· Letter ·

Chemical Constituents in Charred *Sanguisorbae Radix*

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Abstracts: **Objective** To study the chemical constituents in the effective fractions of charred *Sanguisorbae Radix*. **Methods** The compounds were isolated and purified by column chromatography and their structures were identified on the basis of physicochemical properties and spectral analysis. **Results** Five compounds were isolated and identified as 3β-hydroxy-28-norurs-17,19,21-trien (**1**), 3β-hydroxy-28-norurs-12,17-dien (**2**), 3β,19α-dihydroxyurs-13(18)-en-28-oic acid (**3**), 3β-[(α-*L*-arabin-opyranosyl) oxy]-28-norurs-12,17-dien (**4**), and pomolic acid (**5**). **Conclusion** Compounds **1**, **3**, and **4** are novel compounds belong to triterpenoids and triterpenoid saponins, named as sanguisorbigenins Z, Y_1, and Y_2, respectively.

Key words: charred *Sanguisorbae Radix*; pomolic acid; sanguisorbigenin Y_1; sanguisorbigenin Y_2; sanguisorbigenin Z
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Introduction

Garden burnet root is the dried root of *Sanguisorba officinalis* L. or *S. officinalis* L. var. *longifolia* (Bert.) Yu et Li (Rosaceae) (Pharmacopoeia Committee of P. R. China, 2010). It distributes widely in China. It has shown some therapeutic activities, such as cooling blood and detoxification. Currently, *Sanguisorbae Radix* (SR) and charred *Sanguisorbae Radix* (CSR) are the most commonly-used processed products in clinic (The Traditional Chinese Medicine Preparation Standards, 1988). After being charred, SR became CSR, and its hemostatic effect was enhanced, and its main efficacy was changed to convergence bleeding (Guo, Jia, and Xu, 2001). In order to explore the material basis for the enhanced role to stop bleeding, we conducted a study on the chemical compositions in the EtOAc and $CHCl_3$ fractions then identified them as the effective fractions with hemostatic activity. In preliminary study, we have already isolated and purified 11 compounds from EtOAc fraction and six compounds from $CHCl_3$ fraction (Xia *et al*, 2010a; 2010b). To further study the material basis of hemostatic activity of CSR, we continued to study the chemical constituents in $CHCl_3$ fraction.

By column chromatography, preparative TLC, and other means, five compounds were isolated and identified as 3β-hydroxy-28-norurs-17,19,21-trien (**1**), 3β-hydroxy-28-norurs-12,17-dien (**2**), 3β,19α-dihydroxyurs-13(18)-en-28-oic acid (**3**), 3β-[(α-*L*-arabin-opyranosyl) oxy]-28-norurs-12,17-dien (**4**), and pomolic acid (**5**). Compounds **1**, **3**, and **4** are novel compounds belong to triterpenoids and triterpenoid saponins, named as sanguisorbigenins Z, Y_1, and Y_2, respectively. By studying the influence of processing on the content of sanguisorbigenin Z, we found that it could be an inex component to control the quality and processing degree of CSR (Dai *et al*, 2011). We also have confirmed that sanguisorbigenins Y_1 and Y_2 showed anticancer activity (Sun *et al*, 2011a; 2011b).

Materials and methods

Apparatus and reagents

Melting points were determined using a Stuart Smp3

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Melting Point Apparatus. MS spectra were recorded with Agilent Trap VL Mass Spectrometer. IR spectra were obtained with a Nicolet Avatar—330 FT-IR. UV spectra were measured on a Shimadiu UV—2201 UV-Vis Spectrophotometer. ^{1}H-NMR (400 MHz) and ^{13}C-NMR (100 MHz) spectra were recorded with Bruker Advanced 400 NMR Spectrometers.

Column chromatography was performed with silica gel (Qingdao Haiyang Chemical Co., Ltd., China). Ethanol, (60 — 90) ℃ petroleum ether, acetone, chloroform, and other reagents were of analytical grade.

Plant materials

Sanguisorbae Radix was collected from Longxi in Gansu province (China), identified by Prof. LIN Hui-bin in Shandong Academy of Chinese Medicine, and processed to obtain charred *Sanguisorbae Radix*. A voucher specimen was deposited in the Unit for Research of Processing in Shandong Academy of Chinese Medicine.

Extraction and isolation

The dried and powdered CSR (12 kg) was extracted with 80% ethanol (120 L) twice (2 h each time) under reflux. The extract was combined and concentrated under reduced pressure, and was subsequently partitioned successively with petroleum ether, $CHCl_3$, EtOAc, and *n*-butanol. The $CHCl_3$ fraction (214.8 g) was subjected to column chromatography on silica gel (1200 g, 160—200 mesh) eluted with petroleum ether-acetone (100:0→5:5).

Compounds **1** (84.6 mg) and **2** (281.2 mg) were obtained from the petroleum ether-acetone (95:5) fraction. Compound **3** (15.9 mg) was obtained from the petroleum ether-acetone (8:2) fraction. Compound **4** (22.9 mg) was obtained from the petroleum ether-acetone (75:25) fraction.

Based on TLC analysis, petroleum ether-acetone (8:2) and petroleum ether-acetone (75:25) fractions were emerged and then were further separated on a silica gel H eluting with $CHCl_3$-MeOH. Compound **5** (17.3 mg) was obtained eluting with $CHCl_3$-MeOH (93:7).

Results and discussion

Compound **1**: white crystal (EtOAc), mp 244—246 ℃. $[\alpha]_D^{20}$ + 0.39° (*c* 0.054, MeOH). UV λ_{max}^{MeOH} (nm): 215.7, 273.0. IR ν_{max}^{KBr} (cm^{-1}): 3471 (OH), 2937, 1468, 1449 (C=C, benzene ring). HR-ESI-MS (negative) *m*/*z*: 407.3315 $[M - H]^+$ ($C_{29}H_{43}O$, calcd. 407.3308). ^{1}H-NMR (400 MHz, C_5D_5N) δ: 0.94 (3H, s, 25-CH_3), 0.95 (3H, s, 27-CH_3), 1.03 (3H, s, 26-CH_3), 1.08 (3H, s, 24-CH_3), 1.26 (3H, s, 23-CH_3), 2.21 (3H, s, 30-CH_3), 2.23 (3H, s, 29-CH_3), 6.94 (1H, d, *J* = 7.6 Hz, H-22), 7.01 (1H, d, *J* = 7.6 Hz, H-21), 3.49 (1H, m, H-3). The ^{13}C-NMR spectrum of compound **1** (Table 1) exhibited seven tertiary methyl groups, four quaternary olefinic carbons, two olefinic methines, and one hydroxyl methine. The ^{1}H-NMR and ^{13}C-NMR spectral data of compound **1** showed two *o*-positioned aromatic protons δ_H 6.94 (1H, d, *J* = 7.6 Hz, H-22), 7.01 (1H, d, *J* = 7.6 Hz, H-21), with six typical aromatic carbon signals at δ_C 136.0, 140.0, 135.9, 134.8, 127.0, and 126.3. By analyzing the 2D NMR spectra, compound **1** was revealed to have a 28-nor-ursane skeleton. HMBC correlations are observed from Me-29 to quaternary C-18 and 20, as well as from Me-30 to quaternary C-19 and 21. One of the two aromatic protons H-21 shows HMBC correlations with quaternary C-17, C-19, and Me-30. H-22 shows HMBC correlations with quaternary C-18, C-20, and methylene C-16. Through analyzing HSQC and HMBC, the hydroxymethine proton was assigned as H-3. NOESY correlation between H-3 and H_3-23/H-5 indicated the α-orientation of H-3. Therefore, the structure of compound **1** was assigned as 3β-hydroxy-28-norurs-17,19,21-trien. It is a new compound named sanguisorbigenin Z (Fig. 1).

Compound **2**: white crystal (EtOAc), mp 205—207 ℃. $[\alpha]_D^{20}$ + 0.11° (*c* 0.102, MeOH). UV λ_{max}^{MeOH} (nm): 300.0, 243.9, 236.6. IR ν_{max}^{KBr} (cm^{-1}): 3464 (OH), 2938 (saturated C-H), 1452, 1386 (CH_3). HR-ESI-MS (negative) *m*/*z*: 409.3474 $[M - H]^-$ ($C_{29}H_{45}O$, calcd. 409.3465). ^{1}H-NMR (400 MHz, C_5D_5N) δ: 0.95 (3H, s, 26-CH_3), 1.02 (3H, s, 25-CH_3), 1.05 (3H, s, 27-CH_3), 1.08 (3H, s, 24-CH_3), 1.27 (3H, s, 23-CH_3), 0.88 (3H, d, *J* = 6.9 Hz, 29-CH_3), 0.96 (3H, d, *J* = 7.9 Hz, 30-CH_3), 3.50 (1H, m, H-3), 5.71 (1H, brs, H-12). The ^{13}C-NMR spectrum of compound **2** (Table 1) exhibited seven methyl carbons, three quaternary olefinic carbons, one olefinic methane, and one hydroxyl methine. The ^{1}H-NMR and ^{13}C-NMR spectral data showed an olefinic proton [δ 5.71 (1H, brs, H-12)], with four typical olefinic carbon signals at δ_C 117.5, 137.6, 128.9, and 133.5. By analyzing the 2D NMR spectra, compound **2** was revealed to have a 28-nor-ursane

skeleton. HMBC correlations were observed from H-11 to an olefinic methine C-12 and a quaternary olefinic carbon C-13, from Me-27 to a quaternary olefinic carbon C-13, from Me-29 to a quaternary olefinic carbon C-18, as well as from H-15 to a quaternary olefinic carbon C-17. Through analyzing HSQC and HMBC, the hydroxymethine proton was assigned as H-3. NOESY correlation between H-3 and H_3-23/H-5 indicated the α-orientation of H-3. Therefore, compound **2** was assigned as 3β-hydroxyl-28-norurs-12,17-dien.

Compound **3**: white powder (EtOAc), mp 257—260 ℃, $[\alpha]_D^{20}$ + 57.7° (*c* 0.017, MeOH). UV λ_{max}^{MeOH} (nm): 287.2, 243.7, 236.6. IR ν_{max}^{KBr} (cm^{-1}): 3421 (OH), 2938 (saturated C-H), 1450, 1388 (CH_3), 1065 (C-O). HR-ESI-MS (negative) *m*/*z*: 541.3891 $[M - H]^-$ ($C_{34}H_{53}O_5$, calcd. 541.3888). ^{1}H-NMR (400 MHz, C_5D_5N) *δ*: 0.91 (3H, s, 26-CH_3), 0.94 (3H, s, 25-CH_3), 0.94 (3H, s, 27-CH_3), 1.05 (3H, s, 24-CH_3), 1.07 (3H, s, 23-CH_3), 0.85 (3H, d, *J* = 6.1 Hz, 29-CH_3), 0.96 (3H, d, *J* = 5.9 Hz, 30-CH_3), 3.30 (1H, m, H-3), 5.68 (1H, brs, H-12), 5.59 (1H, d, *J* = 2.0 Hz, Ara-H-1′). The ^{13}C-NMR spectrum of compound **3** (Table 1) exhibited seven methyl carbons, three quaternary olefinic carbons, one olefinic methine, one oxymethine, and one anomeric carbon. The ^{1}H-NMR and ^{13}C-NMR spectra showed an olefinic proton [δ_H 5.68 (1H, brs, H-12)], with four typical olefinic carbon signals at δ_C 117.4, 137.6, 128.9, and 133.5. By analyzing the 2D NMR spectra, the aglycone of compound **3** is revealed to have a 28-nor-ursane skeleton. HMBC correlations were observed from H-11 to an olefinic methine C-12 and a quaternary olefinic carbon C-13, from Me-27 to a quaternary olefinic carbon C-13, from Me-29 to a quaternary olefinic carbon C-18, as well as from H-15 to a quaternary olefinic carbon C-17. Through analyzing HSQC and HMBC, the hydroxymethine proton was assigned as H-3. NOESY correlation between H-3 and H_3-23/H-5 indicated the α-orientation of H-3. Based on the above evidences, the aglycone of compound **3** was elucidated as 3β-hydroxy-28-norurs-12, 17-dien, same as compound **2**. The ^{1}H-NMR spectrum showed one anomeric proton resonating at δ_H 5.59, which was correlated with δ_C 87.3 from the HMQC spectrum. The difference between compounds **2** and **3** was that the C-3 signal of compound **3** shifted from *δ* 78.2 to *δ* 87.3. HMBC correlations were also observed from an anomeric proton Ara-H-1′ to the oxymethine carbon C-3. The coupling constant of Ara-H-1′ (d, *J* = 2.0 Hz) confirmed the α-glycosidic linkage for the *L*-arabinose units. The ^{13}C-NMR spectrum confirmed that the *L*-arabinose was α-*L*-arabinofuranoside. After acid hydrolysis, the sugar units were confirmed to be *L*-arabinose by gas chromatographic (GC) analysis. Therefore, compound **3** was assigned as 3β-[(α-*L*-arabinofuranosyl)oxy]-28-norurs-12,17-dien. It is a new compound named as sanguisorbin Y_1 (Fig. 1).

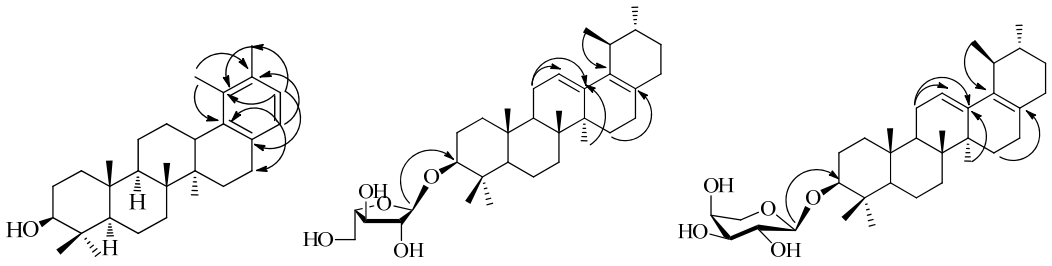
Compound **4**: white crystal (EtOAc), $[\alpha]_D^{20}$ + 0.44° (*c* 0.132, MeOH). UV λ_{max}^{MeOH} (nm): 242.9, 235.7, IR ν_{max}^{KBr} (cm^{-1}): 3484 (OH), 2936 (saturated C-H), 1452, 1388 (CH_3), 1090 (C-O). HR-ESI-MS (negative) *m*/*z*: 541.3892 $[M - H]^-$ ($C_{34}H_{53}O_5$, calcd. 541.3888). ^{1}H-NMR (400 MHz, C_5D_5N) *δ*: 0.91 (3H, s, 26-CH_3), 0.97 (3H, s, 25-CH_3), 1.01 (3H, s, 27-CH_3), 1.07 (3H, s, 24-CH_3), 1.33 (3H, s, 23-CH_3), 0.85 (3H, d, *J* = 6.9 Hz, 29-CH_3), 0.95 (3H, d, *J* = 6.9 Hz, 30-CH_3), 3.30 (1H, m, H-3), 5.70 (1H, brs, H-12), 4.79 (1H, d, *J* = 7.0 Hz, Ara-H-1′). The ^{13}C-NMR spectrum of compound **4** (Table 1) exhibited seven methyl carbons, three quaternary olefinic carbons, one olefinic methine, one oxymethine, and one anomeric carbon. The ^{1}H-NMR and ^{13}C-NMR spectra showed an olefinic proton δ_H 5.70 (1H, brs, H-12), with four typical olefinic carbon signals at δ_C 117.4, 137.5, 128.9, and 133.4. By analyzing the 2D NMR spectra, the aglycone of compound **4** was revealed to have a 28-nor-ursane skeleton. HMBC correlations were observed from H-11 to an olefinic methine C-12 and a quaternary olefinic carbon C-13, from Me-27 to a quaternary olefinic carbon C-13, from Me-29 to a quaternary olefinic carbon C-18, as well as from H-15 to a quaternary olefinic carbon C-17. Through analyzing HSQC and HMBC, the hydroxymethine proton was assigned as H-3. NOESY correlation between H-3 and H_3-23/H-5 indicated the α-orientation of H-3. Based on the above evidences, the aglycone of compound **4** is elucidated as 3β-hydroxy-28-norurs-12,17-dien, same as compound **2**. The ^{1}H-NMR spectrum showed one anomeric proton resonating at δ_H 4.79, which was correlated with carbon at δ_C 88.7 from the HMQC spectrum. The ^{13}C-NMR spectrum showed a difference between compounds **2** and **4** and the C-3 signal of compound **4** shifted from *δ* 78.2 to *δ* 88.7. HMBC correlations were also observed

from an anomeric proton Ara-H-1′ to the oxymethine carbon C-3. The coupling constant of Ara-H-1′ (d, J = 2.0 Hz) confirmed the α-glycosidic linkage for the *L*-arabinose units. The ^{13}C-NMR spectrum confirmed the *L*-arabinose was α-*L*-arabinopyranoside. After acid hydrolysis, the sugar units were confirmed to be *L*-arabinose by GC analysis. Therefore, compound **4** was assigned as 3β-[(α-*L*-arabinopyranosyl) oxy]-28-norurs-12,17-dien. It is a new compound named as sanguisorbin Y_2 (Fig. 1).

Compound **5**: white powder (EtOAc). The positive ESI-MS exhibited $[M + Na]^+$ at m/z 495.1, exhibited $[M-H]^-$ at m/z 471.1. ^{1}H-NMR (600 MHz, C_5D_5N) δ: 1.11 (3H, d, J = 6.0 Hz, 30-CH_3), 0.90, 1.02, 1.11, 1.23, 1.45, 1.73 (18H, s, 6 × CH_3), 3.06 (1H, brs, H-18), 3.43 (1H, m, H-3), 5.61 (1H, brs, H-12). The spectrum data were in accordance with those of pomolic acid (Ju *et al*, 2003).

Table 1 ^{13}C-NMR (100 MHz) spectral data of compounds 1—4 (C_5D_5N, δ)

Positions	1	2	3	4	Positions	1	2	3	4
1	39.3	39.4	39.1	39.2	19	135.9	32.4	32.4	32.3
2	28.4	28.2	27.4	27.4	20	134.8	33.0	33.0	33.0
3	78.1	78.2	87.3	88.7	21	127.0	24.9	24.9	24.9
4	39.6	39.5	39.3	39.6	22	126.3	32.3	32.3	32.3
5	56.3	56.1	56.1	56.1	23	28.6	28.8	28.6	28.6
6	18.9	18.9	18.7	18.6	24	16.3	16.6	17.0	17.0
7	34.2	34.4	34.3	34.3	25	16.9	16.3	16.2	16.2
8	41.3	39.1	39.0	39.0	26	16.0	17.2	17.1	17.1
9	41.8	47.8	47.7	47.7	27	17.2	20.9	20.8	20.8
10	37.6	37.3	37.0	37.0	28	—	—	—	—
11	22.1	23.9	23.9	23.9	29	19.2	13.4	13.4	13.4
12	26.9	117.5	117.4	117.4	30	20.7	19.8	19.8	19.8
13	41.6	137.6	137.6	137.5	3-*O*-Ara				
14	52.1	41.1	41.1	41.0	1′			111.9	107.5
15	29.0	27.4	26.5	26.8	2′			84.0	73.0
16	28.3	28.6	28.2	28.2	3′			78.8	74.7
17	136.0	128.9	128.9	128.9	4′			85.3	69.5
18	140.0	133.5	133.5	133.4	5′			62.9	66.7



1 **3** **4**

Fig. 1 Key HMBC correlations of compounds 1, 3, and 4

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