A New Triterpenoid Saponin from Aidi Injection

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Abstract: **Objective** To investigate the chemical constituents from Aidi Injection. **Methods** The chemical constituents were isolated by chromatography on Sephadex LH-20 gel columns and reverse phase semi-preparative HPLC repeatedly. Their structures were identified by spectroscopic analysis (NMR and MS). **Results** Twenty-two compounds were isolated and identified to be 3-*O*-3′,4′-diacetyl-β-*D*-xylopyranosyl-6-*O*-β-*D*-glucopyranosyl-cycloastragenol (**1**), astragaloside IV (**2**), astragaloside II (**3**), astragaloside I (**4**), isoastragaloside I (**5**), acetylastragaloside I (**6**), ginsenosid Re (**7**), ginsenoside Rf (**8**), ginsenoside Rg_1 (**9**), ginsenoside Rb_3 (**10**), notoginsenoside R_4 (**11**), ginsenoside Rb_1 (**12**), ginsenoside Rc (**13**), ginsenoside Rb_2 (**14**), ginsenoside Rd (**15**), lucyoside H (**16**), 3-*O*-β-*D*-glucopyranosyl(1→4)-β-*D*-glucopyranosyl(1→3)-α-*L*-rhamnopyranosyl (1→2)-α-*L*-arabinopyranosyl oleanolic acid 28-*O*-α-*L*-rhamnopyranosyl(1→4)-β-*D*-glucopyranosyl(1→6)-β-*D*-glucopyranoside (**17**), 3-*O*-β-*D*-glucopyranosyl(1→3)-α-*L*-rhamnopyranosyl [β-*D*-glucopyranosyl-(1→4)]-(1→2)-α-*L*-arabinopyranosyl oleanolic acid 28-*O*-α-*L*-arabinopyranosyl(1→4)-β-*D*-glucopyranosyl(1→6)-β-*D*-glucopyranoside (**18**), syringin (**19**), elentheroside E (**20**), 4-(1,2,3-trihydroxypropyl)-2,6-dimethoxyphenyl-1-*O*-β-*D*-glucopyranoside (**21**), and coniferin (**22**). **Conclusion** Compounds **1**－**6** are originated from *Astragalus membranceus*, compounds **7**－**18** are originated from *Panax ginseng*, and compounds **19**－**22** are originated from *Acanthopanax senticosus* by LC-MS analysis. Compound **1** is a new compound.

Key words: Aidi Injection; astragaloside I; astragaloside II; astragaloside IV; 3-*O*-3′,4′-diacetyl-β-*D*-xylopyranosyl-6-*O*-β-*D*-glucopyranosyl-cycloastragenol
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Introdution

Aidi Injection (ADI), made by extraction from Renshen (*Ginseng Radix* et *Rhizoma*), Huangqi (*Astragali Rdix*), Ciwujia (*Acanthopanacis Senticosi Radix* et *Rhizoma* seu *Caulis*), and Banmao (*Mylabris*), is used for the clinical treatment of cancer. It is reported that ADI could inhibit the growth of tumor with the mechanism of inducing apoptosis, decreasing the side effect of radiotherapy, and increasing immune function, which could also improve life quality of cancer patients (Duan, Fan, and Hou, 2005; Xu, Luo, and Li, 2005). To discover the active agents from ADI, the chemical constituents were systematically investigated. Twenty-two compounds including a new compound, 3-*O*-3′, 4′-diacetyl-β-*D*-xylopyranosyl-6-*O*-β-*D*-glucopyranosyl-cycloastragenol were isolated. The new compound was originated from *A. membranceus* by LC-MS analysis.

Materials and methods

Equipments

^{1}H-NMR and ^{13}C-NMR spectra were recorded on a Bruker ZAB-HS Spectrometer; HR-ESI-MS data were obtained on QSTAR Elite Instrument; ODS was purchased from Qingdao Marine Chemical Factory; Sephadex LH-20 was purchased from Beijing Marine Chemical Factory.

Plant material

ADI (20100801) and plant material [the roots and rhizomes of *Panax ginseng* C. A. Mey., the roots of *Astragalus membranceus* (Fisch.) Bge., and the roots and rhizomes of *Acanthopanax senticosus* (Rupr. et Maxim.) Harms] were provided by Guizhou Yibai Medicine Co., Ltd.

Extraction and isolation

ADI (10 L) was evaporated under reduced

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pressure. The extract was subjected to MPLC on ODS (20－40 μm) column and eluted with gradient mixture of MeOH-H_2O to afford six fractions (Frs. 1－6). All these fractions were further purified by semi-preparative HPLC on a Zorbax SB-ODS column at a flow rate of 2.0 mL/min. Fr. 1 was eluted by MeOH-H_2O (80:20) and yielded pure compounds **1**, **5**, and **6**; Further purification of Fr. 2 using MeOH-H_2O (65:35) as eluant yielded compounds **2**－**4**; Further purification of Fr. 3 using MeOH-H_2O (70:30) as eluant yielded compounds **7**－**10**; Fr. 4 was eluted by MeOH-H_2O (75:25→85:15) gradient elution, yielded compound **11**; Further purification of Fr. 5 using MeOH-H_2O (68:32) as eluant yielded compounds **12**－**18**; Fr. 6 was eluted by MeOH-H_2O (30:70) and yielded compounds **19**－**22**.

Most structures of those compounds were identified by comparison of spectrum data with those in literatures. Twenty compounds were identified to be 3-*O*-3′,4′-diacetyl-β-*D*-xylopyranosyl-6-*O*-β-*D*-glucopyranosyl-cycloastragenol (**1**, 23.0 mg), astragaloside IV (**2**, 104.2 mg, Liu, Wang, and Liang, 2008), astragaloside II (**3**, 31.1 mg), and astragaloside I (**4**, 13.1 mg), isoastragaloside I (**5**, 22.1 mg), acetyl-astragaloside I (**6**, 8.2 mg, Liu, Wang, and Liang, 2008), ginsenosid Re (**7**, 15.2 mg), ginsenoside Rf (**8**, 12.1 mg), ginsenoside Rg_1 (**9**, 78.3 mg), ginsenoside Rb_3 (**10**, 12.4 mg, Wang, Wang, and Xu, 1991), notoginsenoside R_4 (**11**, 14.2 mg), ginsenoside Rb_1 (**12**, 12.0 mg), ginsenoside Rc (**13**, 156.3 mg), ginsenoside Rb_2 (**14**, 35.3 mg), ginsenoside Rd (**15**, 123.5 mg), lucyoside H (**16**, 156 mg, Du and Gao, 2006), 3-*O*-β-*D*-glucopyranosyl (1→4)-β-*D*-glucopyranosyl (1→3)-α-*L*-rhamnopyranosyl (1→2)-α-*L*-arabinopyranosyl oleanolic acid 28-*O*-α-*L*- rhamnopyranosyl (1→4)-β-*D*-glucopyranosyl (1→6)-β- *D*-glucopyranoside (**17**, 17.2 mg, Xia *et al*, 2004), 3-*O*-β-*D*-glucopyranosyl (1→3)-α-*L*-rhamnopyranosyl [β-*D*-glucopyranosyl-(1→4)]-(1→2)-α-*L*-arabinopyranosyl oleanolic acid 28-*O*-α-*L*-arabinopyranosyl (1→4)-β-*D*-glucopyranosyl (1→6)-β-*D*-glucopyranoside (**18**, 15.8 mg), syringin (**19**, 210.3 mg, Zhang, Ye, and Yan, 2000), elentheroside E (**20**, 304.5 mg, Wang, 1980), 4-(1, 2, 3-trihydroxypropyl)-2,6-dimethoxyphenyl-1-*O*-β-*D*-glucopyranoside (**21**, 25.2 mg, Kong and luo, 1990), and coniferin (**22**, 22.0 mg, Li *et al*, 2005).

Results

Compound **1** was obtained as white amorphous powder. $[\alpha]_D^{18}$ +18.1° (*C* 0.05, MeOH). Its molecular formula was assigned as $C_{45}H_{72}O_{16}$ by HR-ESI-MS at *m/z*: 891.4718 $[M + Na]^+$, with the degree of unsaturation of 10. IR spectra of compound **1** suggested the presence of hydroxyl and carbonyl groups at 3410, 1740, 1230, and 1050 cm^{-1}. Compound **1** was inferred to be a triterpenoid saponin by the fact of positive Molish and Liebermann-Burchard reactions.

The ^{1}H-NMR (500 MHz, CD_3OD) data of compound **1** (Table 1) showed seven singlets belonging to seven tertiary methyl groups at δ 1.00 (3H, s, CH_3-30), 1.01 (3H, s, CH_3-29), 1.12 (3H, s, CH_3-27), 1.19 (3H, s, CH_3-28), 1.24 (3H, s, CH_3-18), 1.25 (3H, s, CH_3-21), and 1.27 (3H, s, CH_3-26) and two doublet signals at δ 0.26 (1H, d, J = 4.0 Hz) and 0.59 (1H, d, J = 4.0 Hz) corresponding to H-19, which suggested that compound **1** belonged to the cycloartane-type triterpenoid saponin. Close inspection of the ^{1}H-NMR spectrum revealed the presences of two sugar anomeric protons at δ 4.43 (1H, d, J = 7.5 Hz) and 4.32 (1H, d, J = 7.5 Hz) attributed to Xyl-1′ and Glc-1″, respectively, as well as two methyl groups at δ 2.07 (3H, s) and 1.98 (3H, s) attributed to acetyl groups. In comparison with ^{1}H-NMR and ^{13}C-NMR spectral data of isoastragaloside I (**5**), the two sugar groups could be located at C-3 and C-6 based upon the down-field shifts of C-3 at δ 90.6 and C-6 at δ 80.6, while the acetyl groups were established at C-3′ and C-4′ on the basis of the down field shifts of H-3′ at δ 5.00 and H-4′ at δ 4.80.

The proposed structure of compound **1** was verified by HMBC experiment (Fig. 1). Thus the structure of compound **1** was established as 3-*O*-3′, 4′-diacetyl-β-*D*-xylopyranosyl-6-*O*-β-*D*-glucopyranosyl-cycloastragenol (Fig. 1).

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Table 1 ^{13}C-NMR (125 MHz, CD_3OD) of compounds 1 and 5 and ^{1}H-NMR (500 MHz, CD_3OD) of compound 1

No.	1		5	No.	1		5
	δ_C	δ_H	δ_C		δ_C	δ_H	δ_C
1	34.6	1.69 (m); 1.62 (m)	34.5	23	27.5	1.9 (m); 1.36 (m)	27.3
2	33.4	1.61 (m); 1.36 (m)	33.1	24	83.1	3.65 (t)	82.9
3	90.6	3.24 (m)	90.5	25	72.9	—	72.8
4	43.6	—	43.1	26	29.0	1.27, 3H (s)	28.8
5	53.7	1.62 (m)	53.5	27	27.2	1.12, 3H (s)	28.7
6	80.6	3.42 (m)	80.4	28	29.0	1.19, 3H (s)	30.1
7	35.9	2.61, 2H, dd	35.8	29	17.1	1.01, 3H (s)	16.9
8	47.2	1.83 (m)	47.1	30	20.7	1.00, 3H (s)	20.5
9	22.6	—	22.4	C3-			
10	30.8	—	30.6	Xyl-1′	107.4	4.43 (d, J = 7.5 Hz)	105.2
11	30.4	1.91 (m); 1.69 (m)	27.0	2′	73.8	3.42 (m)	75.8
12	35.6	1.84, 2H (m)	35.5	3′	76.3	5.00 (t, J = 8.5 Hz)	73.5
13	46.5	—	46.3	4′	71.6	4.80 (m)	73.3
14	47.6	—	46.5	5′	63.6	3.98 (m); 3.35 (m)	63.7
15	46.7	2.02 (s); 1.4 (m)	47.4	2′,3′-OAc	172.6	2.07 (s)	172.3
					21.4		21.7
16	75.1	4.64 (m)	75.0	4′-OAc	172.2	1.98 (s)	172.0
					21.1		21.0
17	59.4	2.35 (d , J = 8.0 Hz)	59.3	C6-			
18	21.9	1.24, 3H (s)	21.5	Glc-1″	105.4	4.32 (d, J = 7.5 Hz)	105.3
19	30.2	0.26 (d, J = 4.0 Hz);	30.2	2″	76.1	3.18 (m)	76.0
		0.59 (d, J = 4.0 Hz)		3″	79.1	3.38 (m)	78.9
20	88.9	—	88.7	4″	72.3	3.29 (m)	72.1
21	28.1	1.25, 3H (s)	27.9	5″	78.2	3.30 (m)	78.0
22	43.3		43.1	6″	63.5	3.84 (m); 3.64 (m)	63.3

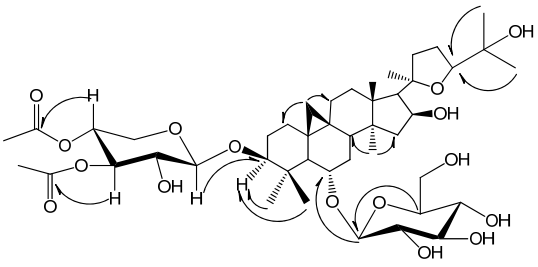

Fig. 1 Structure and key HMBC correlation of compound 1

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