.7	3	75.3	75.4
4	39.2	34.9	4	79.9	79.9
5	141.1	45.4	5	76.2	76.2
6	121.7	29.0	6	60.6	60.6
7	31.5	32.5	Glc I-1	105.0	105.0
8	30.9	35.9	2	81.3	81.3
9	50.1	52.8	3	86.9	86.9
10	37.2	35.9	4	70.8	70.8
11	30.1	21.1	5	78.7	77.8
12	79.0	32.3	6	63.0	63.0
13	46.3	45.4	Glc II-1	104.8	104.8
14	55.5	54.3	2	75.1	75.1
15	32.2	31.6	3	77.8	77.6
16	81.2	90.3	4	70.5	70.5
17	62.8	89.9	5	77.6	77.6
18	11.1	17.4	6	62.6	62.5
19	19.4	12.4	Xyl-1	105.1	105.1
	25*R*/25*S*		2	75.6	75.6
20	43.1/43.5	44.8	3	78.7	78.7
21	14.2/14.3	9.45	4	71.1	71.1
22	109.6/110.0	110.3	5	67.4	67.3
23	32.0/26.5	26.6			
24	29.4/26.3	25.8			
25	30.7/27.6	27.4			
26	66.9/65.2	65.0			
27	17.4/16.3	16.3			

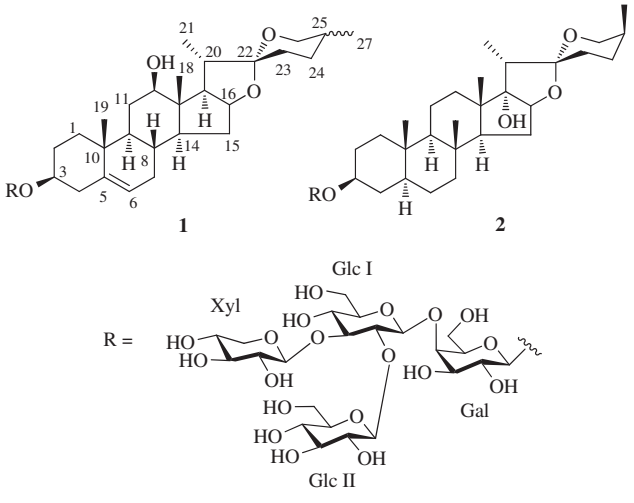

Figure 1 Structures of steroidal saponins **1** and **2** from *Smilacina atropurpurea*.

structure of **1** was assigned to be (25*R,S*)-12β-hydroxy-spirost-5-ene-3β-y1-*O*-β-*D*-glucopyranosyl-(1→2)-[β-*D*-xylopyranosyl-(1→3)]-β-*D*-glucopyranosyl-(1→4)-β-*D*-galactopyranoside. The tetrasaccharide moiety of glucopyranosyl-(1→2)-[xylopyranosyl-(1→3)]-glucopyranosyl-(1→4)-galactopyranosyl is widely present in the genus *Polygonatum*[8–15], *Yucca*[16], *Allium*[17], *Agave*[18,19] and *Polianthes*[20], and appeared to be the functional group in an antifungal assay for tigogenin glycosides[21].

Atropuroside I (**2**) was obtained as a white amorphous powder. The negative ion HR-ESI-MS (*m/z* 1049.5183 [M–H]$^-$; calcd. for 1049.5168) and ^{13}C NMR (DEPT) spectral data indicated a molecular formula of $C_{50}H_{82}O_{23}$ for **2**. The ^{1}H NMR spectrum of **2** displayed four methyl proton signals of a typical steroidal skeleton at δ_H 0.64 (s, Me-18), 0.92 (s, Me-19), 1.04 (d, *J*=7.0 Hz, M-27), 1.21 (d, *J*=6.7 Hz, Me-21). The ^{13}C NMR and DEPT spectra showed that the Me-21 signal was shifted up-field to δ_C 9.45, while the chemical shift of C-16 (CH) and C-17 (C) were shifted downfield to δ_C 90.3 and 89.9, respectively. These observations indicated the presence of one α-oriented hydroxyl group at C-17. Comparing the ^{13}C NMR data of the aglycone of **2** with those of the spirostanol type of pardarinoside B (22-*O*-methyl-26-*O*-acetyl-(25*R*)-5α-furost-17α-hydroxy-3β-yl-*O*-α-*L*-rhamnopyranosyl-(1→2)-β-*D*-glucopyranoside)[22] indicated that they were identical in the A–E rings, except for the configuration at C-25. The carbon signals from C-23 to C-27 at δ_C 26.6, 25.8, 27.4, 65.0 and 16.3 for F-ring of **2** were related to the *S*-configuration at C-25. Thus, the aglycone of **2** was deduced as a new sapogenin, (25*S*)-5α-spirost-3β,17α-diol. In the sugar part of **2**, it displayed identity to **1**, and was supported by NMR data. The identification of the four sugar units and their linkage sites were determined as with **1** by HMQC-TOCSY and HMBC spectra. Therefore, the structure of **2** was determined to be (25*S*)-5α-spirost-17α-hydroxy-3β-yl-*O*-β-*D*-glucopyranosyl-(1→2)-[β-*D*-xylo pyranosyl- (1→3)]-β-*D*-glucopyranosyl-(1→4)-β-*D*-galactopyranoside.

3. Materials and methods

3.1. General

NMR spectra were recorded on a Bruker DRX-500 instrument using pyridine-d_5 as solvent and TMS as an internal standard. The optical rotations were measured by a SEPA-3000 automatic digital polarimeter. FAB-MS (negative ion mode) and HR-ESI-MS (negative ion mode) spectra were recorded on VG AutoSpe 3000 and API Qstar Pulsar LC/TOF spectrometers, respectively. Column chromatography was carried out on silica gel (200–300 mesh and 10–40 μm, Qingdao Marine Chemical Factory) and reversed phase silica gel RP-8 (40–63 μm, Merck Co., Inc.) media. TLC was carried out on silica gel G pre-coated plates (Qingdao Haiyang Chemical Co.) with chloroform ($CHCl_3$): methanol (MeOH):water (H_2O) (7:3:0.5) as the eluant. Spots were detected by spraying with 10% of sulfuric acid, followed by heating.

3.2. Plant material

The fresh rhizome of *S. atropurpurea* was collected from Zhongdian, Yunnan, China, and the voucher specimen (Kun No. 0222958) is deposited at the Herbarium of Kunming Institute of Botany, Chinese Academy of Science.

3.3. Extraction and isolation

As our previous work reported[3], the *n*-BuOH fraction (100 g) of the fresh rhizomes (20 kg) of *S. atropurpurea* was subjected to a silica gel column chromatography (CC), eluting with $CHCl_3$:MeOH:H_2O (7:3:0.5) to give six fractions (Frs. 1–6). After isolating the major saponin of atropuroside F (155 mg) from Fr. 5 (12 g) by silica gel ($CHCl_3$:MeOH:H_2O, 8:2:0.2–7:3:0.5) CC, the remaining impure fractions from Fr. 5 were combined and further subjected to repeated CC over silica gel ($CHCl_3$:MeOH:H_2O, 7:3:0.5) and RP-8 (MeOH:H_2O, 4:6–1:0) to afford compounds **1** (6 mg) and **2** (4 mg).

3.4. Atropuroside H (*1*)

White amorphous powder, $[\alpha]_D^{26}=-66.0^\circ$ (*c*=0.24, MeOH). Negative ion FAB-MS *m/z*: 1048 [M]$^-$, 885 [M–162–H]$^-$, 915 [M–132–H]$^-$, 753 [M–162–132–H]$^-$, 591 [M–162–131–162–H]$^-$. Negative ion HR-ESI-MS *m/z*: 1047.5003 [M–H]$^-$ (calcd. for 1047.5012). ^{1}H NMR (500 MHz, pyridine-d_5): δ_H 0.65 (3H, d, *J*=6.0 Hz, Me-27), 0.87 (3H, s, Me-19), 0.91–0.92 (1H, m, Ha-1), 1.00–1.02 (1H, m, H-9), 1.04 (3H, s, Me-18), 1.07–1.09 (1H, m, H-14), 1.41 (3H, d, *J*=6.4 Hz, Me-21), 1.82–1.84 (1H, m, Hb-1), 2.11–2.14 (1H, m, H-17), 2.15–2.17 (1H, m, H-20), 2.36–2.37 (1H, m, Ha-4), 2.63 (1H, br d, *J*=13.5 Hz, Hb-4), 3.51–3.53 (1H, m, H-12), 3.65 (1H, t, *J*=10.2 Hz, Xyl Ha-5), 3.80–3.82 (1H, m, Glc I H-4), 3.90–3.94 (2H, m, H-3, Glc II H-2), 4.02–4.05 (2H, m, Glc I Ha-6, Xyl H-2), 4.07–4.10 (2H, m, Xyl H-4, Gal H-3), 4.12–4.15 (2H, m, Glc I H-3, Gal Ha-6), 4.17–4.19 (1H, m, Glc II H-4), 4.20–4.22 (1H, m, Xyl Hb-5), 4.31–4.32 (1H, m, Glc II Ha-6), 4.36–4.39 (2H, m, Glc I H-2, Gal H-2), 4.49 (1H, br d, *J*=10.6 Hz, Glc I Hb -6), 4.53–4.56 (2H, m, Glc II Hb-6, H-16), 4.57 (1H, br s, Gal H-4), 4.64 (1H, t, *J*=10.4 Hz, Gal Hb-6), 4.85 (1H, d, *J*=7.5 Hz, Gal H-1), 5.16 (1H, d, *J*=7.7 Hz, Glc I H-1), 5.21 (1H, d, *J*=7.6 Hz, Xyl H-1), 5.27 (1H, br s, H-6), 5.55 (1H, d, *J*=7.1 Hz, Glc II H-1). ^{13}C NMR (125 MHz, pyridine-d_5): see Table 1.

3.5. Atropuroside I (*2*)

White amorphous powder, $[\alpha]_D^{25}=-1.1^\circ$ (*c*=0.15, MeOH). Negative ion FAB-MS *m/z*: 1049 [M–H]$^-$, 887 [M–162–H]$^-$, 756 [M–162–131–H]$^-$, 593 [M–162–131–163–H]$^-$. Negative ion HR-ESI-MS *m/z*: 1049.5183 [M–H]$^-$ (calcd. for 1049.5168). ^{1}H NMR (500 MHz, pyridine-d_5): δ_H 0.64 (3H, s, Me-18), 0.92 (3H, s, Me-19), 1.04 (3H, d, *J*=7.0 Hz, Me-27), 1.21 (3H, d, *J*=6.7 Hz, Me-21), 3.65 (1H, t, *J*=10.2 Hz, Xyl Ha-5), 3.81–3.83 (1H, m, Glc I H-4), 3.94–3.96 (1H, m, Glc II H-2), 4.03–4.07 (3H, m, Glc I Ha-6, Xyl H-2, Gal H-3), 4.08–4.10 (2H, m, Xyl H-4, Glc I H-3), 4.12–4.13 (1H, m, Gal Ha-6), 4.15 (1H, t, *J*=8.4 Hz, Glc II H-4), 4.20–4.22 (1H, m, Xyl Hb-5), 4.30 (1H, br d, *J*=10.6 Hz, Glc II Ha-6), 4.36–4.38 (1H, m, Glc I H-2), 4.40–4.42 (1H, m, Gal H-2), 4.52 (1H, t, *J*=10.3 Hz, Glc I Hb-6), 4.54 (1H, br d, *J*=10.6 Hz, Glc II Hb-6), 4.56 (1H, br s, Gal H-4), 4.58 (1H, t, *J*=10.2 Hz, Gal Hb-6), 4.86 (1H, d, *J*=7.6 Hz, Gal H-1), 5.17 (1H, d, *J*=7.7 Hz, Glc I H-1), 5.22 (1H, d, *J*=7.8 Hz, Xyl H-1), 5.55 (1H, d, *J*=7.4 Hz, Glc II H-1). ^{13}C NMR (125 MHz, pyridine-d_5): see Table 1.

Acknowledgment

The authors are grateful to members of the analytical group in State Key Laboratory of Phytochemistry and Plant Resources in West China, Kunming Institute of Botany, for measuring all the spectral data. This work was supported by the 973 Program of Ministry of Science and Technology of China (2011CB915503).

References

1. Kunming Institute of Botany. Chinese Academy of Sciences. Flora Yunnanica. Beijing: Science Press 1997, pp. 748.
2. State Administration of Traditional Chinese Medicine. *Zhong Hua Ben Cao*. Shanghai: Shanghai Press of Science and Techniques; 1999, pp. 156.
3. Zhang Y, Li HZ, Zhang YJ, Jacob MR, Khan SI, Li XC, et al. Atropurosides A–G, new steroidal saponins from *Smilacina atropurpurea*. *Steroids* 2006;**71**:712–9.
4. Kiyosawa S, Hutoh M. Detection of proto-type compounds of diosgenin- and other spirostanol-glycosides. *Chem Pharm Bull* 1968;**16**:1162–4.
5. Espejo O, Lavot CJ, Jung H, Giral F. Spirostanic diosgenin precursors from *Dioscorea composite* tubers. *Phytochemistry* 1982;**21**:413–6.
6. Agrawal PK, Jain DC, Gupta RK, Thakur RS. ^{13}C NMR spectroscopy of steroidal sapogenins and steroidal saponins. *Phytochemistry* 1985;**24**:2479–96.
7. Serota S, Koob RP, Wall ME. Steroidal sapogenins, LXIII. Chiapagenin, a new normal (25 L) Δ-12β-hydroxysapogenin. *J Org Chem* 1960;**25**:1768.
8. Ono M, Shoyama K, Nohara T. The constituents of Chinese *Polygonati officinalis* rhizome and *Polygonati* rhizome. *Shoyakugaku Zasshi* 1988;**42**:135–42.
9. Lin HW, Han GY, Liao SX. Studies on the active constituents of the Chinese traditional medicine *Polygonatum odoratum* (MILL.) Druce. *Acta Pharm Sin* 1994;**29**:215–22.
10. Ahn MJ, Kim CY, Yoon KD, Yoon KD, Ryu MY, Cheong JH, et al. Steroidal saponins from the rhizomes of *Polygonatum sibiricum*. *J Nat Prod* 2006;**69**:360–4.
11. Son KH, Do JC. Steroidal saponins from the rhizomes of *Polygonatum sibiricum*. *J Nat Prod* 1990;**53**:333–9.
12. Sun LR. Studies on chemical constituents and biological activitives of Polygonatum sibiricum [dissertation]. Shenyang: Shenyang Pharmaceutical University; 1999.
13. Sugiyama M, Nakano K, Tomimatsu T, Nohara T. Five steroidal components from the rhizomes of *Polygonatum odoratum* var. *pluriflorum*. *Chem Pharm Bull* 1984;**32**:1365–72.
14. Huang PL, Gan KH, Wu RH, Lin CN. Benzoquinones, a homoisoflavanone and their constituents from *Polygonatum altelobatum*. *Phytochemistry* 1997;**44**:1369–73.
15. Yesilada E, Houghton PJ. Steroidal saponins from the rhizomes of *Polygonatum orientale*. *Phytochemistry* 1991;**30**:3045–9.
16. Nakano K, Matsuda E, Tsurumi K, Yamasaki T, Murakami K, Takaishi Y, et al. The steroidal glycosides of the flowers of *Yucca gloriosa*. *Phytochemistry* 1988;**27**:3235–9.
17. Inoue T, Mimaki Y, Sashida Y, Nishino A, Satomi Y, Nishino H. Steroidal glycosides from *Allium macleanii* and *A. senescens*, and their inhibitory activity on tumour promoter-induced phospholipid metabolism of Hela cells. *Phytochemistry* 1995;**40**:521–5.
18. Jin JM, Liu XK, Yang CR. Three new hecogenin glycosides from fermented leaves of *Agave americana*. *J Asian Nat Prod Res* 2003;**5**:95–103.
19. Jin JM, Liu XK, Teng RW, Yang CR. Two new steroidal glycosides from fermented leaves of *Agave americana*. *Chin Chem Lett* 2002;**13**:629–32.
20. Jin JM, Zhang YJ, Yang CR. Spirostanol and furostanol glycosides from the fresh tubers of *Polianthes tuberosa*. *J Nat Prod* 2004;**67**:5–9.
21. Yang CR, Zhang Y, Jacob MR, Khan SI, Zhang YJ, Li XC. Antifungal activity of C-27 steroidal saponins. *Antimicrob Agents Chemother* 2006;**50**:1710–4.
22. Shimomura H, Sashida Y, Mimaki Y. 26-*O*-acylated furostanol saponins pardarinoside A and B from the bulbs of *Lilium pardarinum*. *Chem Pharm Bull* 1988;**36**:3226–9.